eppendorf

Reach Your 100%

Your success is our purpose

Your time is precious not only in terms of cost. However, a drug's journey from concept to patient takes years. You need to rely on products and services that ensure maximum efficiency at every step.

Our high-quality solutions are all engineered around the kind of functionality and intuitive performance necessary to speed up workflows, secure reproducibility, and accelerate the desired results.

Let's work together to make our world a better place.

www.eppendorf.com/pharma

Received: 28 June 2021 | Revised: 20 August 2021 | Accepted: 12 September 2021

DOI: 10.1002/ddr.21886

RESEARCH ARTICLE

DRUG DEVELOPMENT RESEARCH WILEY

Innovative antimicrobial substances based on uracil S-derivatives

Svetlana Meshcheryakova[2] | Alina Shumadalova[2] | Ozal Beylerli[3] | Ilgiz Gareev[3] | Shiguang Zhao[1] | Jianing Wu[1]

[1]Department of Neurosurgery, Shenzhen University General Hospital, Guangdong, China

[2]Department of General Chemistry, Bashkir State Medical University, Ufa, Russia

[3]Central Research Laboratory, Bashkir State Medical University, Ufa, Russia

Correspondence
Jianing Wu, MD, PhD, Department of Neurosurgery,Shenzhen University General Hospital, Xueyuan AVE 1098, Nanshan District, Shenzhen, Guangdong, China.
Email: wujning@hotmail.com

Abstract

The problem of antimicrobial resistance is an important global public health challenge. We propose that a development of new antibiotic compounds around known natural substances is a solution to this problem. We investigate reengineer natural products into potent antibiotics. Uracil fragment is abundant in nature and significant to treat infectious diseases due to its affection to the replication of the bacterial chromosome. 12 new uracil S-derivatives were obtained and tested for their in vitro antimicrobial properties. N^3-(thietan-3-yl)- and N^3-(1,1-dioxothietan-3-yl)uracils derivatives were synthesized by thietanylation of 6-methyluracil with 2-chloromethylthiirane and subsequent oxidation of the thietan ring. A method of their alkylation with ethyl-2-chloroacetate was developed and acetohydrazides containing 3-(thietan-3-yl)- and 3-(1,1-dioxothietan-3-yl)uracilyls fragments in the acetyl group were obtained by hydrazinolysis of 2-(thietanyluracil-1-yl)acetic acid ethyl esters. Their interaction with β-dicarbonyl compounds, anhydride of mono- and dicarboxylic acids was studied. Antimicrobial activity was determined by the agar diffusion method on test organisms: *S. aureus*, *E. coli*, *P. vulgaris*, *K. pneumoniae*, *C. diversus*, *E. aerogenes*, *P. aeruginosa*, *S. abosit*. *N*-acyl-5-hydroxypyrazolines and *N*,*N*′-diacylhydrazines of 6-methyluracil thietanyl- and dioxothietanyl derivatives showed high antimicrobial activity, which is consistent with the results of structure activity relationship analysis (MIC 0.1–10 μg/ml).

KEYWORDS
antibacterial and antifungal activities, pyrimidine, thietan

1 | INTRODUCTION

Infectious diseases are the main causes of morbidity and mortality worldwide. Nowadays many infections are caused by multi-resistant microorganisms resulting in difficult to treat diseases. The emergence of antimicrobial drug resistance poses a serious threat to public health (Pieri, et al., 2020; Spellberg & Gilbert, 2014). Natural sources are attracted the attention of pharmaceutical and scientific communities, and an evidence is demonstrated the promising potential of alternative antimicrobials (Martelli & Giacomini, 2018; Rossiter, Fletcher, & Wuest, 2017). We develop synthetic approaches aiming to reengineer natural substances into potent antibiotics and propose that the development of new antibiotics around known natural compounds is the best short-term solution to the rising crisis of antibiotic resistance.

Uracil fragment is one of the constituents of the ribonucleic acid. It is significant to treat infectious diseases due to its affection to the replication of the bacterial chromosome. Its pyrimidine skeleton exists in many natural products such as vitamin B_1 (thiamine) and many synthetic compounds, that posses antibacterial, antifungal, antileishmanial, anticancer, anti-inflammatory, and analgesic activities hence, they attract considerable attention in the design of biologically active molecules (Sharma, Rane, & Gurram, 2014; Agarwal, et al., 2002; Agarwal, et al., 2000; Ram, Haque, & Guru, 1992; Prachayasittikul, et al., 2017; Xie, Zhao, H., Zhao, L., Lou, & Hu, 2009; Amir, Javed, & Kumar, 2007;

Sondhi, Jain, Dwivedi, Shukla, & Raghubir, 2008; Vega, Alonso, Diaz, & Junquera, 1990). Our research group investigate innovative thietan derivatives as they posses a broad spectrum of bioactivities: antimicrobial, broncholytic and wound healing (Kataev, Meshcheryakova, Lazarev, & Kuznetsov, 2013; Kataev, et al., 2018; Meshcheryakova, Kataev, Fattakhova, Nikolaeva, & Bulgakov, 2015; Meshcheryakova, Kataev, Munasipova, Shumadalova, & Bulgakov, 2017; Meshcheryakova, Kataev, & Galimova, 2013; Gabdrakhmanova, et al., 2019; Khaliullin, et al., 2021; Spasov, et al., 2017; Smirnova, et al., 2021). Structural modification of uracil by adding thietan fragment is an efficient way to get new antimicrobial compounds. The aim of this study was to obtain substances based on uracil S-derivatives and to investigate their antibacterial and antifungal properties.

2 | MATERIALS AND METHODS

The reagents used in the experiments were all commercially available without further purification. Each reaction was monitored by thin layer chromatography (TLC) on Silufix and Sorbfil plates using ethanol (A) and acetone-acetonitrile mixtures [volume ratio 1:1, (B)]. Visualization on TLC was achieved by 254 nm ultraviolet (UV) light or iodine indicator.

Melting points (m.p.) were recorded on PTP-M (Russia) melting point apparatus uncorrected. Infrared (IR) spectra were recorded in KBr disks or in vaseline oil on an Infralum FT-02 (Russia) spectrophotometer. ^{1}H nuclear magnetic resonance (NMR) spectra were recorded on Bruker AMX-300300 MHz in deuterated dimethyl sulfoxide (DMSO-d_6) using tetramethylsilane as internal standard (chemical shifts were expressed as δ-values, J in hertz). CHNS elemental analyses were performed on a Hekatech Euro-EA (Germany) elemental analyzer. The results indicate a perfect agreement between the experimental and calculated percentages of the C, H, N, and S atoms in all compounds, confirming their chemical structure (Table 1).

2-[6-methyl-2,4-dioxo-3-(thietan-3-yl)-1,2,3,4-tetrahydropyrimidin-1-yl]acetohydrazide (1) was synthesized as previously described (Meshcheryakova, et al., 2014a).

2-[3-(1,1-Dioxothietan-3-yl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]acetohydrazide (6) was synthesized according to the procedure [Meshcheryakova, Kataev & Nikolaeva, 2014b.

1-[2-(5-Hydroxy-3-methyl-5-phenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl]-6-methyl-3-(thietan-3-yl)pyrimidine-2,4 (1*H*,3*H*)-dione (2). 0.65 g (4 mmol) of 1-phenylbutane-1,3-dione was added to a solution of 0.54 g (2 mmol) of compound **1** in 15 ml of ethanol and the mixture was refluxed for 3 h. The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol, dried, and finally crystallized from propan-2-ol (*i*-PrOH).

1-{2-[5-Hydroxy-3-methyl-5-(4-chlorophenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]-2-oxoethyl}-6-methyl-3-(thietan-3-yl)pyrimidine-2,4 (1*H*,3*H*)-dione (3) was obtained as like compound **2**, using 1-(4-chlorophenyl)butane-1,3-dione. Recrystallization from isobutanol (*i*-BuOH).

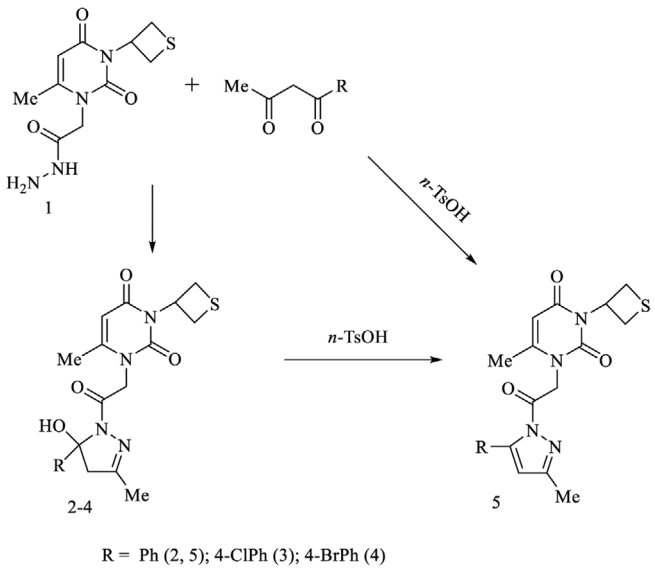

FIGURE 1 Synthetic route of the *N*-acyl-5-hydroxypyrazolines **2–4** and *N*-acylpyrazole **5**

TABLE 1 Yields and physicochemical characteristics of the synthesized compounds

Compound	Yields, % (method)	M.p., (°C)	R*f* (system)	Gross formula
2	64	159–161	0.71 (B)	$C_{20}H_{22}N_4O_4S$
3	69	223–225	0.70 (A)	$C_{20}H_{21}ClN_4O_4S$
4	60	210–211	0.77 (A)	$C_{20}H_{21}BrN_4O_4S$
5	59 (a), 76 (b)	245 with decomp	0.66 (A); 0.80 (B)	$C_{20}H_{20}N_4O_3S$
7	91	205–207	0.30 (B)	$C_{12}H_{16}N_4O_4S$
8	76	197–198	0.59 (A)	$C_{12}H_{16}N_4O_6S$
9	90	135–137	At the start line (A)	$C_{14}H_{18}N_4O_6S$
10	85	240 with decomp	0.10 (B)	$C_{14}H_{16}N_4O_6S$
11	73	193–195	0.03 (B)	$C_{14}H_{18}N_4O_8S$
12	89	206–207	0.04 (B)	$C_{14}H_{16}N_4O_8S$

TABLE 2 Chemical shifts of protons in the ^{1}H NMR spectra of the synthesized compounds (300 MHz, DMSO-d_6, δH, ppm)

Compound	6-CH_3s, 3H	H^5s, 1H	$S(CH_2)_2$m, 2H	$S(CH_2)_2$m, 2H	NCHm, 1H	1-CH_2COs, 2H	Chemical shifts of protons β-dicarbonyl and acyl fragments
2	2.07	5.61	3.04–3.18 (+2H, $C^{4'}H_2$ pyrazole)	4.11–4.19	6.00–6.06	4.93	2.05 s (3H, 3′-CH_3pyrazole); 7.03 s (1H, 5′-OH); 7.24–7.37 m ($5H_{arom}$)
3	2.07	5.61	3.03–3.12 (+2H, $C^{4'}H_2$ pyrazole)	4.10–4.18	6.00–6.05	4.91	2.04 s (3H, 3′-CH_3pyrazole); 7.16 s (1H, 5′-OH); 7.37 wid s ($4H_{arom}$)
4	2.07	5.61	3.05–3.12 (+2H, $C^{4'}H_2$ pyrazole)	4.11–4.18	6.00–6.06	4.91	2.04 s (3H, 3′-CH_3pyrazole); 7.17 s (1H, 5′-OH); 7.31 d ($2H_{arom}$, ^3J 7.5 Hz); 7.51 d ($2H_{arom}$, ^3J 7.5 Hz)
5	2.21	5.69	3.06–3.12	4.10–4.16	6.00–6.07	5.41	2.33 s (3H, 3′-CH_3pyrazole); 6.59 s (1H, $H^{4'}$ pyrazole); 7.37–7.46 m ($5H_{arom}$)
8	2.22	5.77	4.29–4.36	4.83–4.90	5.63–5.69	4.57	1.85 s (3H, CH_3); 9.89 wid s (1H, NH); 10.13 wid s (1H, NH)
9	2.18	5.67	3.06–3.12	4.14–4.20	5.97–6.08	4.54	2.37–2.44 m (4H, $2CH_2$); 9.92 wid s (1H, NH); 10.18 wid s (1H, NH); 11.89 wid s (1H, OH)
10	2.18	5.68	3.07–3.12	4.15–4.20	6.02–6.08	4.58	6.26 d (1H, CH, ^3J 12.1 Hz); 6.36 d (1H, CH, ^3J 12.1 Hz); 10.52 wid s (1H, NH); protons COOH and NHCO exchange

1-{2-[5-(4-Bromophenyl)-5-hydroxy-3-methyl-4,5-dihydro-1*H*-pyrazol-1-yl]-2-oxoethyl}-6-methyl-3-(thietan-3-yl)pyrimidine-2,4(1*H*,3*H*) -dione (4) was obtained as like compound **2**, using 1-(4-bromophenyl) butane-1,3-dione. Recrystallization from propan-1-ol (PrOH).

6-Methyl-1-[2-(3-methyl-5-phenyl-1*H*-pyrazol-1-yl)-2-oxoethyl]-3-(thietan-3-yl)pyrimidine-2,4(1*H*,3*H*)dion (5). *Method a*. 0.65 g (4 mmol) of 1-phenylbutane-1,3-dione and 0.008 g (2 mol%) of *p*-toluenesulfonic acid were added to a suspension of 0.54 g (2 mol%) of compound **1** in 15 ml of ethanol, and the mixture was refluxed for 9 h. The reaction mixture was cooled; the precipitate was filtered off, washed with ethanol, and dried.

Method b. 0.008 g (2 mmol) of *p*-toluenesulfonic acid was added to a solution of 0.83 g (2 mmol) of compound **2** in 15 ml of ethanol, boiled for 5 h. The reaction mixture was cooled, the precipitate was filtered off, washed with ethanol, and dried. Recrystallization from PrOH.

***N′*-Acetyl-2-[6-methyl-2,4-dioxo-3-(thietan-3-yl)-1,2,3,4-tetrahydropyrimidin-1-yl] acetohydrazide (7)**. 0.81 g (3 mmol) of hydrazide **1** was dissolved by heating in 40 ml of 1,4-dioxane, the solution was cooled to 25°C, and 0.55 g (6 mmol) of acetic anhydride was added, stirred for 2 h. The precipitate was filtered off, washed with 1.4-dioxane, dried. Recrystallization from *i*-PrOH.

***N′*-Acetyl-2-[3-(1,1-dioxothietan-3-yl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]acetohydrazide (8)** was obtained as like compound **7** using compound **6**. Recrystallization from ethanol (EtOH).

4-(2-{2-[6-Methyl-2,4-dioxo-3-(thietan-3-yl)-1,2,3,4-tetrahydropyrimidin-1-yl]acetyl}hydrazinyl)-4-oxobutanoic acid (9). 0.81 g (3 mmol) of hydrazide **1** was dissolved by heating in 40 ml of 1,4-dioxane, the solution was cooled to 25°C and 0.33 g (3.3 mmol) of succinic anhydride was added, stirred for 2 h. The reaction mixture was left for 12 h, then the precipitate was filtered off, washed with 1,4-dioxane, and dried. Recrystallization from ethyl acetate.

4-(2-{2-[6-Methyl-2,4-dioxo-3-(thietan-3-yl)-1,2,3,4-tetrahydropyrimidin-1-yl]acetyl}hydrazinyl)-4-oxobut-2-enoic acid (10) was prepared as like compound **9** using maleic anhydride. Recrystallization from EtOH.

4-(2-{2-[3-(1,1-Dioxothietan-3-yl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]acetyl}hydrazinyl)-4-oxobutanoic acid (11) was obtained as like compound **9** from compound **6**. Recrystallization from EtOH.

4-(2-{2-[3-(1,1-Dioxothietan-3-yl)-6-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl]acetyl}hydrazinyl)-4-oxobut-2-enoic acid (12) was obtained as like compound **10** from compound **6**. Recrystallization from methanol (MeOH).

2.1 | Biological studies

Antibacterial and antifungal activities of the novel pyrimidine-2,4(1*H*,3*H*)-dione thietan-containing derivatives were analyzed using the agar diffusion and the tenfold broth (pH 7.2–7.4) dilution methods

(Mironov, 2012). Microbial strains of the department of Microbiology and Virology, Bashkir State Medical University deposited at L.A. Tarasevich State Institute of Standardization and Control of Biomedical Preparations, the Ministry of Health of the Russian Federation were used as test organisms: *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Klebsiella pneumoniae*, *Citrobacter diversus*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Serratia abosit* and lower fungi *Candida albicans*. Ceftriaxone (Lumi LLC, Russia) and pimafucin (natamycin, Astellas, Netherlands) were taken as reference standards. Test compounds (100 mg) and reference standards were weighed and dissolved in 1 ml DMSO. These solutions were diluted in beef extract broth to achieve a final concentration of 10 mg/ml (stock solution). The nutrient broth inoculated with 2.0×10^6 colony forming units (c.f.u)/ml, was used. The cultures were incubated for 72 h at 37°C and for 48 h at 25°C, then the growth was monitored visually. The lowest concentration (highest dilution) required to arrest the growth of bacteria was regarded as minimum inhibitory concentration (MIC).

3 | RESULTS

3.1 | Chemistry

We have studied the interaction of acetohydrazide containing 3-(thietan-3-yl)-2,4-dioxotetrahydropyrimidin-1-yl fragment in the acetyl group with β-dicarbonyl compounds - aromatic acids acylacetones. The interaction of acid hydrazides with β-dicarbonyl compounds, depending on the structures of the *N*-acyl substituent in the hydrazine component and the β-dicarbonyl component, leads to the formation of pyrazole, 5-hydroxypyrazoline, hydrazone, or enhydrazine derivatives (Pakal'nis, Zerova, & Yakimovich, 2007; Rutavicius, Valulene, & Kuodis, 1997; Rutavicius, 2000; Yakimovich, Zerova, & Pakal'nis, 2008).

Thus, *N*-acyl-5-hydroxypyrazolines **2–4** were obtained in 60%–69% yields by the reaction of hydrazide **1** with aryl acetones in boiling ethanol without the use of acid catalysts (Figure 1).

The structure of the synthesized compounds was established by a complex of NMR spectroscopy methods (Table 2). Raw NMR data presented in Supplementary information.

All the new compounds **1–12** were characterized by IR spectroscopic data (Table 3).

After that we studied the acylation reactions of acetohydrazides containing 3-(thietan-3-yl)- and 3-(1,1-dioxothietan-3-yl)-2,4-dioxotetrahydropyrimidin-1-yl fragments in the acetyl group, with mono- and dicarboxylic acid anhydrides.

Reactions of hydrazides **1** and **6** with acids anhydrides (acetic, succinic and maleic) under mild conditions - in an inert solvent - 1,4-dioxane at a temperature of 20–25°C, proceed regioselectively and lead to the formation of *N*,*N*′-diacyl derivatives **7–12** in yields 73%–91% (Figure 2).

Compounds **2–5**, **7–12** are white crystalline substances, soluble in DMSO, dimethylformamide (DMF), in lower alcohols (on heating), compounds **9–12** are soluble in solutions of hydroxides and

TABLE 3 IR spectral data of synthesized compounds

Compound	Stretching vibrations, ν, cm^{-1}
2	3318 m (O—H), 2971 w (C—H), 1707, 1681, 1665 s (C^2=O, C^4=O, C=O, C=N, C=C), 1457, 1443, 1397 s (C—N, δC—H)
3	3453 m (O—H), 2985 w (C—H), 1706, 1688, 1657 s (C^2=O, C^4=O, C=O, C=N, C=C), 1431, 1414, 1397 s (C—N, δC—H)
4	3382 m (O—H), 2953 w (C—H), 1700, 1664, 1623 s (C^2=O, C^4=O, C=O, C=N, C=C), 1431, 1386 s (C—N, δC—H)
5	2983 w (C—H), 1704, 1662 s (C^2=O, C^4=O, C=O, C=N, C=C), 1464, 1443, 1383 m (C—N, δC—H)
7	3215 m (N—H), 1710 s (C^2=O), 1655, 1616 s (C^4=O, C=O, C=C), 1551 m («amide II»), 1484, 1441 m (C—N, δC—H)
9	3327, 3258 b (O—H, N—H), 1716, 1667, 1634, 1567 s (C^2=O, C^4=O, C=O, C=C), 1568 s, 1524 m («amide II»), 1427, 1400 m (C—N, δC—H), 1296, 1255 m (C—N)
10	3327, 3243 b (O-H, N-H), 1712, 1651, 1601 s (C^2=O, C^4=O, C=O, C=C), 1548 s («amide II»), 1451, 1438, 1412 s (C—N, δC—H)
11	3350, 3278 b (O—H, N—H), 1704, 1655, 1594 s (C^2=O, C^4=O, C=O, C=C), 1443, 1389 m (C—N, δC—H), 1320 (ν_{as}), 1148 (ν_s) s [S(=O)$_2$], 1230 m (C—N)
12	3350, 3292 b (O—H, N—H), 1652, 1611, 1572 s (C^2=O, C^4=O, C=O, C=C), 1566 s («amide II»), 1421 m (C—N, δC—H), 1320 (ν_{as}), 1146 (ν_s) s [S(=O)$_2$], 1298 s, 1230 m (C—N)

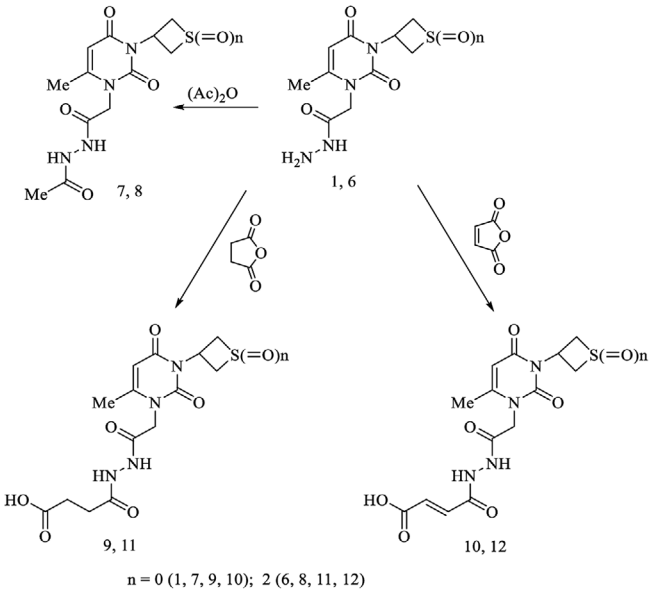

FIGURE 2 Formation of *N*, *N*′-diacyl derivatives **7–12** by reactions of hydrazides **1, 6** with acids anhydrides (acetic, succinic, and maleic)

TABLE 4 Antimicrobial and antifungal activity of new derivatives of thietanylpyrimidine-2,4 (1*H*, 3*H*) –diones

	Minimum inhibitory concentrations (MIC), μg/ml								
Compound	***S. aureus***	***E. coli***	***P. vulgaris***	***Klebsiella pneumoniae***	***C. diversus***	***Enterobacter aerogenes***	***P. aeruginosa***	***S. Abosit***	***C. albicans***
1	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
2	1	10	1	1	1	1	0.1	10	1
3	0.1	1	1	1	1	1	0.1	1	1
4	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	1
5	1	10	10	10	0.1	1	1	10	10
6	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000	>10000
7	10	10	1	1	0.1	1	1	10	1
8	0.1	1	1	1	0.1	0.1	0.1	0.1	0.1
9	0.1	1	1	1	0.1	1	1	1	0.1
10	0.1	10	0.1	0.1	0.1	0.1	0.1	0.1	10
11	0.1	10	0.1	0.1	0.1	0.1	0.1	0.1	10
12	1	1	0.1	1	1	0.1	0.1	1	10
Ceftriaxone	1	0.1	0.1	0.1	1	0.1	10	0.1	0.1
Pimafucin									1

hydrocarbonates of alkali metals. The individuality of the synthesized compounds was confirmed by TLC and m.p.

3.2 | Antimicrobial and antifungal activity

Synthesized compounds were further screened for minimum inhibitory concentration against test organisms: *S. aureus*, *E. coli*, *P. vulgaris*, *K. pneumonia*, *C. diversus*, *E. aerogenes*, *P. aeruginosa*, *S. abosit*, *C. albicans*. Minimum inhibitory concentration values are given in Table 4.

4 | DISCUSSION

New uracil S-derivatives have been successfully obtained and tested as potential antimicrobial and antifungal agents.

In the ^{1}H NMR spectra of the condensation products of hydrazide **1** with aroylacetones, only one set of chemical signals is observed, confirming the regioselectivity of the reaction. The study of these spectra showed that compounds **2–4** have a 5-hydroxypyrazoline structure (Figure 1). Similar to the data of (Rutavicius, Valulene, & Kuodis, 1997), the dehydration of 5-hydroxypyrazolines **2–4** under these conditions is difficult due to the –*I*-effect of the phenyl substituent, which prevents the elimination of the hydroxyl group.

In the spectra of compounds **2–4** (Table 2), the signals of the CH_2 group protons of the pyrazoline system are masked by a pseudotriplet of one of the $S(CH)_2$ groups protons of the thietan ring and are recorded as a combined multiplet in the 3.03–3.18 ppm range. With an intensity of four protons. Singlet signal at 7.03–7.17 ppm belongs to the hydroxyl group proton, the position of which is typical for the proton of the OH group involved in the formation of a strongly conjugated intramolecular hydrogen bond (IMHB) with the carbonyl group, and confirms that 5-hydroxypyrazolines 2–4 in DMSO-d_6 have exclusively *E*-conformational structure relative to the hydrazide bond (Pakal'nis, Zerova, & Yakimovich, 2007; Rutavicius, 2000). The presence in the spectra of compounds **2–4** of the signals of the methyl group protons in the lower-field region at 2.04 or 2.05 ppm, indicates that the condensation occurs at the carbonyl group adjacent to the methyl group.

The 5-hydroxypyrazoline structure of compounds **2–4** is also consistent with the presence of a narrow absorption band in the region of 3453–3318 cm^{-1}, corresponding to the stretching vibrations of the hydroxyl group, and the absence of absorption above 1710 cm^{-1}, characteristic of the non-conjugated carbonyl group of *N*-acylhydrazones and *N*-acylpyrazoles (Table 3).

We have studied the interaction of hydrazide **1** with aromatic acids acetones using an acid catalyst - *p*-toluenesulfonic acid. Spectral studies have shown that *N*-acylpyrazole **5** is the condensation product of hydrazide with benzoylacetone in the presence of *p*-toluenesulfonic acid (2 mol%) (Figure 1), while according to ^{1}H NMR data, the reaction with aroylacetones containing halogen atoms in the phenyl radical yielded substances identical *N*-acyl-5-hydroxypyrazolines **3** and **4**.

N-Acylpyrazole **5** was also obtained by dehydration of *N*-acyl-5-hydroxypyrazoline **2** in the presence of catalytic amounts of *p*-toluenesulfonic acid (Figure 1).

The ^{1}H NMR spectrum of compound **5** (Table 2) shows a singlet signal at 6.59 ppm. Intensity of one proton, assigned to the proton (=CH) in position 4′ of the pyrazole ring, and there are no signals in the region of 3.04–3.18 ppm, which could be attached to the methylene protons of the hydrazone or 5-hydroxypyrazoline structures. In the weak fields region, signals of NH protons of the enhydrazine or hydrazone forms are also not observed. IR spectroscopic data also confirm that compound **5** (Table 3) have a pyrazole structure.

In the high-frequency region above 3000 cm^{-1}, there are no absorption bands of stretching vibrations of the hydroxyl group.

The structure of *N*,*N*′-diacylhydrazines **8, 9,** and **10** is confirmed by ^1H NMR spectra (Table 2), in which, in addition to the characteristic signals of the protons of the 6—CH_3 and H^5 groups of the pyrimidine fragment, thietan (compounds **9** and **10**), thietan-1,1-dioxide (compound **8**) rings, a singlet of the protons of the 1—CH_2CO group in the region of 4.54–4.58 ppm, two broadened singlets of the HN—NH protons of the hydrazide residue in the 9.89–10.52 ppm region are recorded (each of one proton intensity). The spectrum of compound **8** shows a singlet at δH 1.85 ppm of the acetyl residue methyl protons. The protons of the succinic acid residue of compound **9** appear as a multiplet with an intensity of four protons in the 2.37–2.44 ppm range (group CH_2-CH_2) and a broadened singlet at δH 11.89 ppm. (OH group), and the vinyl protons of the maleic acid residue of compound **10** as two doublets with an intensity of one proton each at 6.26 and 6.36 ppm with vicinal spin–spin coupling constant of 12.1 Hz.

In the IR spectra of *N*,*N*′-diacylhydrazines **7**, **9–12**, the region of 3350–3215 cm^{-1} is characterized by the manifestation of one common or two broad absorption bands of stretching vibrations of the H—N—N—H bonds of the hydrazide fragment (Table 3). In the region of 1716–1567 cm^{-1}, a split intense absorption band is observed due to stretching vibrations of multiple bonds (C═O, C═C), and in the region of 1484–1389 cm^{-1} it is less intense band with several maxima associated with stretching vibrations of bonds C—N and deformation vibrations of C—H bonds. The absorption bands in the spectra of *N*,*N*′-diacylhydrazines in the 1566–1524 cm^{-1} range are attached to vibrations of the "amide II" type.

Two strong characteristic absorption bands are observed in the IR spectra of thietan-1,1-oxides **11** and **12**: the band with a frequency in the region 1320 cm^{-1} corresponds to asymmetric vibrations, and with a frequency in the region 1148–1146 cm^{-1} - to symmetric stretching vibrations of bonds $S(=O)_2$.

The PASS predicted biological activity showed that pyrimidine-2,4(1*H*,3*H*)-diones thietan- containing derivatives: *N*-acyl-5-hydroxypyrazolines and *N*,*N*′-diacylhydrazines – can exhibit antimicrobial (P_a-P_i 0.30–0.25) and antifungal (P_a-P_i 0.63–0.29) activity.

The synthesized *N*-acyl-5-hydroxypyrazolines and *N*,*N*′-diacylhydrazines exhibited high antimicrobial activity, which is consistent with the prediction results in PASS system, MIC values were 0.1–10 μg/ml against the test cultures, whereas acetylhydrazides **(1** and **6)** at a concentration of 10,000 μg/ml did not suppress the growth of the strains of microorganisms (Table 4). *N*-Acyl-5-hydroxypyrazoline **4** and *N*,*N*′-diacylhydrazines **(8–11)** exceeded the effect of ceftriaxone against *S. aureus*, *C. diversus*, *P. aeruginosa*. Compounds **4**, **8**, **10**, and **11** (MIC 0.1 μg/ml) are the most active in relation to the most of gram-negative test cultures (Table 4).

N-Acyl-5-hydroxypyrazolines **(2–4)**, *N*,*N*′-diacylhydrazine **(7)** showed antifungal activity against lower fungi *C. albicans* equal to pimafucin (MIC 1 μg/ml), and *N*, *N*′-diacylhydrazines **(8** and **9)** were 10 times more active than pimafucin (MIC 0.1 μg/ml). Compounds **3, 4, 8–11** demonstrated a high inhibition of *E. coli*, *P. aeruginosa*, *S. abosit*.

The structure–activity studies showed that, depending on the oxidation state of the sulfur atom, the newly synthesized compounds exhibit varying degrees of microbial inhibition. Dioxothietan-containig compounds that are obtained by interaction with acetic anhydride and succinic anhydride (compounds **8** and **11**) are generally more effective against tested microorganisms than thietan-containig derivatives (compounds **7** and **9**). Dioxothietan containing derivatives (compounds **8**, **11**, and **12**) are more effective against all tested microorganisms than thietan derivatives (compounds **7**, **9**, and **10**).

The introduction of acetyl (compounds **7** and **8**), 4-oxobut-2-enoic acid (compounds **10** and **12**), 4-oxobutanoic acid (compounds **9** and **11**) fragments improved antibacterial activity in comparison with parent acetohydrazide (compounds **1** and **6**). The presence of π-excessive imidazole heterocycle (compound **5**) decreases antimicrobial activity. Among the 5-hydroxypyrazoline derivatives the inhibitory effect appears to be dependent on the substitution at the pyrazoline fragment. 4-Bromophenyl derivative **4** showed a significant antibacterial activity greater than that of 1-phenyl (compounds **2**) and 4-chlorophenyl (compound **3**) derivatives.

5 | CONCLUSIONS

The newly synthesized pyrimidine-2,4(1*H*,3*H*)-dione thietan-containing derivatives were prepared by interaction of the corresponding acetohydrazides with β-dicarbonyl compounds and with mono- and dicarboxylic acid anhydrides. The synthesized compounds **1–12** were investigated for their in vitro antibacterial and antifungal activities. The synthesized *N*-acyl-5-hydroxypyrazolines, *N*-acylpyrazole, and *N*,*N*′-diacylhydrazines exhibited high antimicrobial activity against the test cultures (MIC values were 0.1–10 μg/ml), whereas acetylhydrazides **(1** and **6)** at a concentration of 10,000 μg/ml did not suppress the growth of the strains of microorganisms.

The obtained results show that it is promising to continue the search for new antimicrobial and antifungal drugs in the series of thietan-containing pyrimidine-2,4(1*H*,3*H*)-diones, the structure of which is fundamentally different from the known antibacterial drugs.

CONFLICT OF INTEREST

The authors declare no potential conflict of interest.

AUTHOR CONTRIBUTIONS

Conceptualization, Svetlana Meshcheryakova, Alina Shumadalova; methodology, Svetlana Meshcheryakova; software, Ozal Beylerli, Ilgiz Gareev; validation, Svetlana Meshcheryakova, Alina Shumadalova; formal analysis, Ozal Beylerli, Ilgiz Gareev; investigation, Svetlana Meshcheryakova, Alina Shumadalova; resources, Svetlana Meshcheryakova, Alina Shumadalova; data curation, Svetlana Meshcheryakova, Alina Shumadalova; writing—original

draft preparation, Svetlana Meshcheryakova, Alina Shumadalova; writing—review and ed-iting, Svetlana Meshcheryakova, Alina Shumadalova; visualization, Svetlana Meshcheryakova, Alina Shumadalova; supervision, Svetlana Meshcheryakova, Jianing Wu; project administration, Shiguang Zhao, Jianing Wu; funding acquisition, Jianing Wu. All authors have read and agreed to the published version of the manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Ilgiz Gareev https://orcid.org/0000-0002-4965-0835

REFERENCES

Agarwal, N., Srivastava, P., Raghuwanshi, S., Upadhyay, D., Sinha, S., Shukla, P., & Ram, J. V. (2002). Chloropyrimidines as a new class of antimicrobial agents. *Bioorganic and Medicinal Chemistry*, *10*, 869–874.

Agarwal, N., Raghuwanshi, S. K., Upadhyay, D. N., Shukla, P. K., & Ram, V. J. (2000). Suitably functionalised pyrimidines as potential antimycotic agents. *Bioorganic and Medicinal Chemistry Letters*, *10*, 703–706.

Amir, M., Javed, S. A., & Kumar, H. (2007). Pyrimidine as antiinflammatory agent: A review. *Indian Journal of Pharmaceutical Sciences*, *68*, 337.

Gabdrakhmanova, A. F., Meshcheryakova, S. A., Gaynutdinova, R., Kil'diyarov, R. F., Kurbanov, S. A., & Khuzhamberdiev, A. K. (2019). Experimental study of the effectiveness of 6-methyl-3-(thietane-3-yl) uracil-containing eye ointment in the treatment of corneal thermal burns. *Kazanskii Meditsinskii Zhurnal*, *100*(2019), 657–661.

Kataev, V. A., Meshcheryakova, S. A., Lazarev, V. V., & Kuznetsov, V. V. (2013). Synthesis of thietanyl-substituted pyrimidine-2,4(1H,3H)-dions. *Russian Journal of Organic Chemistry*, *49*, 743–745.

Kataev, V. A., Meshcheryakova, S. A., Meshcheryakova, E. S., Tyumkina, T. V., Khalilov, L. M., Lazarev, V. V., & Kuznetsov, V. V. (2018). Direction of the reaction of 6-methylpyrimidine-2,4(1H,3H)-dione with 2-chloromethylthiirane: N^1- or N^3-thietanyl derivative? *Russian Journal of Organic Chemistry*, *54*, 918–922.

Khaliullin, F. A., Nikitina, I. L., Klen, E. E., Miftakhova, A. F., Makarova, N. N., Gabidullin, R. A., & Gilmanova, A. G. (2021). Synthesis and antidepressant activity of 4-alkyl-5-bromo-2,4-dihydro-2-(thietan-3-yl)-1,2,4-triazol-3-ones. *Pharmaceutical Chemistry Journal*, *55*, 123–129.

Meshcheryakova, S. A., Kataev, V. A., Fattakhova, I. Y., Nikolaeva, K. V., & Bulgakov, A. K. (2015). Synthesis and antimicrobial activity of thietanylpyrimidin-2,4(1H,3H)-dione acetanilides and acetylhydrazones. *Pharmaceutical Chemistry Journal*, *49*, 598–601.

Meshcheryakova, S. A., Kataev, V. A., Munasipova, D. A., Shumadalova, A. V., & Bulgakov, A. V. (2017). Synthesis, structure, antimicrobial and antifungal activities of diacylhydrazines of thiethanylpyrimidine-2,4(1H,3H)-dione series. *Problems of Biological, Medical and Pharmaceutical Chemistry*, *20*, 20–25.

Meshcheryakova, S. A., Kataev, V. A., & Galimova, R. A. (2013). Synthesis and broncholytic activity of N-alkylpiperazino-substituted uracil and xanthine. *Pharmaceutical Chemistry Journal*, *47*, 247–250.

Meshcheryakova, S. A., Katayev, V. A., Nikolaeva, K. V., Perfilova, V. N., Borodin, D. D., & Tyurenkov, I. N. (2014a). Synthesis, isomery, and hypotensive activity of thiethaneacontaining ylidenehydrazides of uracilylacetic acid. *Bioorganicheskaia Khimiia*, *40*, 327–334.

Meshcheryakova, S. A., Kataev, V. A., & Nikolaeva, K. V. (2014b). Synthesis, isomerism of pyrimidinylacetic acid hydrazides containing 1-oxothietane and 1,1-dioxothietane cycles. *Bashkir Chemical Journal*, *21*, 21–24.

Martelli, G., & Giacomini, D. (2018). Antibacterial and antioxidant activities for natural and synthetic dual-active compounds. *European Journal of Medicinal Chemistry*, *158*, 91–105.

Mironov, A. N. (2012). *Handbook for Preclinical Drug Trials* (pp. 509–513). Grif and K.

Pakal'nis, V. V., Zerova, I. V., & Yakimovich, S. I. (2007). Reaction of aroyl- and hetaroyltrifluoroacetones with acylhydrazines: Regioselectivity and tautomerism of the condensation products. *Russian Journal of General Chemistry*, *77*, 1732–1741.

Pieri, A., Aschbacher, R., Fasani, G., Mariella, J., Brusetti, L., Pagani, E., Sartelli, M., & Pagani, L. (2020). Country income is only one of the tiles: The global journey of antimicrobial resistance among humans, animals, and anvironment. *Antibiotics*, *9*(8), 473.

Prachayasittikul, S., Pingaew, R., Worachartcheewan, A., Sinthupoom, N., Prachayasittikul, V., Ruchirawat, S., & Prachayasittikul, V. (2017). Roles of pyridine and pyrimidine derivatives as privileged scaffolds in anticancer agents. *Mini Reviews in Medicinal Chemistry*, *17*(10), 869–901.

Ram, V. J., Haque, N., & Guru, P. Y. (1992). Chemotherapeutic agents XXV: Synthesis and leishmanicidal activity of carbazolylpyrimidines. *European Journal of Medicinal Chemistry*, *27*, 851–855.

Rossiter, S. E., Fletcher, M. H., & Wuest, W. M. (2017). Natural products as platforms to overcome antibiotic resistance. *Chemical Reviews*, *117*(19), 12415–12474.

Rutavicius, A., Valulene, S., & Kuodis, Z. (1997). Synthesis and structure of hydrazones obtained on the basis of 1,3,4-thiadiazole-2,5-dithioglycolic acid dihydrazide. *Chemistry of Heterocyclic Compounds*, *1*, 137–140.

Rutavicius, A. (2000). Synthesis and structure of hydrazones obtained from hydrazides [5-(pyrid-4-yl)-1,3,4-oxiazol-2-ylthio]acetic or [5-(pyrid-4-yl) -1,3,4-oxiazol-2-ylthio]propionic acid. *Chemistry of Heterocyclic Compounds*, *7*, 966–971.

Sharma, P., Rane, N., & Gurram, V. K. (2014). Synthesis and QSAR studies of pyrimido[4,5-d]pyrimidine-2,5-dione derivatives as potential antimicrobial agents. *Bioorganic and Medicinal Chemistry Letters*, *14*, 4185–4190.

Smirnova, L. A., Spasov, A. A., Khaliullin, F. A., Kucheryavenko, A. F., Ryabukha, A. F., & Abramov, O. K. (2021). Biopharmaceutical study of an injectable dosage form of the new antiaggregant substance 3-methyl-8-(piperazin-1-yl)-7-(thietan-3-yl)-1-ethyl-1H-purine-2,6 (3*H*,7*H*)-dione hydrochloride. *Pharmaceutical Chemistry Journal*, *55*, 14–16.

Sondhi, S. M., Jain, S., Dwivedi, A. D., Shukla, R., & Raghubir, R. (2008). Synthesis of condensed pyrimidines and their evaluation for anti-inflammatory and analgesic activities. *Indian Journal of Chemistry—Section B Organic and Medicinal Chemistry*, *47*, 136–143.

Spasov, A. A., Khaliullin, F. A., Babkov, D. A., Timirkhanova, G. A., Kuznetsova, V. A., Naumenko, L. V., Muleeva, D. R., Maika, O. Y., Prokhorova, T. Y., & Sturova, E. A. (2017). Synthesis and antidiabetic activity of thiazolo[2,3-f]purine derivatives and their analogs. *Pharmaceutical Chemistry Journal*, *51*, 533–539.

Spellberg, B., & Gilbert, D. N. (2014). The future of antibiotics and resistance: A tribute to a career of leadership by John Bartlett. *Clinical Infectious Diseases*, *2*(Suppl 2), S71–S75.

Vega, S., Alonso, J., Diaz, J. A., & Junquera, F. (1990). Synthesis of 3-substituted-4-phenyl-2-thioxo- 1,2,3,4,5,6,7,8-octahydrobenzo[4,5] thieno[2,3-d]pyrimidines. *Journal of Heterocyclic Chemistry*, *27*, 269–273.

Xie, F., Zhao, H., Zhao, L., Lou, L., & Hu, Y. (2009). Synthesis and biological evaluation of novel 2,4,5-substituted pyrimidine derivatives for

anticancer activity. *Bioorganic & Medicinal Chemistry Letters*, *19*(1), 275–278.

Yakimovich, S. I., Zerova, I. V., & Pakal'nis, V. V. (2008). Reactions of 3-ethoxymethylidenepentane-2,4-dione and ethyl 2-ethoxymethylidene-3-oxobutanoate with benzohydrazide. *Russian Journal of Organic Chemistry*, *44*, 625–627.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Meshcheryakova, S., Shumadalova, A., Beylerli, O., Gareev, I., Zhao, S., & Wu, J. (2021). Innovative antimicrobial substances based on uracil S-derivatives. *Drug Development Research*, 1–8. https://doi.org/10.1002/ddr.21886