Full Paper

Synthesis and Antimicrobial Activity of Novel Heterocyclic Sulfamoyl-phenyl-carboximidamides Derived from Clinically Applied Sulfonamides

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A series of novel heterocyclic sulfamoyl-phenyl-carboximidamides were synthesized in satisfactory yields *via* condensation of clinically applied sulfonamides with heterocyclic methyl carbimidates. New structures were confirmed by IR and NMR spectra as well as elemental analyses. All the compounds were screened for their antibacterial, antifungal, and tuberculostatic activities. Preliminary results indicated that some target compounds exhibited promising antibacterial potency. Especially, *N*-[4-(thiazol-2-sulfamoyl)phenyl]pyrazine-2-carboximidamide (**16**) was found to be as potent as clinically applied sulfamethoxypyridazine.

Keywords: Antimicrobial activity / Methyl cabimidates / SAR analysis / Sulfonamides / Synthesis

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Introduction

In the past few decades the emergence and large-scale spread of resistant microorganisms could be observed [1]. Improperly conducted diagnosis, uncontrolled therapy, and drug withdrawal after the termination of infection symptoms lead to the emergence of mutant strains resistant to the medication [2]. Examples include methicillin-resistant *Staphylococcus aureus*, strains of *Mycobacterium tuberculosis* resistant to isoniazid and rifampicin, and azole-resistant strains of *Candida* species [3, 4]. Resistant strains of microorganisms are a major threat especially to immunocompromised patients, and infections caused by them are the most common complication in HIV-positive patients [5]. The emergence of resistant strains creates an urgent need for more effective and safe antimicrobial drugs. For this purpose, completely new chemical groups of effective chemotherapeutics are looked for. Precise knowledge of a molecular target often precedes the exploration of active substances. Another strategy involves a structural modification of known antimicrobial agents to increase their affinity to the target and the extension of the molecular spectrum [6]. In the case of the latter strategy different active structural fragments are often combined with each other in one molecule, each of which acts in an independent manner with the relevant molecular target [7]. In this way, one can get double or even synergistic effect of the drug. This creates an opportunity to overcome the existing resistance and inhibition the phenomenon of the emergence of new resistant strains [8].

Continued interest in sulfonamides known as effective antimicrobial agents is observed. In fact, they exhibit diverse pharmacological activities including carbonic anhydrase inhibitors [9], diabetes [10], antifungal [11], antiviral [12], anticancer and anti-inflammatory [13], and antibacterial activities. Clinically used antibacterial agents as sulfamerazine, sulfamethazine, sulfamethoxypyridazine, and sulfathiazole (Fig. 1) belong to this chemical group. These compounds are sulfanilamide derivatives and possess heterocyclic systems of pyrimidine, pyridazine, or thiazole at the N-1 position. Complexes of these drugs with metals of the transition block, Hg(II), Cu(II), Zn(II), and Ag(I), are very popular among synthetics. Such complexes exhibit interesting antibacterial and antifungal properties [14–16]. Therapeutic efficacy of sulfonamides is also increased

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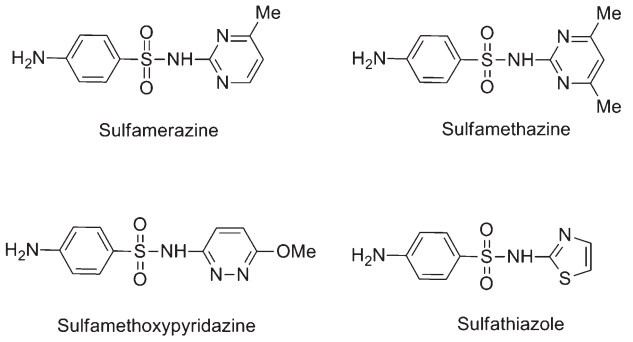


Figure 1. Structures of clinically used sulfonamides.

through the use of combination therapy with other chemotherapeutic agents such as trimethoprim [17].

The amidine functional group is an important structural element of compounds with found pharmacological activity. Amidine derivatives have antidegenerative [18], antitumor [19], and anti-platelet action [20]. They also act as serine protease inhibitors [21] and nitric oxide synthase inhibitors [22]. Compounds with anti-HIV [23], antibacterial, and antifungal activities [24] were also found among them. Moreover, the amidine group may be a perfect linker unit that could connect two pharmacophores, *e.g.*, the sulfanilamide moiety and the pyridine or pyrazine system.

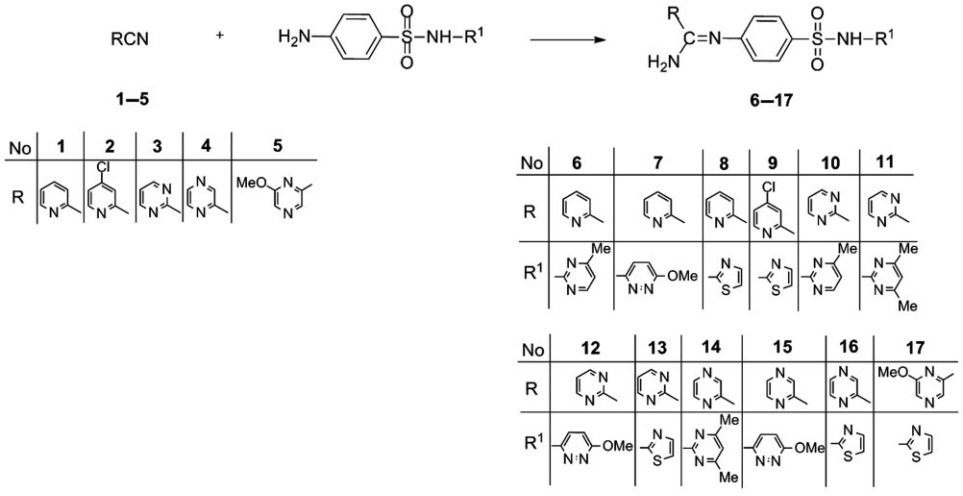
Based on all above considerations and as an extension of our search for effective antimicrobial agents among nitrogen heterocyclic compounds, we took the synthesis of derivatives that were condensates of clinically used sulfonamides with heterocyclic methyl carbimidates. The synthesized compounds were evaluated for their antimicrobial activity *in vitro*: antibacterial, antifungal, and tuberculostatic.

Results and discussion

Chemistry

The object of this study was to investigate how antimicrobial activity *in vitro* of clinically used sulfonamides changed as a result of their reaction with heterocyclic methyl carbimidates. Sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfathiazole (Fig. 1), and heterocyclic methyl carbimidates **1**–**5** were used in the reaction (Scheme 1). Methyl carbimidates **1**–**3** were obtained from the corresponding carbonitriles, which in a methanol solution of DBU (1,8-diazabicyclo[5.4.0]under-7-ene) gave the corresponding carbimidates which, without isolation, underwent further reaction with sulfonamides. Pyrazine-2-carbimidates **4**, **5** were used after the prior isolation. Syntheses of those carbimidates have been previously described by us [25, 26]. These products are formed by the reaction of pyrazine-2-carbonitrile and 6-chloropyrazine-2-carbonitrile with sodium methanolate. As described in this work, the reaction of carbonitrile in a methanol solution of DBU is a much more convenient and more efficient way to receive carbimidate **4**.

Carbimidates easily react influenced by protons in both the alkaline and acid groups, and these occur in the used sulfonamides. It was therefore necessary to decide which of the protons involved in the reactions, the proton of the 4-aminophenyl group or the sulfonamide one. To check the reactivity of the sulfonamide proton the attempt of the reaction of methyl pyrimidine-2-carbimidate **3** with sulfathiazole was



Scheme 1. Reaction conditions and yields: (a) carbonitrile (1 M equiv.), DBU (0.14 M equiv.) MeOH, 0.5 h reflux, sulfonamide (0.9 M equiv.), 3 h reflux, 36–75% (compounds **6**–**13**); (b) methyl carbimidate (1 M equiv.), sulfonamide (0.9 M equiv.), dioxane, 2 h reflux, 46–83% (compounds **14**–**17**).

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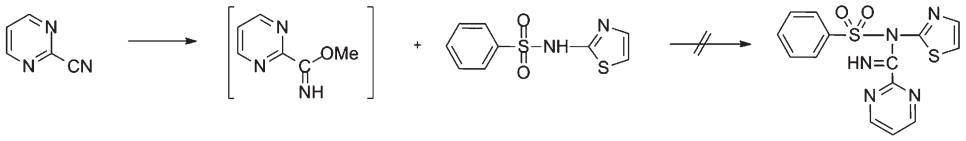


Scheme 2. Reaction conditions: methyl pyrimidine-2-carbimidate **3** (1 M equiv.) sulfathiazole (0.9 M equiv.), DBU (0.7 M equiv.) MeOH, 0.5 h reflux.

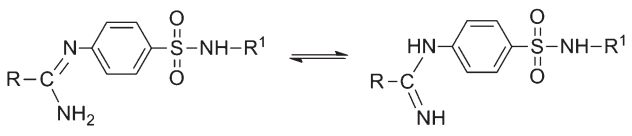
made under the conditions in which the reactions of the other sulfonamides were carried out (Scheme 2). Since the reaction proved negative, it was obvious that the active group in the reactions of sulfonamides with carbimidates was the 4-aminophenyl group.

Synthesis reactions of planned sulfamoyl-phenyl-carboximidamides (**6–17**) were carried out according to Scheme 1. This course of the synthesis was also confirmed in our previous work [27] for the reaction of methyl carbimidates with benzene- and 4-aminobenzenesulfonamide. In that work, we also demonstrated, based on X-ray studies, that the two protons in the resulting amidine group of the formed products are associated with the same nitrogen atom, and not with two different ones (Scheme 3). Compounds of this type may in fact undergo the amino-imine tautomerization. ^{1}H NMR spectra images confirmed the amine structure of the resulting products. The exception was compound **13**, for which the presence of three NH groups at ~7.73, 9.50, and 11.10 ppm in the ^{1}H NMR spectrum was found.

All newly synthesized compounds were characterized by IR and NMR spectra as well as the elemental analysis listed in the Experimental section. The spectral analyses were in accordance with the assigned structures.

Antimicrobial activity

All of the obtained sulfamoyl-phenyl-carboximidamides **6–17** were evaluated for their antibacterial activity against *S. aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853. Antifungal activity was determined with use of two strains: *Candida albicans* ATCC 90028 and *Candida parapsilosis* ATCC. The MIC values were determined as the minimum concentration inhibiting the growth of tested strains in relation to the probe with no tested compound. Sulfamerazine, sulfamethazine, sulfamethoxypyridazine, and sulfathiazole were used as reference compounds. New derivatives were also tested for their *in vitro* tuberculostatic activity against the *M. tuberculosis* H_{37}Rv strain and two ''wild'' strains isolated from tuberculosis patients: one (Spec. 210) resistant to *p*-aminosalicylic acid (PAS), isonicotinic acid hydrazide (INH), etambutol (ETB), and rifampicine (RFP) and another (Spec. 192) fully sensitive to the administered tuberculostatics. Isoniazid (INH) was used as reference drug. The bioactive data are summarized in Table 1.



Scheme 3. Possible tautomeric forms of synthesized compounds.

Antibacterial activity

All compounds, except sulfathiazole used clinically, showed very weak activity against a strain of *P. aeruginosa*, also other clinical sulfonamides. MIC values were 128 μg/mL for sulfathiazole and >256 μg/mL for the other compounds. All the condensates were less active than their respective sulfanilamide precursors. Derivative **16**, having in its structure 2-pyrazine ring linked to the thiazole system by the sulfamoyl-phenyl-carboximidamide moiety was the most active among the synthesized compounds **6–17**. The activity of that derivative against *S. aureus* with MIC value of 128 and 64 μg/mL against *E. coli* was comparable to the activity obtained for clinical sulfamethoxypyridazine. Compounds **15** and **17** which are derivatives of 2-pyrazine and 6-methoxy-2-pyrazine in conjunction with the appropriate 6-methoxy-3-pyridazine (**15**) and thiazole (**17**) showed lower activity. MIC values determined for those compounds were 256 μg/mL against *S. aureus* and 128 μg/mL against *E. coli*. Compounds **6** and **7** with 2-pyridine ring and 4-methyl-2-pyrimidine and 6-methoxy-3-pyridazine, respectively, showed activity against *E. coli* strain with MIC 128 μg/mL. They were not active against *S. aureus*. Derivatives **8** and **9** with 2-pyridine and 4-chloro-2-pyridine rings fused with sulfathiazole showed weak activity against *S. aureus* with MIC value of 256 μg/mL. They were not active against *E. coli*. Compounds **10**, **11**, **13**, and **14** showed no activity against the tested bacterial strains.

Antifungal activity

Unfortunately, all of the tested compounds showed very weak activity against the tested strains of *C. albicans* and *C. parapsilopsis* (MIC >256 μg/mL). The obtained results validated the fact that sulfonamides have no obvious antifungal activity *in vitro*, although the activity of some sulfonamides as inhibitors of carbonic anhydraze from the yeast *Saccharomyces cerevisiae* has been reported [10].

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Table 1. Antimicrobial activity of the newly synthesized sulfamoyl-phenyl-carboximidamides **6**–**17**.

No.	MIC (μg/ML)[a]							
	M. tuberculosis[b]			Bacterial strains			Fungal strains	
	$H_{37}Rv$	Spec. 192	Spec. 210	*S. aureus*	*E. coli*	*P. aeruginosa*	*C. albicans*	*C. parapsilosis*
6	100	50	50	>256	**128**	>256	>256	>256
7	50	50	50	>256	**128**	>256	>256	>256
8	50	50	50	**256**	>256	>256	>256	>256
9	50	50	50	**256**	>256	>256	>256	>256
10	50	50	50	>256	>256	>256	>256	>256
11	50	100	50	>256	>256	>256	>256	>256
12	100	100	50	**256**	>256	>256	>256	>256
13	100	100	50	>256	>256	>256	>256	>256
14	50	50	50	>256	>256	>256	>256	>256
15	50	50	50	**256**	**128**	>256	>256	>256
16	25	50	50	**128**	**64**	>256	>256	>256
17	25	25	50	**256**	**128**	>256	>256	>256
A[c]	100	50	100	128	32	>256	>256	>256
B	100	100	100	128	64	>256	>256	>256
C	100	50	50	64	32	>256	>256	>256
D	50	100	25	128	64	128	>256	>256
INH	0.5	0.5	1.1	–	–	–	–	–

[a] Minimum inhibitory concentrations for bacterial strains were determined by two-fold serial dilution method for microdilution plates and for mycobacterial strains by two-fold classical test-tube method of successive dilution.
[b] *M. tuberculosis* $H_{37}Rv$, Spec. 192, Spec. 210, *S. aureus* (ATCC 25923), *E. coli* (ATCC 25922), *P. aeruginosa* (ATCC 27853).
[c] A, sulfamerazine; B, sulfamethazine; C, sulfamethoxypyridazine; D, sulfathiazole; INH, isoniazid.

Tuberculostatic activity

The tested compounds also showed weak tuberculostatic activity, weaker than the reference isoniazide (MIC 0.5–1.0 μg/mL). For most of the compounds obtained the MIC values ranged from 50 to 100 μg/mL. Compounds **16** and **17** which were derivatives of 2-pyrazine and 6-methoxy-2-pyrazine showed the highest activity with MIC values of 25 μg/mL against the standard strain $H_{37}Rv$, 50 and 25 μg/mL, respectively, toward the sensitive strain 192, and 50 μg/mL against the resistant strain 210. Sulfathiazole was the most active among the clinical sulfonamides again with MIC values of 50 μg/mL ($H_{37}Rv$) and 100 μg/mL (Spec. 192). Interestingly, that drug showed a higher activity against the resistant strain 210, MIC 25 μg/mL.

Conclusion

In conclusion, a series of novel sulfamoyl-phenyl-caboximidamides with different nitrogen heterocyclic systems were synthesized successfully *via* condensation of clinically applied sulfonamides with heterocyclic methyl carbimidates. All these new compounds were confirmed by IR and NMR spectra as well as elemental analyses. Their antimicrobial activities were evaluated against *S. aureus*, *E. coli*, *P. aeruginosa*, *C. albicans*, *C. parapsilosis* as well as *M. tuberculosis*. The results showed that some of the synthesized sulfonamide derivatives exhibited moderate antimicrobial activities *in vitro*. Especially, compounds **15**–**17** bearing the pyrazine ring and methoxypyrimidine or thiazole systems showed the most potent antibacterial activities against *S. aureus* and *E. coli* strains with MIC values ranging from 64 to 256 μg/mL. The activity of compound **16** was at the same level as clinically used sulfamethoxypyridazine. However, the condensates were less active than their respective sulfanilamide precursors. Compounds **16** and **17** exhibited the highest activity against *M. tuberculosis* with MIC values of 25–50 μg/mL which indicated a weaker activity than the reference isoniazid (MIC 0.5–1.0 μg/mL).

Experimental

Chemistry

All materials and solvents were of analytical reagent grade. Sulfamerazine, sulfamethazine, sulfamethoxypyridazine, and sulfathiazole were from Fluka Chemie (Sigma–Aldrich Chemie GmbH, Buchs, Switzerland). Thin-layer chromatography was performed on Merck silica gel $60F_{254}$ plates and visualized with UV. The results of elemental analyses (%C, H, N) for all of the obtained compounds were in agreement with calculated values within ±0.3% range. NMR spectra in $CDCl_3$ or DMSO-d_6 were recorded on Varian Unity Plus (500 MHz) and Varian Gemini (200 MHz) instruments (Varian, Palo Alto, CA). IR spectra were determined as KBr pellets of the solids on a Satellite FT-IR spectrophotometer (Mattson Instruments, Madison, WI). Melting points were determined on a Boethius apparatus (Franz Küstner Nachf. KG, Dresden, Germany) and were uncorrected. Methyl

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6-methoxypyrazine-2-carbimidate (**5**) was obtained according to the method described earlier by Foks and Manowska [26]. Reaction yield and compound characteristics were found to be identical with those described (mp 100–101°C).

*Synthesis of methyl pyrazine-2-carbimidate (**4**)*

Pyrazine-2-carbonitrile (10 mL, 116 mmol) was dissolved in 20 mL of methanol and 1 mL (6.5 mmol) of DBU was added to the solution. The mixture was stirred for 1 min and the product precipitated abundantly. The mixture was cooled and the precipitate was filtered off and recrystallized from methanol yielding 13.5 g (85%) of colorless needles; mp 115–116°C [25].

*General method for the synthesis of sulfamoylphenylcarbimidamides (**6**–**13**)*

Appropriate carbonitrile **1**–**3** (5 mmol) was dissolved in 10 mL of methanol and 0.1 mL (0.7 mmol) of DBU was added. The mixture was refluxed for 0.5 h required for methyl carbimidate formation. Then 4.5 mmol of appropriate sulfonamide was added. The mixture was refluxed for another 3 h. The solvent was evaporated and 20 g of ice was added. The precipitate was filtered off and recrystallized from a suitable solvent.

*N-[4-(4-Methylpyrimidin-2-sulfamoyl)phenyl]pyridine-2-carboximidamide (**6**)*

The crude product was recrystallized from ethylene glycol–ethanol mixture (1:1). Yield 45%, mp 218–220°C; IR (KBr): 3467, 3356 (ν N–H), 3037, 2852 (ν C–H), 1648 (ν C=N), 1568 (ν C=C), 1330, 1157 (ν SO_2), 1090 (δ C–H), 894 (γ C–H), 573 (γ N–H) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.34 (s, 3H CH_3), 6.80 (br s, 2H, NH_2 + D_2O exchangeable), 6.92 (d, 1H, J = 5.0 Hz), 7.00 (d, 2H, J = 8.3 Hz), 7.53–7.59 (m, 1H), 7.93–7.97 (m, 3H), 8.25 (d, 1H, J = 8.0 Hz), 8.35 (d, 1H, J = 5.0 Hz), 8.63 (d, 1H, J = 4.4 Hz), 11.50 (br s, 1H, NH + D_2O exchangeable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 23.62, 115.13, 121.73 (2C), 125.92, 129.71 (2C), 133.64, 137.54, 148.40 (2C), 151.29, 152.41, 155.02, 157.08, 157.90, 168.44. Anal. Calcd for $C_{17}H_{16}N_6O_2S$ (mw 368.41): C, 55.42; H, 4.38; N, 22.81. Found: C, 55.53; H, 4.37; N, 22.76.

*N-[4-(6-Methoxypyridazin-3-sulfamoyl)phenyl]pyridine-2-carboximidamide (**7**)*

This compound was recrystallized from ethylene glycol–ethanol mixture (1:1). Yield 58%, mp 176–178°C; IR (KBr): 3478, 3347, 3280 (ν N–H), 1640 (ν C=N), 1572 (ν C=C), 1332 (ν SO_2), 1297, 1137 (ν SO_2), 1083, 931 (δ C–H), 572 (γ N–H) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 3.85 (s, 3H OCH_3), 6.58 (br s, 2H, NH_2 + D_2O exchangeable), 7.00 (d, 2H, J = 8.2 Hz), 7.30 (d, 1H, J = 9.7 Hz), 7.53–7.59 (m, 1H), 7.74–7.81 (m, 3H), 7.91–7.99 (m, 1H), 8.20 (d, 1H, J = 8.0 Hz), 8.63 (d, 1H, J = 4.4 Hz), 11.50 (br s, 1H, NH + D_2O exchangeable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 54.74, 121.77, 122.14 (2C), 124.83, 125.91, 126.27, 127.97 (2C), 135.88, 137.53, 148.38, 151.31, 152.43, 153.12, 154.33, 158.90. Anal. Calcd for $C_{17}H_{16}N_6O_3S$ (mw 384.41): C, 53.12; H, 4.20; N, 21.86. Found: C, 53.06; H, 4.21; N, 21.97.

*N-[4-(Thiazol-2-sulfamoyl)phenyl]pyridine-2-carboximidamide (**8**)*

This compound was recrystallized from methanol. Yield 67%, mp 191–192°C; IR (KBr): 3487, 3370 (ν N–H), 3108 (ν C–H), 1642 (ν C=N), 1585, 1537 (ν C=C), 1141 (ν SO_2), 1086, 936 (δ C–H), 858, 650 (γ C–H), 573 (γ N–H) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 6.80 (d, 1H, J = 4.4 Hz), 6.83 (br s, 2H, NH_2 + D_2O exchangeable), 7.00 (d, 2H, J = 8.5 Hz), 7.24 (d, 1H, J = 4.7 Hz), 7.53–7.60 (m, 1H), 7.74 (d, 2H, J = 8.5 Hz), 7.91–8.00 (m, 1H), 8.27 (d, 1H, J = 8.0 Hz), 8.63 (d, 1H, J = 4.8 Hz), 11.50 (br s, 1H, NH + D_2O exchangeable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 108.08, 121.76, 121.91 (2C), 125.88, 126.38, 127.52 (2C), 136.51, 137.52, 148.38, 151.36, 152.34, 153.93, 169.02. Anal. Calcd for $C_{15}H_{13}N_5O_2S_2$ (mw 359.43): C, 50.12; H, 3.65; N, 19.48. Found: C, 49.99; H, 3.66; N, 19.51.

*4-Chloro-N-[4-(thiazol-2-sulfamoyl)phenyl]pyridine-2-carboximidamide (**9**)*

This compound was recrystallized from dioxane. Yield 75%, mp 247–249°C; IR (KBr): 3459, 3352 (ν N–H), 1655 (ν C=N), 1534, 1423 (ν C=C), 1284, 1150 (ν SO_2), 1087, 937 (δ C–H), 573 (γ N–H) cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6): δ 6.81 (d, 1H, J = 4.4 Hz), 6.85 (br s, 2H, NH_2 + D_2O exchangeable), 7.05 (d, 2H, J = 7.3 Hz), 7.24 (d, 1H, J = 4.4 Hz), 7.71–7.77 (m, 3H), 8.27 (d, 1H, J = 2.0 Hz), 8.63 (d, 1H, J = 5.3 Hz), 12.6) (br s, 1H, NH + D_2O exchangeable); ^{13}C NMR (500 MHz, DMSO-d_6): δ 108.21, 121.70, 122.01 (2C), 124.86, 125.82, 127.52 (2C), 136.31, 144.17, 150.00, 151.41, 153.30, 168.92. Anal. Calcd for $C_{15}H_{12}ClN_5O_2S_2$ (mw 393.88): C, 45.74; H, 3.07; N, 17.78. Found: C, 45.63; H, 3.07; N, 17.75.

*N-[4-(4-Methylpyrimidin-2-sulfamoyl)phenyl]pyrimidine-2-carboximidamide (**10**)*

This compound was recrystallized from ethylene glycol–ethanol mixture (1:1). Yield 41%, mp 207–208°C; IR (KBr): 3342, 3262 (ν N–H), 1644 (ν C=N), 1594, 1565, 1498 (ν C=C), 1405, 1335, 1161 (ν SO_2), 1090 (δ C–H), 809 (γ C–H), 585 (γ N–H) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.33 (s, 3H, CH_3), 6.88 (d, 1H, J = 4.9 Hz), 7.43 (br s, 2H, NH_2 + D_2O exchangable), 7.56–7.79 (m, 2H), 7.84–8.09 (m, 3H), 8.33 (d, 1H, J = 4.9 Hz), 8.98–9.06 (m, 2H), 11.50 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 23.58, 112.72, 115.06, 119.87, 122.65 (2C), 129.32 (2C), 131.25, 133.22, 153.75, 157.09, 157.86, 157.99 (2C), 168.40. Anal. Calcd for $C_{16}H_{15}N_7O_2S$ (mw 369.40): C, 52.02; H, 4.09; N, 26.54. Found: C, 52.11; H, 4.08; N, 26.59.

*N-[4-(4,6-Dimethylpyrimidin-2-sulfamoyl)phenyl]-pyrimidine-2-carboximidamide (**11**)*

This compound was recrystallized from dioxane. Yield 65%, mp 268–271°C; IR (KBr): 3334 (ν N–H), 3108, 2923 (ν C–H), 1693, 1598 (ν C=N), 1569, 1520 (ν C=C), 1399, 1320, 1159 (ν SO_2), 680 (γ C–H), 589 (γ N–H) cm^{-1}; ^1H NMR (200 MHz, DMSO-d_6): δ 2.23 (s, 6H, $2CH_3$), 6.30 (br s, 2H, NH_2 + D_2O exchangable), 6.72 (s, 1H), 7.76 (t, 1H, J = 4.8 Hz), 7.95–8.06 (m, 4H), 9.00 (d, 2H, J = 5.0 Hz), 11.60 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 23.13 (2C), 113.68, 119.66, 123.67 (2C), 129.36 (2C), 135.96, 142.17, 156.56 (2C), 158.19, 161.71, 167.61 (2C), 169.27. Anal. Calcd for $C_{17}H_{17}N_7O_2S$ (mw 383.43): C, 53.25; H, 4.47; N, 25.57. Found: C, 53.12; H, 4.46; N, 25.51.

*N-[4-(6-Methoxypyridazin-3-sulfamoyl)phenyl]pyrimidine-2-carboximidamide (**12**)*

This compound was recrystallized from dioxane–ethanol mixture (1:1). Yield 53%, mp 204–206°C; IR (KBr): 3320 (ν N–H), 2853 (ν C–H), 1647 (ν C=N), 1586, 1564, 1529, 1469, 1414 (ν C=C), 1296 (ν SO_2), 1266 (ν C–O), 1144 (ν SO_2), 752 (γ C–H), 576 (γ N–H) cm^{-1};

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^{1}H NMR (200 MHz, DMSO-d_6): δ 3.83 (s, 3H, OCH_3), 5.95 (br s, 2H, NH_2 + D_2O exchangable), 7.32–7.39 (m, 1H), 7.45–7.50 (m, 1H), 7.59–7.87 (m, 5H), 9.00 (d, 2H, J = 4.6 Hz), 11.20 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 54.72, 120.39, 122.66 (2C), 124.80, 126.25, 127.42, 127.63 (2C), 128.75, 135.43, 153.10, 157.99 (2C), 158.19, 158.94. Anal. Calcd for $C_{16}H_{15}N_7O_3S$ (mw 385.40): C, 49.86; H, 3.92; N, 25.44. Found: C, 49.98; H, 3.93; N, 25.38.

*N-[4-(Thiazol-2-sulfamoyl)phenyl]pyrimidine-2-carboximidamide (**13**)*

This compound was recrystallized from DMF–dioxane mixture (1:1). Yield 36%, mp 223–225°C; IR (KBr): 3343, 3211 (ν N–H), 3099 (ν C–H), 1597 (ν C=N), 1563, 1530 (ν C=C), 1404, 1271, 1138 (ν SO_2), 1087, 938 (δ C–H), 681, 633 (γ C–H), 583, 559 (γ N–H) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.80 (d, 1H, J = 4.6 Hz), 7.23 (d, 1H, J = 4.6 Hz), 7.48–7.82 (m, 6H, 5H ArH and 1H NH + D_2O exchangable), 9.00 (d, 2H, J = 4.8 Hz), 9.50 (br s, 1H, NH + D_2O exchangeable) 11.10 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 108.18, 120.27, 120.41 (2C), 122.68, 125.43, 127.20 (2C), 127.99, 135.82, 154.38, 157.99 (2C), 168.92. Anal. Calcd for $C_{14}H_{12}N_6O_2S_2$ (mw 360.41): C, 46.65; H, 3.36; N, 23.32. Found: C, 46.57; H, 3.37; N, 23.27.

*General method for the synthesis of sulfamoylphenylimidamides (**14**–**17**)*

Methyl pyrazine-2-carbimidate (**4**) or methyl 6-methoxypyrazine-2-carbimidate (**5**) (2 mmol) and appropriate sulfonamide (1.7 mmol) were refluxed in 10 mL of dioxane for 2 h. Then dioxane was evaporated and 10 g of ice was added to the residue. The precipitate was filtered off and recrystallized from a suitable solvent.

*N-[4-(4,6-Dimethylpyrimidin-2-sulfamoyl)phenyl]pyrazine-2-carboximidamide (**14**)*

This compound was recrystallized from methanol. Yield 66%, mp 223–225°C; IR (KBr): 3485, 3437, 3369 (ν N–H), 2924 (ν C–H), 1646 (ν C=N), 1579, 1487, 1432 (ν C=C), 1332, 1155 (ν SO_2), 868 (γ C–H), 584 (γ N–H) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 2.27 (s, 6H, $2CH_3$), 6.78 (s, 1H), 6.93 (br s, 2H, NH_2 + D_2O exchangeable), 7.05 (d, 2H, J = 8.3 Hz), 7.95 (d, 2H, J = 8.3 Hz), 8.67 (s, 1H), 8.75 (s, 1H), 9.34 (s, 1H), 11.50 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 23.28 (2C), 114.00, 121.55 (2C), 129.95 (2C), 134.10, 143.16, 143.74, 146.47, 146.65, 151.33, 154.27, 156.66 (2C), 167.67. Anal. Calcd for $C_{17}H_{17}N_7O_2S$ (mw 383.43): C, 55.25; H, 4.47; N, 25.57. Found: C, 53.38; H, 4.46; N, 25.63.

*N-[4-(6-Methoxypyridazin-3-sulfamoyl)phenyl]pyrazine-2-carboximidamide (**15**)*

This compound was recrystallized from dioxane–water mixture (1:1). Yield 82%, mp 184–186°C; IR (KBr): 3439, 3332 (ν N–H), 1642 (ν C=N), 1583, 1530, 1470, 1415 (ν C=C), 1296, 1144 (ν SO2), 1091, 1017, 946 (δ C–H), 731 (γ C–H), 577 (γ N–H) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 3.95 (s, 3H, OCH_3), 7.04–7.08 (m, 4H, 2H ArH, and 2H NH_2 + D_2O exchangeable), 7.35 (d, 1H, J = 8.9 Hz), 7.68–7.91 (m, 3H), 8.71 (s, 1H), 8.81 (s, 1H), 9.42 (s, 1H), 12.80 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 54.74, 122.14 (2C), 124.95, 127.96 (2C), 128.75, 136.24, 143.14, 143.74, 146.45, 146.67, 152.87, 153.15, 153.71, 158.82. Anal. Calcd for $C_{16}H_{15}N_7O_3S$ (mw 385.40): C, 49.86; H, 3.92; N, 25.44. Found: C, 49.88; H, 3.91; N, 25.48.

*N-[4-(Thiazol-2-sulfamoyl)phenyl]pyrazine-2-carboximidamide (**16**)*

This compound was recrystallized from dioxane–petroleum ether mixture (1:1). Yield 83%, mp 213–215°C; IR (KBr): 3359 (ν N–H), 3165, 2918, 2811 (ν C–H), 1636 (ν C=N), 1583, 1539, 1427 (ν C=C), 1291, 1139 (ν SO_2), 1090, 950 (δ C–H), 858 (γ C–H), 575, 557 (γ N–H) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 6.82 (d, 1H, J = 4.8 Hz), 6.90 (br s, 2H, NH_2 + D_2O exchangeable), 7.06 (d, 2H, J = 8.4 Hz), 7.25 (d, 1H, J = 4.8 Hz), 7.75 (d, 2H, J = 8.5 Hz), 8.71 (s, 1H), 8.81 (s, 1H), 9.42 (s, 1H), 12.50 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 108.23, 112.72, 122.03 (2C), 124.80, 127.53 (2C), 136.35, 143.13, 143.75, 146.44, 151.35, 153.58, 168.92. Anal. Calcd for $C_{14}H_{12}N_6O_2S_2$ (mw 360.41): C, 46.65; H, 3.36; N, 23.32. Found: C, 46.75; H, 3.35; N, 23.29.

*6-Methoxy-N-[4-(thiazol-2-sulfamoyl)phenyl]pyrazine-2-carboximidamide (**17**)*

This compound was recrystallized from ethanol. Yield 46%, mp 203–204°C; IR (KBr): 3467, 3365 (ν N–H), 3167, 3100, 2807 (ν C–H), 1638 (ν C=N), 1590, 1538, 1439 (ν C=C), 1299, 1143 (ν SO_2), 1088, 941 (δ C–H), 859, 688 (γ C–H), 640, 554 (γ N–H) cm^{-1}; ^{1}H NMR (200 MHz, DMSO-d_6): δ 4.02 (s, 3H, OCH_3), 6.81 (d, 1H, J = 4.7 Hz), 6.90 (br s, 2H, NH_2), 7.03 (d, 2H, J = 8.4 Hz), 7.24 (d, 1H, J = 4.3 Hz), 7.73 (d, 2H, J = 8.4 Hz), 8.44 (s, 1H), 8.93 (s, 1H), 12.60 (br s, 1H, NH + D_2O exchangable); ^{13}C NMR (200 MHz, DMSO-d_6): δ 54.16, 108.18, 121.98 (2C), 127.50, 127.99 (2C), 134.99, 136.70, 143.40, 151.28, 152.48, 153.70, 158.85, 168.92. Anal. Calcd for $C_{15}H_{14}N_6O_3S_2$ (mw 390.44): C, 46.14; H, 3.61; N, 21.52. Found: C, 46.08; H, 3.62; N, 21.56.

Antibacterial and antifungal activity

Antimicrobial activity of newly synthesized compounds was examined. In the study of antibacterial activity, three recommended reference strains, *S. aureus* ATCC 25923, *E. coli* ATCC 25922, and *P. aeruginosa* ATCC 27853, were used [28]. Antifungal activity was determined with use of two strains: *C. albicans* ATCC 90028 and *C. parapsilosis* ATCC 22019 [29]. 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 [28, 29]. The stock solutions of the agents were prepared by dissolving the chemicals in DMSO. The final concentration of the agents in 200 μL of Mueller–Hinton broth (for bacterial strains) or in RPMI 1640 (for fungi) ranged over 0.125–256 μg/mL.

In order to prepare bacterial suspensions, an overnight culture of bacteria in 3% Tryptic Soy Broth was diluted in sterile saline to the final concentration of approximately 10^7 CFU/mL. Aliquats (10 μL) of bacterial suspension were added to each agent solution. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the agent that completely inhibited growth of the bacteria after 18-h incubation in 35°C.

Inocula of *Candida* strains were prepared by the suspension of five colonies picked from 24-h-old cultures on Saburaud Agar in sterile saline to the concentration of 10^6 cells/mL. The final concentration of the working suspension was approximately 10^4 cells/mL. Aliquots (10 μL) of the suspension

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were added to each agar solution. The MIC was defined as the lowest concentration of the agent that completely inhibited growth of the fungi after 48-h incubation in 35°C. The final results were average values from two independent experiments.

Tuberculostatic activity

The newly synthesized sulfamoylcarboximidamides (**6–18**) were examined *in vitro* for their tuberculostatic activity against *M. tuberculosis* H_{37}Rv strain and two ''wild'' strains isolated from tuberculosis patients: one (Spec. 210) resistant to *p*-aminosalicylic acid (PAS), isonicotinic acid hydrazide (INH), etambutol (ETB) and rifampicine (RFP) and the other (Spec. 192) fully sensitive to the administered tuberculostatics. Investigations were performed by a classical test-tube method of successive dilution in Youmans' modification of the Proskauer and Beck liquid medium containing 10% of bovine serum [30, 31]. Bacterial suspensions were prepared from 14-day-old cultures of slowly growing strains and from 48-h-old cultures of saprophytic strains [32, 33]. Solutions of compounds in ethylene glycol were tested. Stock solutions contained 10 mg of compounds in one milliliter. Dilutions (in geometric progression) were prepared in Youmans' medium. The medium containing no investigated substances and containing isoniazid (INH) as reference drug were used for comparison. Incubation was performed at a temperature of 37°C. The MIC values were determined as the minimum concentration inhibiting the growth of the tested tuberculous strains in relation to the probe with no tested compound.

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