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Studies on Pyrazine Derivatives LII: Antibacterial and Antifungal Activity of Nitrogen Heterocyclic Compounds Obtained by Pyrazinamidrazone Usage

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ABSTRACT: *Using reactivity of pyrazinamidrazones and their N′-substituted derivatives* **1–8** *in reaction with sulfonyl chlorides sulfone derivatives* **9–17** *were obtained, with orthoformate cyclized to sulfonyl compounds* **18–20**. *Amidrazones in reaction with pyrazin-iminoesters gave dihydrazidines* **21–23**, *which cyclized to 3,5-dipyrazine derivatives of 1,2,4-triazole* **24–26**. *1-Methyl- or 1-phenyl-3-pyrazine-1,2,4-triazole* **27–38** *was formed in reaction of amidrazones* **1–8** *with orthoformate and orthoacetate or benzoyl chloride. N′-Phenylamidrazones* **3, 8** *in reaction with thionyl chloride were transformed to 1,2,3,5-thiatriazole S-oxides* **39, 40**. *Obtained compounds exhibited low antibacterial activity. Antifungal activity was affirmed for compounds* **1, 3, 4, 5, 8, 37, 39,** *and* **40**, *for which minimal inhibitory concentration (MIC) was in the concentration range of 16–128* μg/*mL*. © 2011 Wiley Periodicals, Inc. Heteroatom Chem 23:49–58, 2012; View this article online at wileyonlinelibrary.com. DOI 10.1002/hc.20751

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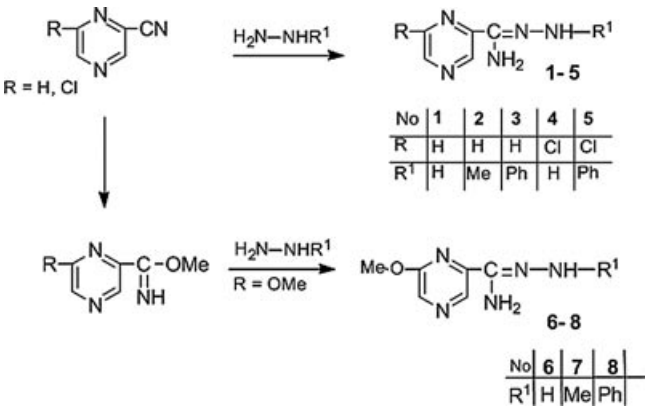
INTRODUCTION

Pyrazine derivatives possess a wide range of biological activities. They are found in a variety of naturally occurring compounds such as aspergillic acid, hydroxyaspergillic acid, and other antibiotics of similar structure, which possess antibacterial activities [1–6]. Emimycin (3-hydroxypyrazine *N*-oxide) has also antibacterial properties [7]. Synthetic pyrazine derivatives exhibit a wide variety of pharmacological properties, including hypoglycemic [8–10] and diuretic [11,12] action. Sulfonamides with pyrazine moiety are known to have high antibacterial activity [13]. Pyrazinamide and its morpholino–methylene derivative act as tuberculostatic agents [14,15]. Nicotinic and isonicotinic amidrazones are also reported in the literature as antibacterial agents [16–18]. They also act as diuretic [19], antimycotic [20], and circulatory system agents [21]. The therapeutic potential activity of amidrazone derivatives with pyrazine moiety can be developed as potential antibacterial drugs.

CHEMISTRY

Pyrazinamidrazones for further reactions were obtained by direct addition of respective hydrazines to pyrazinenitriles or by reaction of pyrazinenitriles



SCHEME 1

with appropriate pyraziniminoesters (Scheme 1) according to the methods described previously [22,23].

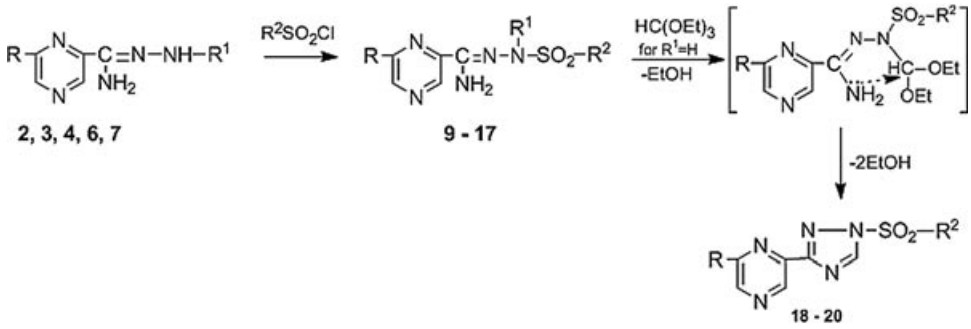
Some of the amidrazones were converted into methane, benzenesulfone, and p-toluenesulfone derivatives. Compounds **9, 12**, and **13** were converted into cyclic derivatives of benzene- and p-toluene-1,2,4-triazole **18–20**, by means of triethyl orthoformate (Scheme 2).

At attempt of getting benzenesulfonylamidrazones **9** and **11** in reaction of pyraziniminoesters with benzenesulfonylhydrazide in water–methanol solution, unexpectedly dihydrazidines were received, their structures were confirmed by means of spectroscopic methods: IR, ^{1}H NMR, and MS. It can be explained on the basis of enhanced sensitivity in the water environment, which has caused decomposition to hydrazine and benzenesulfonic acid. Free hydrazine has reacted with dissolved iminoester, giving corresponding amidrazones. Then hydrazine has acted with the remaining iminoester in the presence of acid ($PhSO_3H$), resulting in symmetric dihydrazidine **21** and **22** with facility. In these conditions, p-toluenesulfonylhydrazide has behaved similarly (Scheme 3).

Such a mechanism confirms obtaining dihydrazidines **21** and **23** in a reaction of iminoesters with amidrazones in the presence of acetic acid. It shows a capacity of synthesis of asymmetric dihydrazidines, using for reaction different amidrazones and iminoesters (Scheme 4).

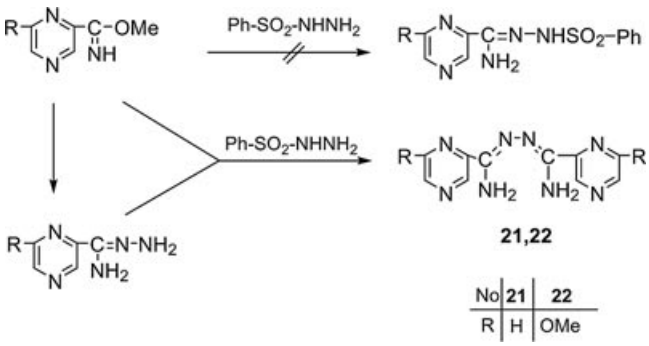
Dihydrazidines **21–23** have easily cyclized to appropriate disubstituted 1,2,4-triazoles **24–26**, giving off ammonia. Cyclization has been carried out by heating in diglyme or glacial acetic acid.

The ^{1}H NMR spectrum of compound **25** shows the splitting of signal at 15.29–15.41 ppm attributable to the proton of the NH group of the triazole ring. It indicates the existence of two structures (Scheme 5). Lack of molecular symmetry causes the formation of two energetic unequal hydrogen bonds between the proton of the triazole NH group and nitrogen atoms of the pyrazine ring. ^{1}H NMR spectrum exhibits that hydrogen bond is more stable with nitrogen of substituted pyrazine with the methoxy group (structure A). It allows the rotation of another pyrazine ring about the single bound with triazole ring. Addition of D_2O causes disappearance of the NH group proton signal and sharpens proton bands of unsubstituted pyrazine. The effect described above is not observed for symmetric molecules of compounds **24** and **26**, for which NMR spectra show characteristic singlets of the triazole ring.



No	9	10	11	12	13	14	15	16	17	18	19	20
R	H	H	OMe	Cl	H	OMe	OMe	H	OMe	H	Cl	OMe
R^1	H	Me	Me	H	H	H	Me	Me	Me	-	-	-
R^2	Ph	Ph	Ph	Ph	4-MePh	4-MePh	4-MePh	Me	Me	Ph	Ph	4-Me-Ph

SCHEME 2

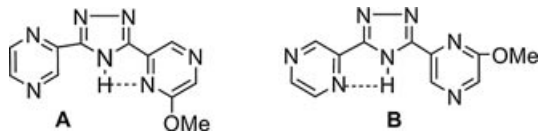


SCHEME 3

N′-Methyl and *N′*-phenyl substituted amidrazones were converted into corresponding di- and tri-substituted derivatives (**27–38**) in reaction with triethyl orthoformate, triethyl acetate, and benzoyl chloride (Scheme 6).

N′-Phenyl substituted amidrazones **3** and **8** in reaction with thionyl chloride in chloroform and anhydrous pyridine below 5°C afforded 2,5-dihydro-1,2,3,5-thiatriazole *S*-oxides **39** and **40** with very low yield (Scheme 7). Analogous synthesis for *N′*-methyl substituted amidrazones was carried out without success.

N′-Substituted amidrazones could be a starting material for synthesis of 1,2,4-triazoles with methyl in ring position 1 and cycloamine in position 4. It could be confirmed by reaction of *N′*-methylpyrazinamidrazone with *S*-methylester of 4-morpholinodithiocarbaminic acid. The analogous product **41** has been obtained from *N′*-methylpyrazinehydrazide in aqueous solution of potassium carbonate with a good yield (Scheme 8).



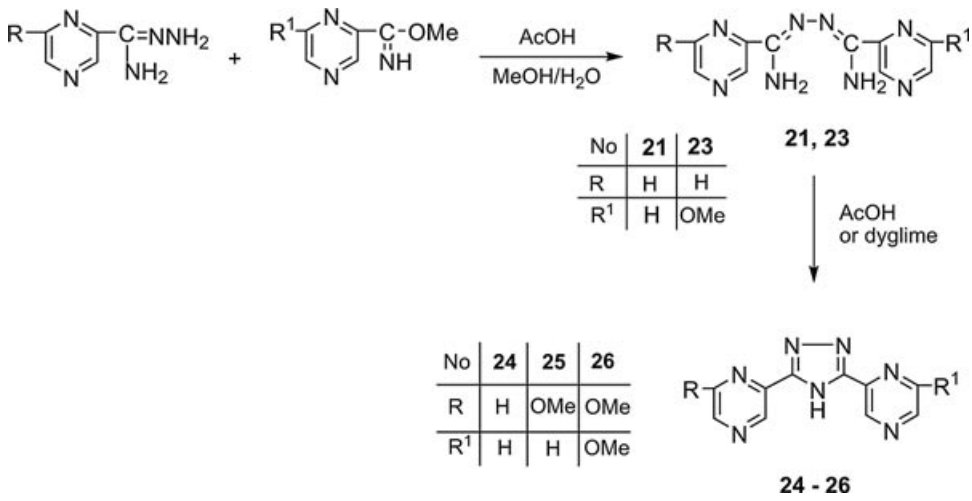
SCHEME 5

Reaction yields, physical constants, and spectral data of newly synthesized compounds are given in Tables 1 and 2.

MICROBIOLOGY

All of obtained pyrazinamidrazone derivatives 1–41 were evaluated for their in vitro antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosae*, and *Candida albicans* strains (Table 3). Gramicidin was used as a reference drug in an antibacterial test. Unfortunately, all tested compounds showed poor or no antibacterial activity as the minimal inhibitory concentration (MIC) obtained for the strains ranged from 128 to 256 μg/mL. Three of 41 derivatives (**3, 5,** and **12**) exhibited low activity against *S. aureus* (MIC 128 μg/mL) and both compounds **2** and **3** were active toward *P. aeruginosae* in concentrations of 128 μ and 256 μg/mL. The remaining agents showed no antibacterial activity within the limits of used concentrations. MIC values determined for all of tested compounds were much higher than for reference gramicidin.

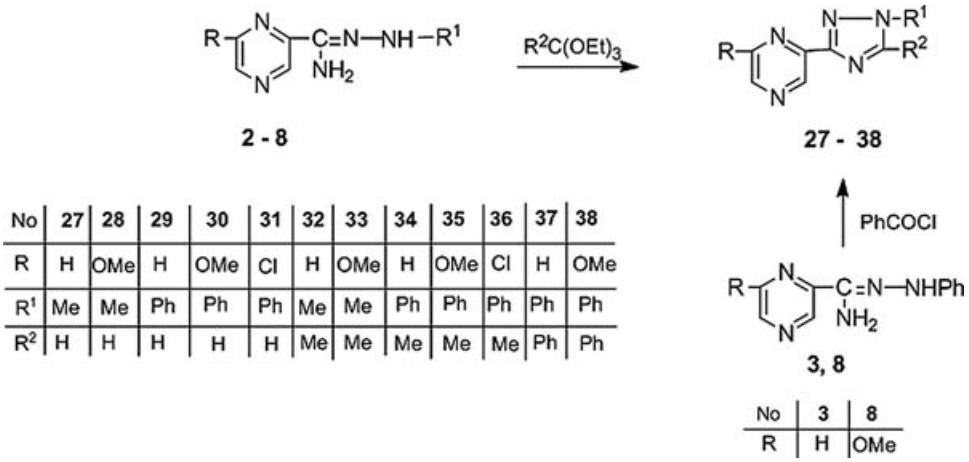
Eight (20%) derivatives (**1, 3, 4, 5, 8, 37, 39, 40**) inhibited growth of the clinical strain of *C. albicans* in a concentration 16–128 μg/mL exhibiting antifungal activity. The most active derivatives were **3** and



SCHEME 4

TABLE 1 Characteristics of Compounds **2**, **7–41**

				Calcd/Found					
				C		H		N	
No.	*Mp[° C] Solvent*	*Molecular Formula MW*	*Yield [%]*						
2	108–109 EtOH	$C_6H_9N_5$ 151.17	38	47.67	47.56	6.00	5.99	46.33	46.28
7	134–135 MeOH	$C_7H_{11}N_5O$ 181.20	58	46.40	46.28	6.12	6.10	38.65	38.68
8	149–150 MeOH	$C_{12}H_{13}N_5O$ 243.26	30	59.25	59.32	5.39	5.38	28.79	28.75
9	191–192 MeOH	$C_{11}H_{11}N_5O_2S$ 277.30	35	47.64	47.62	4.00	4.01	25.26	25.28
10	157–158 MeOH	$C_{12}H_{13}N_5O_2S$ 291.33	60	49.47	49.53	4.50	4.52	24.04	23.97
11	198–199 EtOH	$C_{13}H_{15}N_5O_3S$ 321.35	78	48.59	48.56	4.70	4.71	21.27	21.22
12	184–185 EtOH	$C_{11}H_{10}ClN_5O_2S$ 311.75	20	42.38	42.32	3.23	3.23	22.46	22.48
13	179–180 MeOH	$C_{12}H_{13}N_5O_2S$ 291.33	44	49.47	49.51	4.50	4.49	24.04	24.07
14	167–168 EtOH	$C_{13}H_{15}N_5O_3S$ 321.35	45	48.59	48.63	4.70	4.71	21.79	21.81
15	208–209 EtOH	$C_{14}H_{17}N_5O_3S$ 335.38	28	50.14	50.17	5.11	5.12	20.88	20.90
16	180–181 MeOH	$C_7H_{11}N_5O_2S$ 229.26	16	36.67	36.71	4.84	4.83	30.55	30.57
17	206–207 MeOH	$C_8H_{13}N_5O_3S$ 259.29	48	37.06	37.12	5.05	5.04	27.01	27.03
18	171–172 MeOH	$C_{12}H_9N_5O_2S$ 287.30	79	50.17	50.21	3.16	3.16	24.38	24.41
19	155–157 (dec.) MeOH	$C_{12}H_8ClN_5O_2S$ 321.74	59	44.80	44.83	2.51	2.51	21.77	21.78
20	159–160 MeOH	$C_{14}H_{13}N_5O_3S$ 331.35	73	50.75	50.78	3.95	3.96	21.14	21.18
21	251–255 (dec.) DMF	$C_{10}H_{10}N_8$ 242.24	24	49.58	49.56	4.16	4.17	46.26	46.31
22	252–258 (dec.) DMF	$C_{12}H_{14}N_8O_2$ 302.29	28	47.68	47.71	4.67	4.68	37.07	37.04
23	195–198 (dec.) Dioxan	$C_{11}H_{12}N_8O$ 272.27	64	48.53	48.50	4.44	4.45	41.16	41.13
24	274–276 (dec.) EtOH	$C_{10}H_7N_7$ 225.21	58	53.33	53.29	3.13	3.13	43.54	43.51
25	209–210 MeOH	$C_{11}H_9N_7O$ 255.24	98	51.76	51.78	3.55	3.55	38.41	38.45
26	265–268 (dec.) EtOH	$C_{12}H_{11}N_7O_2$ 285.26	98	50.53	50.47	3.89	3.89	34.37	34.41
27	174–175 MeOH	$C_7H_7N_5$ 161.16	88	52.17	52.21	4.38	4.39	43.45	43.47
28	133–134 EtOH	$C_8H_9N_5O$ 191.19	95	50.26	50.29	4.74	4.75	36.63	36.68
29	139–140 H_2O	$C_{12}H_9N_5$ 223.23	72	64.56	64.63	4.06	4.06	31.37	31.35
30	146–147 MeOH/H_2O (1:1)	$C_{13}H_{11}N_5O$ 253.26	54	61.65	61.62	4.38	4.39	27.65	27.67
31	163–164 MeOH	$C_{12}H_8ClN_5$ 257.68	56	55.93	56.05	3.13	3.13	27.18	27.15
32	173–174 MeOH	$C_8H_9N_5$ 175.19	80	54.85	54.88	5.18	5.19	39.98	39.92
33	82–84 EtOH	$C_9H_{11}N_5O$ 205.22	96	52.67	52.71	5.40	5.41	34.13	34.14
34	125–126 H_2O	$C_{13}H_{11}N_5$ 237.26	77	65.81	65.87	4.67	4.66	29.53	29.51
35	144–145 MeOH/H_2O (1:1)	$C_{14}H_{13}N_5O$ 267.28	73	62.91	62.95	4.90	4.89	26.20	26.24
36	142–144 (dec.) MeOH	$C_{13}H_{10}ClN_5$ 271.70	39	57.47	57.43	3.71	3.71	25.78	25.73
37	177–178 MeOH	$C_{18}H_{13}N_5$ 299.33	28	72.23	72.41	4.38	4.38	23.40	23.42
38	190–191 MeOH	$C_{19}H_{15}N_5O$ 329.36	58	69.29	69.35	4.59	4.59	21.26	21.24
39	204–205 (dec.) MeOH	$C_{11}H_9N_5OS$ 259.29	68	50.95	50.97	3.50	3.50	27.01	27.05
40	179–180 MeOH	$C_{12}H_{11}N_5O_2S$ 289.31	72	49.82	49.71	3.83	3.83	24.21	24.23
41	221–222 EtOH	$C_{11}H_{14}N_6OS$ 278.33	40	47.47	47.52	5.07	5.08	30.19	30.18



No	27	28	29	30	31	32	33	34	35	36	37	38
R	H	OMe	H	OMe	Cl	H	OMe	H	OMe	Cl	H	OMe
R^1	Me	Me	Ph	Ph	Ph	Me	Me	Ph	Ph	Ph	Ph	Ph
R^2	H	H	H	H	H	Me	Me	Me	Me	Me	Ph	Ph

No	3	8
R	H	OMe

SCHEME 6

TABLE 2 IR and ^{1}H NMR Spectra Data of Newly Synthesized Compounds

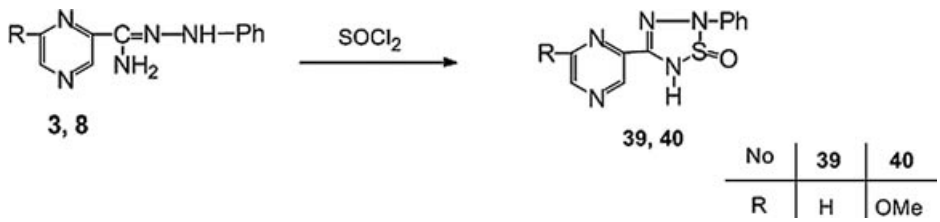
No.	*IR [cm^{-1}]*	*^{1}H NMR δ[ppm] (200 MHz, *500 MHz)*
2	3392, 3314, 3092, 2868, 1651, 1521, 1423, 1365, 1134, 1016, 846, 768	$CDCl_3$: 3.01 (s, 3H, CH_3); 4.16 (s, 1H, NH); 5.03 (s, 2H, NH_2); 8.48 (d, $J = 2.6$ Hz, 1H, CH); 8.40 (d, $J = 2.6$ Hz, 1H, CH); 8.42 (d, $J = 1.5$ Hz, 1H, CH); 9.32 (d, $J = 1.5$ Hz, 1H, CH)
7	3203, 2950, 1634, 1534, 1411, 1369, 1301, 1208, 1030, 1009, 879, 797	$CDCl_3$: 2.97 (s, 3H, CH_3); 3.96 (s, 3H, OCH_3); 4.07 (s, 1H, NH); 4.99 (s, 2H, NH_2); 8.14 (s, 1H, CH); 8.84 (s, 1H, CH)
8	3314, 3194, 1640, 1607, 1512, 1451, 1407, 1297, 1203, 1019, 867, 749, 690	$CDCl_3$: 4.02 (s, 3H, OCH_3); 5.07 (s, 2H, NH_2); 6.58 (s, 1H, NH); 6.91–7.34 (m, 5H, Ph); 8.20 (s, 1H, CH); 9.05 (s, 1H, CH)
9	3352, 3206, 1657, 1333, 1160, 1092, 1020, 727	DMSO-d_6: 6.56 (s, 2H, NH_2); 7.62–7.93 (m, 5H, Ph); 8.60–9.02 (m, 3H, CH); 9.76 (s, 1H, NH)
10	3295, 3254, 3191, 1648, 1575, 1446, 1339, 1162, 1018, 776, 683	$CDCl_3$: 2.86 (s, 3H, CH_3); 6.59 (s, 2H, NH_2); 7.55–7.95 (m, 5H, Ph); 8.56 (s, 1H, CH); 8.70 (s, 1H, CH); 9.39 (s, 1H, CH)
11	3315, 1647, 1578, 1449, 1377, 1336, 1311, 1164, 824, 730	$CDCl_3$. 2.84 (s, 3H, CH_3); 4.02 (s, 3H, OCH_3); 6.52 (s, 2H, NH_2); 7.72-7.92 (m, 5H, Ph); 8.35 (s, 1H, CH); 8.92 (s, 1H, CH)
12	3329, 3202, 1645, 1451, 1331, 1165, 1091, 1010, 868, 734	DMSO-d_6: 6.54 (s, 2H, NH_2); 7.63–7.92 (m, 5H, Ph); 8.80 (s, 1H, CH); 8.96 (s, 1H, CH); 9.86 (s, 1H, NH)
13	3333, 3051, 2812, 1661, 1330, 1160, 815, 692	DMSO-d_6: 2.35 (s, 3H, CH_3); 6.53 (s, 2H, NH_2); 7.40–7.80 (m, 4H, Ph); 8.63 (d, $J = 9.7$ Hz, 2H, CH); 9.03 (s, 1H, CH); 9.69 (s, 1H, NH)
14	3382, 3066, 2949, 1641, 1584, 1538, 1413, 1378, 1301, 1209, 1162, 1087, 1008, 813, 735	$CDCl_3$: 2.48 (s, 3H, CH_3); 4.03 (s, 3H, OCH_3); 6.21 (s, 2H, NH_2); 7.32-7.90 (m, 4H, Ph); 8.30 (s, 1H, CH); 8.79 (s, 1H, CH)
15	3310, 1640, 1577, 1543, 1447, 1377, 1333, 1210, 1159, 813, 720, 664	$CDCl_3$: 2.47 (s, 3H, CH_3); 2.82 (s, 3H, CH_3); 4.02 (s, 3H, OCH_3); 6.46 (s, 2H, NH_2); 7.38 (d, $J = 8.2$ Hz, 2H, Ph); 7.78 (d, $J = 8.2$ Hz, 2H, Ph); 8.34 (s, 1H, CH); 8.90 (s, 1H, CH)
16	3298, 3252, 3024, 2973, 1641, 1586, 1326, 1143, 791	$CDCl_3$: 3.03 (s, 3H, CH_3); 3.05 (s, 3H, CH_3); 6.62 (s, 2H, NH_2); 8.57–9.53 (m, 3H, CH)
17	3347, 3244, 3181, 1642, 1335, 1154, 1008, 823, 757	$CDCl_3$: = 2.99 (s, 3H, CH_3); 3.02 (s, 3H, CH_3); 4.01 (s, 3H, OCH_3); 6.40 (s, 2H, NH_2); 8.35 (s, 1H, CH); 9.02 (s, 1H, CH)
18	3064, 1489, 1395, 1222, 1118, 763, 729	$CDCl_3$: 7.59–8.19 (m, 5H, Ph); 8.65 (d, $J = 2.5$ Hz, 1H, CH); 8.71 (d, $J = 2.5$ Hz, 1H, CH); 8.88 (s, 1H, CH); 9.38 (s, 1H, CH)
19	3127, 3064, 1391, 1188, 1172, 1112, 1008, 731	$CDCl_3$: 7.70–8.17 (m, 5H, Ph); 8.66 (s, 1H, CH); 8.90 (s, 1H, CH); 9.27 (s, 1H, CH)
20	3065, 1593, 1510, 1439, 12227, 1196, 1178, 701	$CDCl_3$: 2.45 (s, 3H, CH_3); 7.40–8.03 (m, 4H, Ph); 8.64 (s, 1H, CH); 8.69 (s, 1H, CH); 8.80 (s, 1H, CH); 9.37 (s, 1H, CH)
21	3308, 1607, 1572, 1516, 1470, 1429, 1156, 1019, 862	DMSO-d_6: 6.90 (s, 4H, NH_2); 7.94–8.67 (m, 8H, CH)
22	3338, 1615, 1534, 1429, 1299, 1205, 1007, 876	DMSO-d_6: 4.05 (s, 6H, OCH_3); 6.80 (s, 4H, NH_2); 8.34 (s, 2H, CH); 9.27 (s, 2H, CH)
23	3306, 2854, 1611, 1538, 1426, 1304, 1209, 1116, 1018, 871	DMSO-d_6: 4.04 (s, 3H, OCH_3); 6.82 (s, 4H, NH_2); 8.32–9.74 (m, 5H, CH)
24	3059, 2916, 2807, 1524, 1378, 1141, 1018, 984, 861, 762	DMSO-d_6:* 8.80 (d, $J = 12.6$ Hz, 4H, CH); 9.38 (s, 2H, CH); 15.48 (s, 1H, NH)
25	3069, 3037, 2956, 1539, 1469, 1430, 1370, 1319, 1277, 1167, 1039, 1002, 894, 858, 764	DMSO-d_6:* 4.08 (s, 3H, OCH_3); 8.45-9.34 (m, 5H, CH); 15.29 (s, 1H, NH)
26	3037, 3004, 2954, 1540, 1471, 1368, 1045, 1005, 901, 878, 766	DMSO-d_6:* 4.06 (s, 6H, OCH_3); 8.44 (s, 2H, CH); 8.92 (s, 2H, CH); 15.19 (s, 1H, NH)
27	3091, 1495, 1390, 1334, 1223, 1157, 1017, 858, 761	$CDCl_3$*: 4.06 (s, 3H, CH_3); 8.23 (s, 1H, CH); 8.62 (s, 1H, CH); 8.68 (s, 1H, CH); 9.38 (s, 1H, CH)
28	3094, 2951, 1542, 1385, 1306, 1194, 1032, 1007, 873, 764	DMSO-d_6: 2.48 (s, 3H, CH_3); 2.49 (s, 3H, OCH_3); 8.34 (s, 1H, CH); 8.63 (s, 1H, CH); 8.78 (s, 1H, CH)

(Continued)

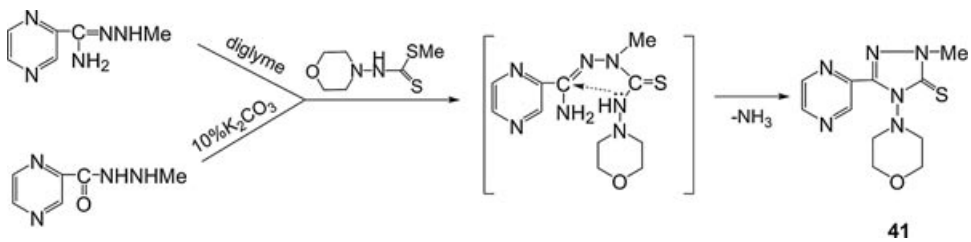
TABLE 2 Continued

No.	*IR [cm^{-1}]*	*^{1}H NMR δ[ppm] (200 MHz, *500 MHz)*
29	3100, 1598, 1510, 1498, 1479, 1332, 1253, 1126, 761, 688	$CDCl_3$: 7.43–7.82 (m, 5H, Ph); 8.60-8.69 (m, 3H, CH); 9.49 (s, 1H, CH)
30	3084, 2941, 1537, 1381, 1303, 1237, 1080, 1040, 1007, 758, 686	$CDCl_3$*: 4.16 (s, 3H, OCH_3); 7.47–7.80 (m, 5H, Ph); 8.32 (s, 1H, CH); 8.70 (s, 1H, CH); 9.04 (s, 1H, CH)
31	3096, 2888, 1596, 1509, 1376, 1336, 1153, 1128, 818, 760, 689	$CDCl_3$*: 7.49–7.81 (m, 5H, Ph); 8.67 (s, 1H, CH); 8.71 (s, 1H, CH); 9.38 (s, 1H, CH)
32	2949, 1537, 1384, 1354, 1140, 1018, 858, 761	$CDCl_3$: 2.54 (s, 3H, CH_3); 3.91 (s, 3H, CH_3); 8.56 (d, J = 2.6 Hz, 1H, CH); 8.63 (d, J = 2.6 Hz, 1H, CH); 9.31 (s, 1H, CH)
33	3013, 2947, 1542, 1388, 1304, 1175, 1007, 898	$CDCl_3$: 2.56 (s, 3H, CH_3); 3.92 (s, 3H, CH_3); 4.11 (s, 3H, OCH_3); 8.23 (s, 1H, CH); 8.87 (s, 1H, CH)
34	3066, 2937, 1534, 1388, 1360, 1179, 1131, 1015, 864, 789, 679, 674	$CDCl_3$: 2.64 (s, 3H, CH_3); 7.49–7.56 (m, 5H, Ph); 8.62 (d, J = 2.5 Hz, 1H, CH); 8.69 (s, 1H, CH); 9.30 (s, 1H, CH)
35	2979, 1536, 1416, 1360, 1292, 1171, 1050, 1034, 776, 697, 679	$CDCl_3$*: 2.67 (s, 3H, CH_3); 4.16 (s, 3H, OCH_3); 7.50–7.59 (m, 5H, Ph); 8.29 (s, 1H, CH); 8.97 (s, 1H, CH)
36	3056, 1510, 1494, 1437, 1376, 1331, 1168, 1139, 1008, 825, 768, 697	$CDCl_3$*: 2.66 (s, 3H, CH_3); 7.52–7.58 (m, 5H, Ph); 8.64 (s, 1H, CH); 9.32 (s, 1H, CH)
37	3309, 3068, 1641, 1347, 1167, 688	$CDCl_3$: 7.21–7.71 (m, 10H, Ph); 8.53 (q, J = 2.5 Hz, 1H, CH); 8.69 (d, J = 2.6 Hz, 1H, CH), 9.47 (s, 1H, CH)
38	3044, 3012, 2941, 1536, 1453, 1383, 1335, 1305, 1210, 1005, 781, 759, 702	$CDCl_3$: 4.16 (s, 3H, OCH_3); 7.33–7.61 (m, 10H, Ph); 8.31 (s, 1H, CH); 9.07 (s, 1H, CH)
39	3011, 1596, 1494, 1391, 1291, 1144, 1049, 747, 686	DMSO-d_6*: 7.19–7.60 (m, 5H, Ph); 8.79 (s, 1H, CH); 9.39 (s, 1H, CH); 12.26 (s, 1H, NH)
40	2947, 1593, 1539, 1466, 1381, 1317, 1142, 1125, 755, 687	DMSO-d_6: 4.12 (s, 3H, OCH_3); 7.50-7.63 (m, 5H, Ph); 8.47 (s, 1H, CH); 8.95 (s, 1H, CH); 11.97 (s, 1H, NH)
41	2970, 2854, 1475, 1458, 1384, 1325, 1212, 1107, 847, 729	$CDCl_3$: 2.95 (d, J = 10.5 Hz, 2H, CH_2); 3.66 (t, J = 11.4 Hz, 2H, CH_2); 3.84 (s, 3H, CH_3); 3.93 (d, J = 10.5 Hz, 2H, CH_2); 4.85 (t, J = 11.4 Hz, 2H, CH_2)8.75 (d, J = 5.6 Hz, 2H, CH); 9.20 (s, 1H, CH)

[1]HNMR spectra for compounds 24, 27, 30, 31, 35, 36 and 39 were performed at frequency 500 MHz.



SCHEME 7



SCHEME 8

8, which inhibited growth of *C. albicans* in a concentration of 16 μg/mL.

Analyzing the structure–activity relationship, it has been maintained that more active compounds possessed an amidrazone group (**3–5, 8**). Cyclization to 1,2,4-triazole caused loss of antibacterial activity. Only 2,5-dihydro-1,2,3,5-thiatriazole *S*-oxides **39** and **40** exhibited tuberculostatic activity in concentrations 62 and 31 μg/mL, respectively.

TABLE 3 *In vitro* Antimicrobial Activity of Pyrazinamidrazone Derivatives **1–41** [a,b,c]

	MIC [μg/mL]			
No.	*S. aureus*	*E. coli*	*P. aeruginosae*	*C. albicans*
1	>256	>256	>256	**256**
2	>256	**256**	**128**	>256
3	**128**	>256	**256**	**32**
4	>256	>256	>256	**128**
5	**128**	>256	>256	**32**
6	>256	>256	>256	>256
7	>256	>256	>256	>256
8	>256	>256	>256	**16**
9	>256	>256	>256	>256
10	>256	>256	>256	>256
11	>256	>256	>256	>256
12	128	>256	>256	>256
13	>256	>256	>256	>256
14	>256	>256	>256	>256
15	>256	>256	>256	>256
16	>256	>256	>256	>256
17	>256	>256	>256	>256
18	>256	>256	>256	>256
19	>256	>256	>256	>256
20	>256	>256	>256	>256
21	>256	>256	>256	>256
22	>256	>256	>256	>256
23	>256	>256	>256	>256
24	>256	>256	>256	>256
25	>256	>256	>256	>256
26	>256	>256	>256	>256
27	>256	>256	>256	>256
28	>256	>256	>256	>256
29	>256	>256	>256	>256
30	>256	>256	>256	>256
31	>256	>256	>256	>256
32	>256	>256	>256	>256
33	>256	>256	>256	>256
34	>256	>256	>256	>256
35	>256	>256	>256	>256
36	>256	>256	>256	>256
37	>256	>256	>256	**128**
38	>256	>256	>256	>256
39	>256	>256	>256	**32**
40	>256	>256	>256	**64**
41	>256	>256	>256	>256
Gm	0.5	0.5	2	–

[a]Minimum inhibitory concentrations for microbial strains were determined by a two-fold classical test-tube method of successive dilution.
[b]**Gm**, gramicidin.
[c]*S. aureus* ATCC 25923, *E.coli* ATCC 25922, *P. aeruginosae* ATCC 27853, *C. albicans* clinical strain.

EXPERIMENTAL

All materials and solvents were of analytical reagent grade. Thin-layer chromatography was performed on Merck silica gel $60F_{254}$ plates and visualized with UV. Melting points were determined with Boethius apparatus and were uncorrected. IR spectra were taken on Satellite FT-IR spectrophotometer (KBr pellets). ^{1}H NMR spectra were recorded on Varian Gemini (200 MHz) and Varian Unity Plus (500 MHz) instruments. Mass spectra (MS) for compounds **21** and **22** were taken on Finigan MAT 95 (70 eV). The results of elemental analysis (% C, H, N) for all of obtained compounds were in agreement with calculated values within the $\pm$ 0.3% range.

Syntheses of amidrazones **1, 3–5** were described elsewhere [24,25]. Reaction yields, physical constants, and spectral data of the compounds are given in Tables 1 and 2.

*N′-Methylpyrazinamidrazone (**2**)*

Cyanopyrazine (4.27 mL, 0.05 mol) was dissolved in 10 mL of anhydrous ethanol. Then 5.3 mL (0.1 mol) of methylhydrazine was added. The mixture was refluxed for 15 min and then left at room temperature for 24 h. The product was filtered in the form of long yellow needles and washed with cold ethanol.

*6-Methoxypyrazinamidrazone (**6**)*

6-Methoxypyrazine-2-iminoester [23] (3.3 g,0.02 mol) was dissolved in 15 mL of methanol. Then 2 mL (0.04 mol) of 98% hydrazine hydrate was added. The mixture was heated to boiling. Product crystals were filtered after cooling.

*N′-Methyl-6-methoxypyrazinamidrazone (**7**)*

To a solution of 3.3 g (0.02 mol) of 6-methoxypyrazine-2-methyliminoester [23] in 15 mL of methanol, 2.1 mL (0.04 mol) of methylhydrazine was added. The mixture was heated for 1 min and left for crystallization.

*N′-Phenyl-6-methoxypyrazinamidrazone (**8**)*

6-Methoxypyrazine-2-iminoester [23] (1.67 g, 0.01 mol) was dissolved in 10 mL of methanol. After addition of freshly distilled phenylhyrazine (1 mL, 10 mmol) the mixture was heated for 1 min and left for crystallization. Precipitate was filtered and washed with cold water.

*N′-Arylsulfonylpyrazinamidrazones (**9–15**)*

General Procedure. 5 mmol of appropriate pyrazine compound **3, 4,** or **6** was dissolved in 10 mL of dioxane in the presence of 2 mL (14 mmol) of triethylamine. Then 5 mmol of benzenesulfonyl chloride was added and mixture was refluxed for 2 h. The solvent was evaporated in vacuo and ice was added to oily residue. The precipitate was filtered and recrystallized.

*N′-Methyl-N′-methanesulfonylpyrazinamidrazone (**16,17**)*

Appropriate pyrazinamidrazone (5 mmol) **2** or **7** was dissolved in 4 mL of anhydrous pyridine. Then 5 mmol of methanesulfonyl chloride was added and the mixture was heated for 1 min. The mixture was left for over 1 h and evaporated under reduced pressure. The residue was washed with water, filtered, and recrystallized.

*3-Pyrazine-1H-[1,2,4]triazoles (**18–20, 27–36**)*

General Procedure. To appropriate amidrazone **2–8**, **9–17** (5 mmol), 5 mL of triethyl orthoformate was added. The mixture was refluxed for 3 h. After cooling, the precipitate was filtered and washed with ethyl ether.

*1,5-Diphenyl-3-pyrazine- and 1,5-Diphenyl-3-(6-methoxypyrazine-2-yl)-1H-[1,2,4]triazoles (**37,38**)*

N′-Phenylpyrazinamidrazone **5** or **8** (5 mmol) was refluxed with 0.6 mL (5 mmol) of benzoyl chloride in 5 mL of anhydrous pyridine for 6 h. Then the mixture was evaporated to dryness. The oily residue was stirred with ice and the precipitate was filtered and recrystallized.

*Bis-(α-aminomethylenepyrazin-2-yl)hydrazine (**21**)*

Method A. 0.861 g (5 mmol) of benzenesulfonylhydrazide was dissolved in 15 mL of methanol–water mixture (1:1). Then 0.685 g (5 mmol) of pyrazin-iminoester was added. The mixture became yellow and an intensively yellow crystalline product precipitated. The product was filtered and recrystallized.

Method B. 0.861 g (5 mmol) of pyrazinamidrazone and 0.685 g (5 mmol) pyrazinemethylester were dissolved in the methanol–water mixture (1:1). The mixture was acidified with acetic acid and a yellow product precipitated. The product was filtered, washed with water, and recrystallized. MS: *m/z* 243.1 (100 MH^+).

*Bis-(α-aminomethylene-6-methoxypyrazin-2-yl) hydrazine (**22**)*

It was synthesized according to the procedure for **21** in *method A* from 0.861 g (5 mmol) of benzenesulfonylhydrazide and 0.835 g (5 mmol) of 6-methoxypyrazine-2-iminomethylester. MS: *m/z* 303.1 (100 MH^+).

*1,4-Diamine-(6-methoxypyrazin-2-yl)-4-pyrazine-2,3-diazabuta-1,3-diene (**23**)*

It was synthesized according to the procedure for **21** in *method B* from 0.861 g (5 mmol) of

pyrazinamidrazone and 0.835 g (5 mmol) of 6-methoxypyrazine-2-iminomethylester.

*3,5-Dipyrazine-2-yl-4H-[1,2,4]triazoles (**24–26**)*

Appropriate bis-(α-aminomethylenepyrazine)hydrazine **21** or **22** (1 mmol) was heated for 1 h in 5 mL of glacial acetic acid. In the meantime, the mixture became colorless. Then ice was added to the mixture and product precipitated. It was filtered and recrystallized.

*2-Phenyl-4-pyrazin-2-yl-2,5-dihydro-[1,2,3,5] thiatriazole 1-oxide (**39, 40**)*

Appropriate amidrazone **3** or **8** (5 mmol) was dissolved in 4 mL of anhydrous pyridine and 5 mL of chloroform. Then the solution of 0.92 mL (13 mmol) of thionyl chloride in 5 mL of anhydrous chloroform was added dropwise to the stirred mixture at a temperature below 5°C. The mixture was stirred at room temperature overnight, then concentrated to dryness and stirred with ice. The precipitate was filtered and recrystallized.

*2-Methyl-4-morpholin-2-yl-5-pyrazin-2-yl-2,4-dihydro-[1,2,4]triazole-3-thione (**41**)*

Method A. *N′*-methylpyrazinamidrazone **2** (0.38 g, 2.5 mmol) was dissolved in 10 mL of mixture: dioxane and 10% potassium carbonate water solution (1:1). Then 0.48 g (2.5 mmol) of morpholin-4-yl-dithiocarbamic acid methyl ester was added. The mixture was refluxed for 8 h and left overnight. The precipitate was filtered and recrystallized.

Method B. Pyrazinecarboxylic acid *N′*-methylhydrazide (0.38 g, 2.5 mmol) was dissolved in 10 mL of mixture: dioxane and 10% potassium carbonate water solution (1:1). Then 0.48 g (2.5 mmol) of morpholin-4-yl-dithiocarbamic acid methyl ester was added. The mixture was refluxed for 8 h and left overnight. The filtered product was heated in 1 M solution of sodium hydroxide for 5 min. After cooling, the precipitate was filtered and washed with cold water until the alkaline reaction disappeared.

Antimicrobial Assay

Antimicrobial activity of the chemical agents against three recommended reference strains [26]: *S. aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosae* ATCC 27853, and clinical strain *C. albicans* was examined. The susceptibility of the microorganisms to the agents was determined by the broth microdilution assay according to the procedures outlined by the National Committee for Clinical Laboratory Standards [26]. The final concentration of the agents prepared in 200 μL of Mueller–Hinton broth (or Sabouraud's medium for *C. albicans*) ranged over 0.125–256 μg/mL. To prepare bacterial suspension, overnight a culture of bacteria in 3% Trypticase Soy Broth (or Sabouraud's medium for *C. albicans*) was diluted in sterile saline to the final concentration of approximately 10^7 CFU/mL of bacteria. Aliquots (5 μL) of bacterial suspension were added to each agent solution. The MIC was defined as the lowest concentration of the agent that completely inhibited growth of the bacteria after 24 h incubation in 37°C. The final results were the average values from the two independent experiments.

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