Synthesis of mycostatics based on 4-aryldiazenyl-3,5-dimethylpyrazoles*

O. G. Khudina,[a] A. E. Ivanova,[a] Ya. V. Burgart,[a] N. A. Gerasimova,[b] N. P. Evstigneeva,[b] and V. I. Saloutin[a]★

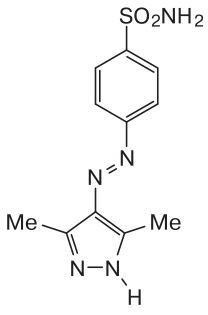
[a]*I. Ya. Postovsky Institute of Organic Synthesis, Ural Branch of the Russian Academy of Sciences, 22/20 ul. S. Kovalevskoi, 620137 Ekaterinburg, Russian Federation. Fax: +7 (343) 374 5954. E-mail: saloutin@ios.uran.ru*

[b]*Ural Research Institute of Dermatovenereology and Immunopathology, 8 ul. Shcherbakova, 620076 Ekaterinburg, Russian Federation*

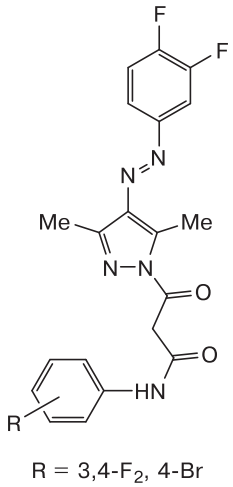
The condensation of 3-arylhydrazinylidenepentane-2,4-diones with hydrazine hydrate, 2-hydroxyethylhydrazine, benzylhydrazine hydrochloride, and 4-hydrazinylbenzenesulfonamide hydrochloride gave 4-aryldiazenyl-3,5-dimethylpyrazoles. An alternative route to the synthesis of *N*-substituted 4-aryldiazenylpyrazoles is based on the alkylation of NH-pyrazoles with haloalkanes. The synthesized compounds were tested for antimicrobial activity against eight pathogenic dermatophytes, yeast-like fungi of the *Candida* genus and the bacteria *Neisseria gonorrhoeae*. The structure—activity relationship analysis showed that 4-tolyldiazenylpyrazoles bearing H, $AcO(CH_2)_4$, or $HO(CH_2)_4$ substituents at the N(1) atom have the highest mycostatic activity against all the dermatophyte strains under study. However, 4-aryldiazenyl-3,5-dimethylpyrazoles proved to be quite cytotoxic against the *McCoy B* cell line.

Key words: 3-arylhydrazinylidenepentane-2,4-dione, condensation, hydrazines, 4-aryldiazenyl-3,5-dimethylpyrazoles, alkylation, antimycotic and antigonorrhoeae activities, cytotoxicity.

The 4-aryldiazenyl-3,5-dimethylpyrazole scaffold has a great potential for the design of antimicrobial agents.[1—4] For example, 4-[(3,5-dimethyl-1*H*-pyrazol-4-yl)diazenyl]-benzenesulfonamide exhibited stronger antifungal activity against the yeast-like fungi *Candida albicans* compared to nystatin.[1] This compound showed also high antibacterial activity against the Gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*. 3-{4-[(3,4-Difluorophenyl)diazenyl]-3,5-dimethyl-1*H*-pyrazol-1-yl}-3-oxo-*N*-arylpropanamides were shown to be able to effectively inhibit the growth of the Gram-negative bacteria *Escherichia coli* and the Gram-positive bacteria *Staphylococcus aureus*, and they also exhibited high antifungal activity against *Aspergillus niger*.[2] 4-Aryldiazenyl-3,5-dimethylpyrazoles containing an 4-arylthiazole substituent at the N(1) atom possess fungicidal activity against *Candida*

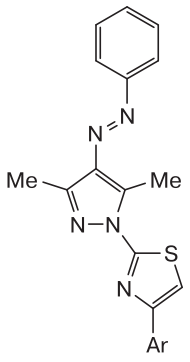


Antibacterial and antimycotic activity

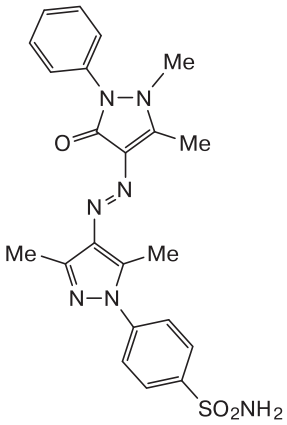


R = 3,4-F_2, 4-Br

Antibacterial and antimycotic activity



Antimycotic activity



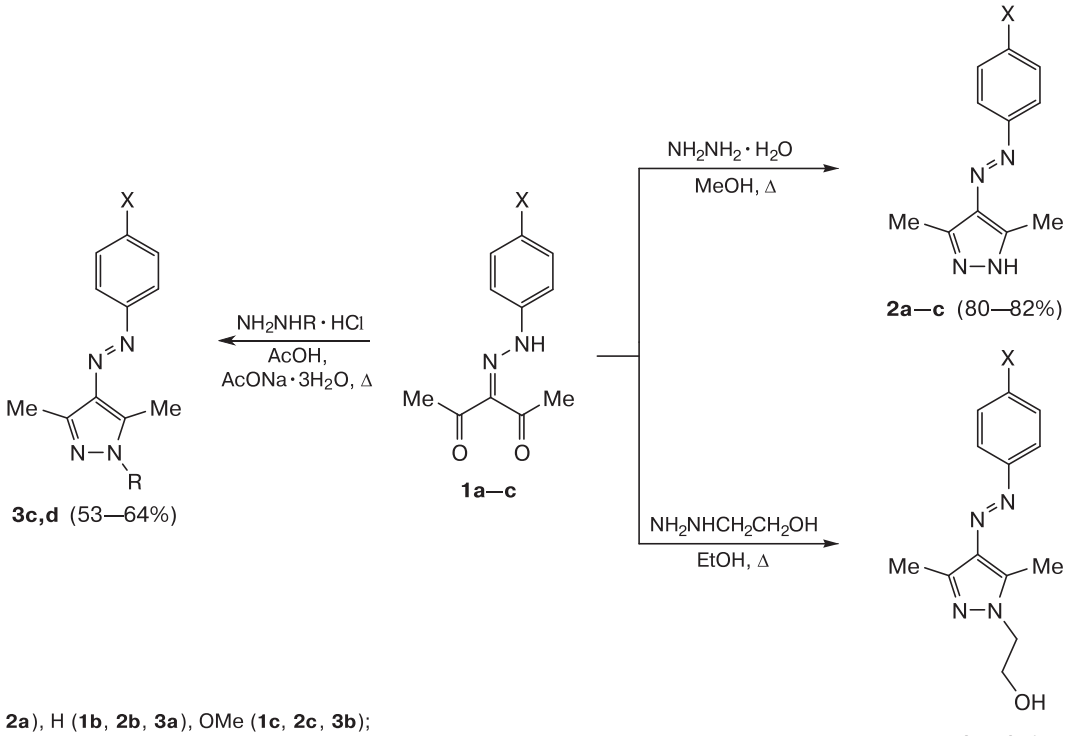
Antibacterial activity

* Dedicated to Academician of the Russian Academy of Sciences V. N. Charushin on the occasion of his 70th birthday.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 6, pp. 1124—1130, June, 2021.

1066-5285/21/7006-1124

Scheme 1



X = Me (**1a**, **2a**), H (**1b**, **2b**, **3a**), OMe (**1c**, **2c**, **3b**);
X = OMe, R = CH_2Ph (**3c**); X = OMe, R = $C_6H_4SO_2NH_2$-4 (**3d**)

albicans.[3] 4-Hetaryldiazenyl-3,5-dimethylpyrazole containing the antipyrine moiety and the *N*-benzenesulfonamide substituent exhibited, apart from antibacterial activity, an anti-inflammatory effect comparable to that of indomethacin, but did not have an ulcerogenic side effect.[4]

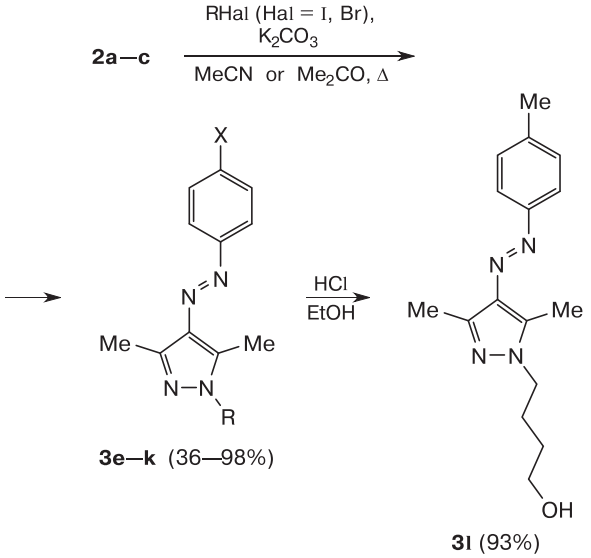
The prospects of the use of the 4-aryldiazenyl-3,5-dimethylpyrazole scaffold for the design of new antimycotics have provided the impetus for us to synthesize new derivatives of this series and evaluate their antifungal activity.

The condensation of 3-arylhydrazinylidenepentane-2,4-diones **1a—c** with hydrazine hydrate in methanol at 50 °C afforded N(1)-unsubstituted 4-aryldiazenyl-3,5-dimethylpyrazoles **2a—c** (Scheme 1), which have been previously[5] tested for antibacterial activity against *Escherichia coli*, *Bacillus cereus*, and *Proteus vulgaris*. We synthesized these compounds in order to evaluate their antimycotic and antigonorrhoeae effects.

The heating of arylhydrazones **1b,c** with 2-hydroxyethylhydrazine under reflux in ethanol gave derivatives of pyrazoles **2a—c**, heterocycles **3a,b** containing the 2-hydroxyethyl substituent at the N(1) atom. Pyrazole **3a** was prepared previously[6] in acetic acid; however only the melting point of this compound was reported, whereas the spectroscopic data were absent. We fully characterized this compound. Pyrazoles **3c**,**d** were synthesized by the condensation of arylhydrazone **1c** with benzylhydrazine and 4-hydrazinylbenzenesulfonamide hydrochlorides in acetic acid in the presence of sodium acetate (see Scheme 1).

A series of 4-aryldiazenyl-3,5-dimethylpyrazoles **3e—k** substituted at the N(1) atom were synthesized by the alkylation of *N*-unsubstituted pyrazoles **2** with haloalkanes in the presence of K_2CO_3 (Scheme 2). Pyrazole **3e** was

Scheme 2



3	R	X	**3**	R	X
e	$(CH_2)_4OAc$	Me	**i**	$(CH_2)_3CF_3$	H
f	$(CH_2)_4OAc$	OMe	**j**	$(CH_2)_3CF_3$	OMe
g	Et	H	**k**	$(CH_2)_2CF_3$	OMe
h	Et	OMe			

prepared previously[7] by the alkylation of *N*-unsubstituted pyrazole **2a** with 4-bromobutyl acetate followed by the removal of the acyl protecting group under acidic conditions and the formation of 4-hydroxybutylpyrazole **3l** described previously.[7] N(1)-Substituted 4-aryldiazenylpyrazoles **3f—h** were synthesized by the alkylation of NH-pyrazoles **2b,c** with 4-bromobutyl acetate and 1-iodoethane.

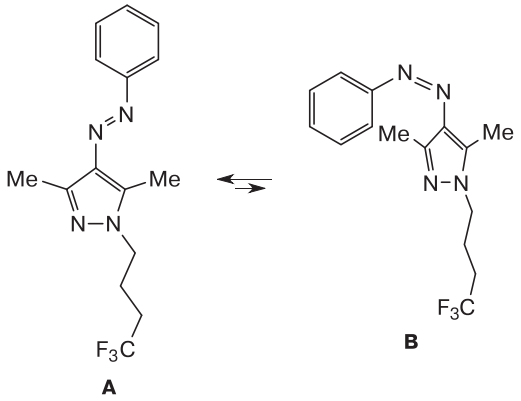
Organofluorine compounds have attracted increasing interest because electron-withdrawing fluorine atoms in substrates modulate various biological activities,[8,9] in particular antimycotic activity, as evidenced by the use of fluorine-containing agents (fluconazole and flucytosine) for the treatment of different fungal diseases.

Therefore, we introduced fluorine-containing substituents into 4-aryldiazenyl-3,5-dimethylpyrazoles. Thus, the alkylation of pyrazoles **2b,c** with 1-iodo-4,4,4-trifluorobutane and 1-iodo-3,3,3-trifluoropropane gave N(1)-trifluoroalkyl-substituted 4-aryldiazenylpyrazoles **3i—k** (see Scheme 2).

Products **3** can exist in the *trans*-azo and *cis*-azo forms. The ^{1}H and ^{19}F NMR spectroscopic studies showed that, immediately after the dissolution in $CDCl_3$, pyrazoles **3** exist in the sterically most favorable *trans*-azo form **A** (Scheme 3). However, after the storage in $CDCl_3$ for 3 h or longer, the ^{1}H NMR spectrum of compound **3i** shows the presence of 4.8% of the *cis*-azo form **B** (see the Experimental).

In the ^{1}H NMR spectra of the *cis*-azo form, the signals of all protons are significantly shifted upfield compared to

Scheme 3



the *trans*-azo form. The largest upfield shifts are observed for the *o*-protons of the aromatic ring (by 0.76 ppm) and the Me group (by 0.83 and 0.58 ppm). This may be due to the effect of the aromatic ring current on the Me groups and the anisotropic field effect, induced by the pyrazole ring, on the *o*-protons of Ph groups.

We evaluated the antimycotic and antibacterial activity of pyrazoles **2** and **3** against eight test strains of pathogenic dermatophyte fungi, the yeast-like fungi *Candida albicans* and the obligate bacterial pathogen *Neisseria gonorrhoeae* (Table 1). Fluconazole and spectinomycin were used as the positive control for the antimycotic and antibacterial assessment.

Table 1. Antimycotic and antibacterial activity of pyrazoles **2** and **3** (MIC is the minimum inhibitory concentration)

Compound	MIC/μg mL^{-1}									
	T. rubrum	*T. gypseum*	*T. tonsurans*	*T. violaceum*	*T. interdigitale*	*T. schoenleinii*	*E. floccosum*	*M. canis*	*C. albicans*	*N. gonorrhoeae*
2a	6.25	6.25	6.25	12.50	25.00	12.50	6.25	25.00	50.00	—[a]
2b	25.00	25.00	50.00	25.00	25.00	25.00	25.00	50.00	50.00	250.00
2c	7.80	31.20	7.80	31.20	—[a]	62.50	7.80	7.80	1000.00	125.00
3a	50.00	>200.00	100.00	100.00	50.00	50.00	100.00	50.00	>200.00	125.00
3b	100.00	100.00	>200.00	>200.00	—[a]	>200.00	50.00	100.00	>200.00	—[a]
3c	>200.00	>200.00	>200.00	>200.00	—[a]	>200.00	>200.00	>200.00	>200.00	—[a]
3d	>200.00	>200.00	>200.00	>200.00	>200.00	>2000	>200.00	>200.00	>200.00	>250.00
3e	3.12	12.50	12.50	12.50	12.50	12.50	12.50	6.25	>200.00	—[a]
3f	100.00	100.00	100.00	100.00	—[a]	>200.00	50.00	100.00	>200.00	—[a]
3g	25.00	50.00	50.00	25.00	25.00	25.00	25.00	50.00	200.00	125.00
3h	200.00	25.00	12.50	12.50	—[a]	>200.00	12.50	25.00	>200.00	—[a]
3i	200	50.00	>200.00	125.00	50.00	25.00	100.00	>200.00	>200.00	62.50
3j	>200	>200.00	>200.00	>200.00	—[a]	>200.00	200.00	>200.00	>200.00	—[a]
3k	>200.00	>200.00	>200.00	>200.00	—[a]	>200.00	200.00	>200.00	>200.00	—[a]
3l	3.12	12.50	25.00	12.50	25.00	6.25	12.50	12.50	50.00	—[a]
Fluconazole	3.12	6.25	6.25	1.95	0.78	1.56	1.56	3.12	1.56	—[b]
Spectinomycin	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	16

[a] Not tested.
[b] Data are absent.

Table 2. Anti-Candida activity of pyrazoles **2a** and **3l**

Compound	MIC/μg mL^{-1}					
	C. crusei	*C. glabrata*	*C. parapsilosis*	*C. dubliniensis*	*C. guilliermondii*	*C. tropicalis*
2a	>200	200	>200	50	100	200
3l	100	100	200	50	100	100
Fluconazole	100	200	3.12	>200	25	>200

The evaluation of the antifungal activity of pyrazoles **2** and **3** showed that pyrazoles **2a,c** and **3e,l** possess high or moderate antimycotic activity against strains of pathogenic dermatophyte fungi. Thus, pyrazoles **3e,l** were found to be able to suppress the growth of the strain of the fungi *T. rubrum* comparable to that of fluconazole with the minimum inhibitory concentration (MIC) of 3.12 μg mL^{-1}; compounds **2a,c**, with MIC of 6.25—7.8 μg mL^{-1}. The inhibitory activity of pyrazole **2a** against *T. gypseum* is comparable to that of the reference sample (MIC = = 6.25 μg mL^{-1}), whereas analogues **3e,l** were less effective (MIC = 12.5 μg mL^{-1}). The activity against *T. Tonsurans*, comparable to that of fluconazole, was found for pyrazoles **2a,c** (MIC = 6.25—7.8 μg mL^{-1}), whereas compounds **3e,h** showed lower activity (MIC = 12.5 μg mL^{-1}). Compounds **2** and **3** were less effective against *T. violaceum*, *T. interdigitale*, *T. schoenleinii*, *E. floccosum*, *M. canis*, and *C. albicans* than fluconazole. However, it should be noted that pyrazoles **2a,c** exhibited high activity (MIC = 6.25—7.8 μg mL^{-1}) against *E. floccosum*; compound **3l**, against *T. schoenleinii*; heterocycles **2c** and **3e**, against *M. canis*. In the series of the synthesized pyrazoles, compounds **2a** and **3e,l**, were found to be the most active (MIC = 12.5 μg mL^{-1}) as inhibitors of the growth of the fungi *T. violaceum*; product **3e** exhibited the highest inhibitory activity against *T. interdigitale*.

The structure—activity relationship analysis showed that pyrazoles **2a** and **3e,l** containing the tolyl substituent along with H, AcO(CH$_2$)$_4$, or HO(CH$_2$)$_4$ groups at the N(1) atom are the most active compounds (MIC = = 3.12—25 μg mL^{-1}) against all the dermatophyte strains under study. The absence of a substituent at the N(1) atom is favorable for the antimycotic activity, because all pyrazoles **2a—c** were found to be able to inhibit the growth of most of the dermatophyte strains with MIC from high to moderate. Their activity decreases in the following series of derivatives: tolyl (**2a**), methoxyphenyl (**2b**), and phenyl (**2c**). Pyrazoles **3g,h** containing the Et group at the N(1) atom in the ring and H or OMe substituents in the aromatic moiety displayed moderate antimycotic activity against some fungi. Compounds **3a**,**b**,**f**,**i** bearing phenyl and methoxyphenyldiazenyl substituents along with HO(CH$_2$)$_2$, AcO(CH$_2$)$_4$, or CF$_3$(CH$_2$)$_3$ groups at the pyrazole ring exhibited weak activity. The presence of CF$_3$(CH$_2$)$_3$, CF$_3$(CH$_2$)$_2$, PhCH$_2$, and 4-NH$_2$SO$_2$C$_6$H$_4$ substituents in methoxyphenyldiazenylpyrazoles **3c**,**d**,**j**,**k** leads to the loss of fungistatic activity.

4-Hydroxybutyl-substituted pyrazole **3l** and *N*-unsubstituted pyrazoles **2a**,**b** also displayed weak activity (MIC = 50 μg mL^{-1}) against the yeast-like fungi *C. albicans* (see Table 1). For compounds **2a** and **3l**, the range was extended by additional six strains of clinically significant species of yeast-like fungi of the *Candida* genus (Table 2). It was found that these compounds retained weak activity only against the strain *C. dubliniensis*.

The evaluation of antibacterial activity of pyrazoles **2** and **3** against the bacteria *Neisseria gonorrhoeae* (see Table 1) revealed only weak activity of compound **3i**.

The assessment of the cytotoxic effect of pyrazoles **2a—c** and **3a,d,g,i,l** using the *McCoy B* cell line (Table 3) demonstrated that pyrazoles **2c** and **3l** exhibiting high fungistatic activity against strains of pathogenic dermatophyte fungi are highly toxic and cause the death of 50% of *McCoy B* cells in the concentration range of 0.98—15.6 μg mL^{-1}. In the series of the tested compounds, pyrazoles **2a** and **3a,d** exhibited the lowest cytotoxicity. At a concentration of 125 μg mL^{-1}, these compounds caused the death of 50% of *McCoy B* cells.

In summary, we performed the synthesis of 4-aryldiazenyl-3,5-dimethylpyrazoles by the condensation of 3-arylhydrazinylidenepentane-2,4-diones with hydrazines and by the alkylation of *N*-unsubstituted pyrazoles with haloalkanes. The biological evaluation demonstrated that

Table 3. Cytotoxicity of pyrazoles **2** and **3**

Compound	IC_{90}[a]	IC_{50}[a]	IC_{10}[a]
2a	250	125	62.5
2b	125	62.5	31.2
2c	1.9	0.98	0.49
3a	250	125	62.5
3d	250	125	62.5
3g	62.5	31.2	15.6
3i	62.5	46.8	31.2
3l	31.2	15.6	7.8
Spectinomycin	—[b]	—[b]	>500

[a] IC_{90}, IC_{50}, and IC_{10} are the concentrations that induce 90, 50, and 10% cell death, respectively.
[b] Data are absent.

3,5-dimethyl-4-[(4-methylphenyl)diazenyl]-1*H*-pyrazole (**2a**) has the optimal activity—toxicity relationship.

Experimental

The NMR spectra were recorded on a Bruker AVANCE III 500 spectrometer in $CDCl_3$ and DMSO-d_6 (^{1}H, 500 MHz relative to $SiMe_4$; ^{19}F, 470 MHz relative to C_6F_6; ^{13}C, 126 MHz relative to the signal of the solvent (δ_C 77 for $CDCl_3$, δ_C 39.5 for DMSO-d_6)). The IR spectra were measured on a Perkin—Elmer Spectrum Two Fourier-transform infrared spectrometer equipped with an attenuated total reflectance diamond crystal (ATR) accessory in a range of 4000—400 cm^{-1}. Electrospray-ionization mass spectra were obtained on a MaXis Impact HD quadrupole time-of-flight mass spectrometer (Bruker Daltonik GmbH) for solutions in $CHCl_3$—MeOH (0.2 : 1.6) or CH_2Cl_2—MeOH (0.05 : 1.6) at a flow rate of 180 μL^{-1} h^{-1} using a modified pre-set method of small-molecule infusion. The mass calibration was performed using external HPC (high precision calibration) and a G1969-85000 calibration solution (Agilent Technologies). The melting points were measured in open capillary tubes using a Stuart SMP30 melting point apparatus. Column chromatography was performed on Macherey-nagel silica gel 60 (0.063—0.2 mm). The progress of the reactions was monitored by TLC on ALUGRAM® Xtra SIL G/UV_{254} plates.

3,5-Dimethyl-4-[(4-methylphenyl)diazenyl]-1*H*-pyrazole (**2a**), 3,5-dimethyl-4-phenyldiazenyl-1*H*-pyrazole (**2b**), and 3,5-dimethyl-4-[(4-methoxyphenyl)diazenyl]-1*H*-pyrazole (**2c**) were synthesized by known procedures.[5] 4-{3,5-Dimethyl-4-[(4-methylphenyl)diazenyl]-1*H*-pyrazol-1-yl}butyl acetate (**3e**) and 4-{3,5-dimethyl-4-[(4-methylphenyl)diazenyl]-1*H*-pyrazol-1-yl}butan-1-ol (**3l**) were synthesized by procedures described previously.[7]

Synthesis of pyrazoles 3a,b. A mixture of 3-arylhydrazinylidenepentane-2,4-dione **1b**,**c** (2 mmol) and 2-hydroxyethylhydrazine (0.167 g, 2.2 mmol) in EtOH (10 mL) was refluxed for 12—14 h. The reaction mixture was concentrated. The products were purified by column chromatography using $CHCl_3$—EtOH (45 : 1) as the eluent.

2-{3,5-Dimethyl-4-phenyldiazenyl-1*H*-pyrazol-1-yl}ethan-1-ol (3a). Yellow powder, yield was 97%, m.p. 105—107 °C (MeCN) (*cf.* lit. data[6]: m.p. 105—106 °C). IR, ν/cm^{-1}: 3249 (OH), 2866 (C—H); 1555, 1508 (C=C). ^{1}H NMR ($CDCl_3$), δ: 2.50 (s, 3 H, CH_3); 2.60 (s, 3 H, CH_3); 3.41 (br.s, 1 H, OH); 4.04 (t, 2 H, OCH_2, J = 4.9 Hz); 4.13 (t, 2 H, NCH_2, J = 4.9 Hz); 7.37 (tt, 1 H, Ph, J_1 = 7.2 Hz, J_2 = 1.1 Hz); 7.4 (t, 2 H, Ph, J = 7.7 Hz); 7.78 (dd, 2 H, Ph, J_1 = 8.3 Hz, J_2 = 1.1 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.80 (CH_3), 13.98 (CH_3), 50.13 (NCH_2), 61.45 (OCH_2), 121.77, 128.88, 129.41, 134.96, 139.40, 142.83, 153.52. MS, found: *m/z* 245.1397 $[M + H]^+$. Calculated for $C_{13}H_{17}N_4O^+$: 245.1397.

2-{3,5-Dimethyl-4-[(4-methoxyphenyl)diazenyl]-1*H*-pyrazol-1-yl}ethan-1-ol (3b). Yellow powder, yield was 67%, m.p. 109—111 °C. IR, ν/cm^{-1}: 3250 (OH), 2944—2868 (C—H); 1603, 1584, 1556, 1499 (C=C). ^{1}H NMR ($CDCl_3$), δ: 2.49 (s, 3 H, CH_3); 2.58 (s, 3 H, CH_3); 3.43 (br.s, 1 H, OH); 3.87 (s, 3 H, OCH_3); 4.03 (t, 2 H, OCH_2, J = 4.9 Hz); 4.13 (t, 2 H, NCH_2, J = 4.9 Hz); 6.97 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.77 (d, 2 H, C_6H_4, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.75 (CH_3), 13.88 (CH_3), 50.11 (NCH_2), 55.47 (OCH_3), 61.39 (OCH_2), 113.99, 123.29, 134.69, 138.61, 142.56, 147.80, 160.78. MS, found: *m/z* 275.1508 $[M + H]^+$. Calculated for $C_{14}H_{19}N_4O_2^+$: 275.1503.

Synthesis of pyrazoles 3c,d. A mixture of arylhydrazone **1c** (2 mmol), benzylhydrazine dihydrochloride (410 mg, 2.1 mmol) or 4-hydrazinylbenzenesulfonamide hydrochloride (470 mg, 2.1 mmol), and AcONa·$3H_2O$ (300 mg, 2.2 mmol) in AcOH (10 mL) was refluxed for 12—14 h. The reaction products were precipitated with water, filtered off, washed with water, and dried. Pyrazole **3c** was purified by column chromatography using ethyl acetate—hexane (5 : 1) as the eluent. Compound **3d** was washed with MeCN and recrystallized from EtOH.

1-Benzyl-4-[(4-methoxyphenyl)diazenyl]-3,5-dimethyl-1*H*-pyrazole (3c). Yellow powder, yield was 64%, m.p. 111—113 °C. IR, ν/cm^{-1}: 2923 (C—H); 1600, 1581, 1553, 1498 (C=C). ^{1}H NMR ($CDCl_3$), δ: 2.50 (s, 3 H, CH_3); 2.53 (s, 3 H, CH_3); 3.87 (s, 3 H, OCH_3); 5.28 (s, 2 H, CH_2); 6.97 (d, 2 H, C_6H_4, J = 9.1 Hz); 7.14 (d, 2 H, Ph, J = 8.2 Hz); 7.27—7.35 (m, 3 H, Ph); 7.77 (d, 2 H, C_6H_4, J = 9.1 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.97 (CH_3), 13.96 (CH_3), 53.00 (NCH_2), 55.48 (OCH_3), 114.00, 123.29, 126.69, 127.72, 128.79, 135.28, 136.41, 138.21, 142.49, 147.88, 160.76. MS, found: *m/z* 321.1712 $[M + H]^+$. Calculated for $C_{19}H_{21}N_4O^+$: 321.1710.

4-{4-[(4-Methoxyphenyl)diazenyl]-3,5-dimethyl-1*H*-pyrazol-1-yl}benzenesulfonamide (3d). Yellow powder, yield was 53%, m.p. 267—268 °C. IR, ν/cm^{-1}: 3289, 3181 (NH_2); 3023 (C—H); 1599, 1584, 1558, 1508 (C=C). ^{1}H NMR (DMSO-d_6), δ: 2.49 (s, 3 H, CH_3); 2.71 (s, 3 H, CH_3); 3.85 (s, 3 H, OCH_3); 7.10 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.52 (br.s, 2 H, NH_2); 7.80 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.86 (d, 2 H, C_6H_4, J = 8.6 Hz); 7.99 (d, 2 H, C_6H_4, J = 8.6 Hz). ^{13}C NMR (DMSO-d_6), δ: 11.02 (CH_3), 14.09 (CH_3), 55.52 (OCH_3), 114.41, 123.35, 124.23, 126.87, 135.69, 139.56, 141.16, 142.69, 142.89, 147.03, 160.92. MS, found: *m/z* 386.1280 $[M + H]^+$. Calculated for $C_{18}H_{20}N_5O_3S^+$: 386.1281.

Synthesis of pyrazoles 3f—k (general procedure). A mixture of pyrazole **2b**,**c** (1.5 mmol), haloalkane (3 mmol), and K_2CO_3 (0.31 g, 2.25 mmol) in MeCN (or acetone in the synthesis of compound **3k**) (10 mL) was refluxed for 6—12 h. The precipitate was filtered off, and the mother liquor was concentrated. The products were purified by column chromatography using a hexane—ethyl acetate mixture (4 : 1) as the eluent.

4-{4-[(4-Methoxyphenyl)diazenyl]-3,5-dimethyl-1*H*-pyrazol-1-yl}butyl acetate (3f). Orange oil, yield was 78%. IR, ν/cm^{-1}: 2954 (C—H), 1734 ($MeCO_2$); 1600, 1501 (C=C). ^{1}H NMR ($CDCl_3$), δ: 1.65—1.71 (m, 2 H, CH_2); 1.89—1.95 (m, 2 H, CH_2); 2.05 (s, 3 H, CH_3CO_2); 2.49 (s, 3 H, CH_3); 2.57 (s, 3 H, CH_3); 3.87 (s, 3 H, OCH_3); 4.06 (t, 2 H, OCH_2, J = 7.2 Hz); 4.09 (t, 2 H, NCH_2, J = 6.5 Hz); 6.97 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.77 (d, 2 H, C_6H_4, J = 9.0 Hz). MS, found: *m/z* 345.1923 $[M + H]^+$. Calculated for $C_{18}H_{25}N_4O_3^+$: 345.1921.

1-Ethyl-3,5-dimethyl-4-phenyldiazenyl-1*H*-pyrazole (3g). Brown powder, yield was 63%, m.p. 38—39 °C. IR, ν/cm^{-1}: 2987 (C—H); 1548, 1503 (C=C). ^{1}H NMR ($CDCl_3$), δ: 1.43 (t, 3 H, CH_3, J = 7.3 Hz); 2.51 (s, 3 H, CH_3); 2.58 (s, 3 H, CH_3); 4.08 (q, 2 H, NCH_2, J = 7.3 Hz); 7.36 (t, 1 H, Ph, J =7.2 Hz); 7.45 (t, 2 H, Ph, J = 7.5 Hz); 7.78 (dd, 2 H, Ph, J_1 = 8.0 Hz, J_2 = 0.9 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.73 (CH_3), 13.94 (CH_3), 15.18 (CH_3), 43.92 (NCH_2), 121.71, 128.86, 129.20, 135.13, 137.92, 142.38, 153.65. MS, found: *m/z* 229.1449 $[M + H]^+$. Calculated for $C_{13}H_{17}N_4^+$: 229.1448.

1-Ethyl-4-[(4-methoxyphenyl)diazenyl]-3,5-dimethyl-1*H*-pyrazole (3h). Orange powder, yield was 52%, m.p. 72—74 °C.

IR, ν/cm^{-1}: 2968 (C—H); 1600, 1579, 1551, 1501 (C=C). ^{1}H NMR ($CDCl_3$), δ: 1.43 (t, 3 H, CH_3, J = 7.3 Hz); 2.50 (s, 3 H, CH_3); 2.56 (s, 3 H, CH_3); 3.86 (s, 3 H, OCH_3); 4.08 (q, 2 H, NCH_2, J = 7.3 Hz); 6.97 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.77 (d, 2 H, C_6H_4, J = 9.0 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.69 (CH_3), 13.87 (CH_3), 15.19 (CH_3), 43.85 (NCH_2), 55.47 (OCH_3), 113.97, 123.20, 134.87, 137.12, 142.11, 147.93, 160.63. MS, found: m/z 259.1553 $[M + H]^+$. Calculated for $C_{14}H_{19}N_4O^+$: 259.1553.

3,5-Dimethyl-4-phenyldiazenyl-1-(4,4,4-trifluorobutyl)-1*H*-pyrazole (3i). Brown oil, yield was 68%. IR, ν/cm^{-1}: 2953 (C—H); 1555, 1503 (C=C); 1148—1130 (C—F). Immediately after the dissolution in $CDCl_3$, the *trans*-azo form was observed. ^{1}H NMR ($CDCl_3$), δ: 2.11—2.20 (m, 4 H, $(CH_2)_2$); 2.50 (s, 3 H, CH_3); 2.60 (s, 3 H, CH_3); 4.12 (t, 2 H, NCH_2, J = 6.5 Hz); 7.38 (tt, 1 H, Ph, J_1 = 7.4 Hz, J_2 = 1.1 Hz); 7.47 (t, 2 H, Ph, J = 7.5 Hz); 7.78 (dd, 2 H, Ph, J_1 = 8.3 Hz, J_2 = 1.1 Hz) (*trans*). ^{19}F NMR ($CDCl_3$), δ: 95.67 (t, CF_3, J = 10.4 Hz) (*trans*). ^{13}C NMR ($CDCl_3$), δ: 9.74 (CH_3); 13.95 (CH_3); 22.50 (q, $\underline{C}H_2CH_2CF_3$, J = 3.0 Hz); 30.96 (q, $CH_2\underline{C}H_2CF_3$, J = 29.3 Hz); 47.27 (NCH_2); 121.76; 126.8 (q, CF_3, J = 276.0 Hz); 128.89; 129.42; 135.14; 138.48; 142.81; 153.53 (*trans*). After 3 h, a mixture of *cis*/*trans*-azo forms (1 : 20) was observed. ^{1}H NMR ($CDCl_3$), δ: 1.67 (s, 3 H, CH_3); 2.02 (s, 3 H, CH_3); 2.01—2.04 (m, 4 H, $(CH_2)_2$); 3.97 (t, 2 H, NCH_2, J = 6.5 Hz); 7.02 (dd, 2 H, Ph, J_1 = 8.3 Hz, J_2 = 1.1 Hz); 7.19 (tt, 1 H, Ph, J_1 = 7.5 Hz, J_2 = 1.1 Hz); 7.34 (t, 2 H, Ph, J = 7.5 Hz) (*cis*). ^{19}F NMR ($CDCl_3$), δ: 95.62 (t, CF_3, J = 10.4 Hz) (*cis*). MS, found: m/z 311.1476 $[M + H]^+$. Calculated for $C_{15}H_{18}F_3N_4^+$: 311.1478.

4-[(4-Methoxyphenyl)diazenyl]-3,5-dimethyl-1-(4,4,4-trifluorobutyl)-1*H*-pyrazole (3j). Orange powder, yield was 98%, m.p. 77—79 °C. IR, ν/cm^{-1}: 2961 (C—H); 1603, 1583, 1557, 1504 (C=C), 1137 (C—F). ^{1}H NMR ($CDCl_3$), δ: 2.11—2.20 (m, 4 H, $(CH_2)_2$); 2.49 (s, 3 H, CH_3); 2.57 (s, 3 H, CH_3); 3.87 (s, 3 H, OCH_3); 4.10 (t, 2 H, NCH_2, J = 6.5 Hz); 6.97 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.78 (d, 2 H, C_6H_4, J = 9.0 Hz). ^{19}F NMR ($CDCl_3$), δ: 95.69 (t, CF_3, J = 10.4 Hz). ^{13}C NMR ($CDCl_3$), δ: 9.72 (CH_3); 13.86 (CH_3); 22.55 (q, $\underline{C}H_2CH_2CF_3$, J = 3.0 Hz); 30.96 (q, $CH_2\underline{C}H_2CF_3$, J = 29.3 Hz); 47.23 (NCH_2); 55.49 (OCH_3); 114.02; 123.31; 126.8 (q, CF_3, J = 276.0 Hz); 134.92; 137.65; 142.60; 147.84; 160.83. MS, found: m/z 341.1590 $[M + H]^+$. Calculated for $C_{16}H_{20}F_3N_4O^+$: 341.1584.

4-[(4-Methoxyphenyl)diazenyl]-3,5-dimethyl-1-(3,3,3-trifluoropropyl)-1*H*-pyrazole (3k). Yellow powder, yield was 36%, m.p. 75—77 °C. IR, ν/cm^{-1}: 2966—2925 (C—H); 1602, 1581, 1557, 1503 (C=C), 1141 (C—F). ^{1}H NMR ($CDCl_3$), δ: 2.49 (s, 3 H, CH_3); 2.59 (s, 3 H, CH_3); 2.68—2.78 (m, 2 H, CH_2); 3.87 (s, 3 H, OCH_3); 4.27 (t, 2 H, NCH_2, J = 7.2 Hz); 6.97 (d, 2 H, C_6H_4, J = 9.0 Hz); 7.78 (d, 2 H, C_6H_4, J = 9.0 Hz). ^{19}F NMR ($CDCl_3$), δ: 96.07 (t, CF_3, J = 10.4 Hz). MS, found: m/z 327.1433 $[M + H]^+$. Calculated for $C_{15}H_{18}F_3N_4O^+$: 327.1427.

Microbiological evaluation of compounds 2 and 3 was performed at the Ural Research Institute of Dermatovenereology and Immunopathology. The antimicrobial properties were evaluated by the serial dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI, 2014). The following test strains were used: strains of the dermatophyte fungi *Trichophyton rubrum* (RCPF F 1408), *Trichophyton mentagrophytes var. gypseum* (RCPF F 1425), *Trichophyton tonsurans* (RCPF F 1396/228), *Trichophiton violaceum* (RCPF F 1211), *Trichophyton mentagrophytes var. interdigitale* (RCPF F 1459/11044), *Trichophyton schoenleinii* (RCPF F 235/25), *Epidermophyton floccosum* (RCPF F 1659/17), *Microsporum canis* (RCPF F 1643/1585); stains of the yeast-like fungi *Candida albicans* (RCPF Y 401/NCTC 885/653), *Candida krusei* (RCPF Y 1472/310), *Candida glabrata* (RCPF Y 1486/47), *Candida parapsilosis* (RCPF Y 1579/296), *Candida dubliniensis* (RCPF Y 1307), *Candida guilliermondii* (RCPF Y 1373), *Candida tropicalis* (RCPF Y 1513/784) from the Russian Collection of Pathogenic Fungi of the Kashkin Research Institute of Medical Mycology of the North-Western State Medical University named after I. I. Mechnikov (St. Petersburg, Russia); the reference strain *Neisseria gonorrhoeae* (NCTC 12700 /ATCC 49226) from the National Collection of Type Cultures (NCTC, UK).

The agar medium Complegon (Russia) supplemented with a 1% growth additive (Russia, registration certificate number FSR 2007/00163) was used as a culture medium for gonococci; for fungi, the Sabouraud Dextrose Broth (SDB, Research Center of Pharmacotherapy, St. Petersburg, registration certificate number FSR 2012/14080) was used. Microorganisms were identified with an accuracy of 99.9% using a BioMerieux VITEK MS MALDI-TOF Plus mass spectrometer by time-of-flight mass spectrometry of matrix-anchored bacterial proteins. Inocula were prepared using a BioMerieux DensiCHEK Plus densitometer; the optical density (OD) was 0.5 McFarland, which corresponds to $1.5 \cdot 10^8$ CFU mL^{-1}. A suspension of microorganisms for *N. gonorrhoeae* and *C. albicans* was prepared from an overnight culture; for dermatophytes, from a two-week culture pre-homogenized in physiological sterile saline. The inoculum dose for gonococci was 10^5 CFU mL^{-1}; for fungi, 10^6 CFU mL^{-1}. The antimycotic activity of the chemicals was evaluated by a micromethod.[10] The antibacterial activity against *N. gonorrhoeae* was assessed using serial twofold dilutions on agar (gold standard).[11] Agar-based culture medium served as the growth medium; the liquid state of the medium was maintained at 52 °C in a BioSan WB-4 MS water bath-thermostat. The tested chemicals were dissolved in DMSO. The initial 1000 μg mL^{-1} dilution was prepared using sterile distilled water; serial twofold dilutions (from 250—200 μg mL^{-1}) were prepared using growth culture media. Dermatophytes were incubated for up to 14 days at 27 °C; yeast-like fungi of the *Candida* genus, for 24 h; gonococci, for 24 h at 37 °C in a wet chamber of a Sanyo MCO-15AC CO_2 incubator (CO_2 5.0%). In each assay, the positive and negative controls were employed. The minimum inhibitory concentration was assessed visually as the lowest concentration, which inhibits culture growth. The degree of antimicrobial activity of the chemicals was determined according to the recommended criteria.[10] Chemically pure fluconazole and spectinomycin were used as the reference analogues.

Evaluation of cytotoxicity of active compounds using the cell culture *McCoy*. The cytotoxic effect of the newly synthesized chemicals was evaluated using the transplantable fibroblast-like *McCoy B* cells, which were obtained from the Russian Collection of Cell Cultures of Vertebrates (RCCCV) of the Institute of Cytology of the Russian Academy of Sciences (St. Petersburg, Russia). The concentrations of compounds required to inhibit 90, 50, and 10% cell growth (IC_{90}, IC_{50}, and IC_{10}, respectively) were determined.

The cells were grown in DMEM culture medium using HEPES salts and supplemented with L-glutamine (Biolot, Russia) and 10% fetal bovine serum (standard quality (FBS), GE Healthcare, Austria). Sterile 96-well cell culture plates were used. A cell suspension ($3 \cdot 10^4$ cells per well) was seeded in 96-well

plates, and the plates were incubated at 37 °C (5.0% CO_2) in an incubator until a monolayer formed. Serial dilutions of solutions of the chemicals were prepared in sterile 96-well plates at concentrations of 500—0.25 μg mL^{-1}. Dimethyl sulfoxide was used as the solvent for chemicals; DMEM, as the dilution medium. After removal of the incubation medium, solutions of the chemicals were added to the wells, and the plates were incubated for 72 h at 37 °C in a CO_2 incubator. All concentrations were tested in triplicate. A negative control (culture medium) and the DMSO solvent control were used in experiments. The solutions of the tested compounds were removed, the plates were washed with physiological sterile saline, and a solution (200 μL) containing 1 mg mL^{-1} NBT (USA) was added. The incubation was performed for 30 min at 37 °C (5.0% CO_2). The results were visually assessed using a LOMO BIOLAM P2-1 inverted microscope. Purple-colored cells in the monolayer were counted as live; yellow-colored cells, as dead. Apart from the color, the morphology of the cells in the monolayer was evaluated.

The work was performed within the framework of the state assignment (No. AAAA-A19-119011790130-3) using facilities of the Joint Use Center "Spectroscopy and Analysis of Organic Compounds" (JUC SAOC) of the I. Ya. Postovsky Institute of Organic Synthesis of the Ural Branch of the Russian Academy of Sciences.

No human or animal subjects were involved in this research.

The authors declare no conflict of interest, financial or otherwise.

References

1. A. H. Moustafa, H. A. El-Sayed, S. M. El Rayes, H. A. Morsy, S. H. Abd-allah, H. F. Ismail, M. M. Abd El-Aal, *Russ. J. Gen. Chem.,* 2019, **89**, 2314; DOI: 10.1134/S1070363219110240.
2. M. Shukla, D. S. Seth, H. Kulshreshtha, *J. Appl. Chem.*, 2013, **2**, 1484; www.joac.info.
3. K. Kaur, V. Kumar, V. Beniwal, V. Kumar, K. R. Aneja, V. Sharma, S. Jaglan, *Med. Chem. Res.*, 2015, **24**, 3863; DOI: 10.1007/s00044-015-1429-2.
4. A. A. Bekhit, H. M. A. Ashour, A. A. Guemei, *Arch. Pharm. Chem. Life Sci.*, 2005, **338**, 167; DOI: 10.1002/ardp.200400940.
5. S. A. Sankpal, M. B. Deshmukh, P. V. Anbhule, D. K. Salunkhe, K. N. Alsundkar, P. P. Patil, D. R. Chandam, S. D. Jagadale, A. G. Mulik, S. S. Rokade, *J. Chem. Pharm. Res.*, 2010, **2**, 574.
6. M. Bianchi, *Ann. Chim.*, 1966, **56**, 151.
7. A. E. Ivanova, Ya. V. Burgart, V. I. Saloutin, *Chem. Heterocycl. Compd.* (*Engl. Transl.*), 2013, **49**, 1128; DOI:10.1007/s10593-013-1353-7.
8. D. O´Hagan, *J. Fluorine Chem.*, 2010, **131**, 1071; DOI: 10.1016/j.jfluchem.2010.03.003.
9. K. Haranahalli, T. Honda, I. Ojima, *J. Fluorine Chem.*, 2019, **217**, 29; DOI: 10.1016/j.jfluchem.2018.11.002.
10. A. A. Kubanova, Zh. V. Stepanova, T. A. Gus´kova, T. V. Pushkina, L. Yu. Krylova, I. B. Shilova, A. S. Trenin, *Rukovodstvo po provedeniyu doklinicheskikh issledovanii lekarstvennykh sredstv* [*Guidelines for Preclinical Trials of Medicinal Products*], Ed. A. N. Mironov, Grif i K, Moscow, 2012, Part 1, 578 (in Russian).
11. *Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing. 24th Ed. CLSI Document M100-S24*, 2014, **34**, 84.

Received February 15, 2021;
in revised form April 5, 2021;
accepted April 8, 2021