Synthesis, Experimental and Theoretical Study on the Structure of Some Semicarbazides with Potential Antibacterial Activity

Monika Pitucha[a], Zbigniew Karczmarzyk[b], Urszula Kosikowska[c], and Anna Malm[c]

[a] Department of Organic Chemistry, Medical University, Chodźki 4a, 20-093 Lublin, Poland
[b] Department of Chemistry, University of Podlasie, 3 Maja 54, 081-10 Siedlce, Poland
[c] Department of Pharmaceutical Microbiology, Medical University, Chodźki 1, 20-093 Lublin, Poland

Reprint requests to Dr. Monika Pitucha. E-mail: monika.pitucha@umlub.pl

Z. Naturforsch. **2011**, *66b,* 505 – 511; received January 22, 2011

A series of 1,4-disubstituted semicarbazide and 4,4′-bis[1-substituted semicarbazide]diphenylmethane derivatives were synthesized to explore their antibacterial activity. New compounds were characterized by elemental analysis and spectroscopic data. In order to find the tautomeric equilibrium for the molecules energy calculations for each possible tautomeric form of model compound **2**, and for the most antibacterially active compound **7** in the investigated series, were calculated for the gas phase at the RHF/SCF/6-31G** level of theory.

Key words: Semicarbazides, Keto-Enol Tautomerism, Gas Phase, Antibacterial Activity, RHF/SCF/6-31G** Calculations

Introduction

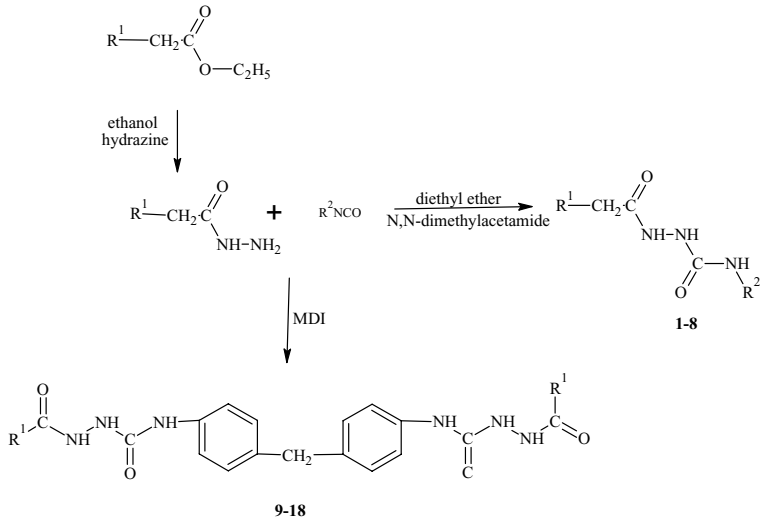
Semicarbazides are important building blocks for the synthesis of five-membered heterocycles [1 – 3]. Biologically active semicarbazide derivatives were studied as anticonvulsant [4] and antitubercular [5] agents. Additionally, derivatives having the pyrrole ring showed antibacterial activity, inhibiting growth of some Gram-positive bacteria, including *Bacillus cereus* and *Micrococcus luteus* [6]. Aryl semicarbazides have been reported to display excellent anticonvulsant activity in mice and rats compared to that of phenytoin [7]. It is known that the biological activity of the molecules is related to their structure and physicochemical properties. The electronic effects and the position of substituents, the pH value and the solvent polarity are major factors that influence this tautomerism [8]. In particular, the proton transfer associated with keto-enol tautomerization has long been of much interest because such interactions may catalyze certain biochemical transformations [9]. Aliphatic carbonyl compounds that contain hydrogens alpha to the carbonyl group undergo keto-enol tautomerism. Therefore, semicarbazide derivatives may exist theoretically in both keto and enol tautomeric forms.

In this paper, we present results of preliminary studies of the antibacterial activity of newly synthesized semicarbazide derivatives. In order to find the tautomeric equilibrium for the molecules, the energy of each possible tautomeric form in the gas phase was calculated.

Results and Discussion

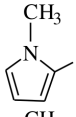
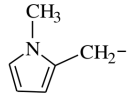
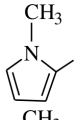
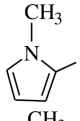
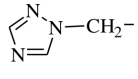
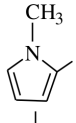
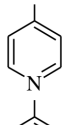
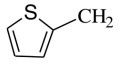
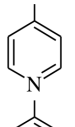
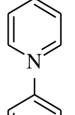
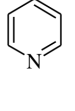
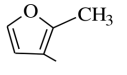
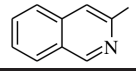
The new semicarbazide derivatives **1** – **18** were synthesized according to Scheme 1. The key hydrazide intermediates were obtained by addition of the corresponding carboxylic acid ester to an excess of hydrazine hydrate in anhydrous ethanol, followed by the method described earlier [10]. The acid hydrazides were reacted with 4-ethoxyphenyl, phenyl, 4-bromophenyl, ethyl, cyclohexyl, and 4-tolyl isocyanate, and 1,4-disubstituted semicarbazides **1** – **8** were obtained. Another series of new 4,4′-bis[1-substituted semicarbazide]diphenylmethane derivatives **9** – **18** were synthesized in the reaction of carboxylic acid hydrazide with 4,4′-diphenylmethane diisocyanate. The reactions were carried out in anhydrous diethyl ether or *N*,*N*-dimethylacetamide at r. t. Only compound **18** was obtained in absolute ethanol. Substituents for newly synthesized compounds are shown in Table 1.

There are 10 possible tautomeric forms of the keto-semicarbazide system (Fig. 1) in compounds **1** – **8** with one semicarbazide spacer and respectively 10^2 tautomeric forms for compounds **9** – **18** with two semi-



Scheme 1. General procedure for the synthesis of title compounds **1**–**18**; MDI = 4,4′-diphenylmethane diisocyanate.

Table 1. Substituents for compounds **1**–**18**.

Compound	R^1	R^2	Compound	R^1
1		4-(C_2H_5O)-C_6H_4	**9**	
2		C_6H_5	**10**	CH_3
3		4-BrC_6H_4	**11**	
4		C_2H_5	**12**	$CH_2C_6H_5$
5		4-(C_2H_5O)-C_6H_4	**13**	
6		C_6H_{11}	**14**	C_6H_5
7		4-BrC_6H_4	**15**	$C_6H_5OCH_2CH_2$
8		4-$CH_3C_6H_4$	**16**	
			17	H
			18	

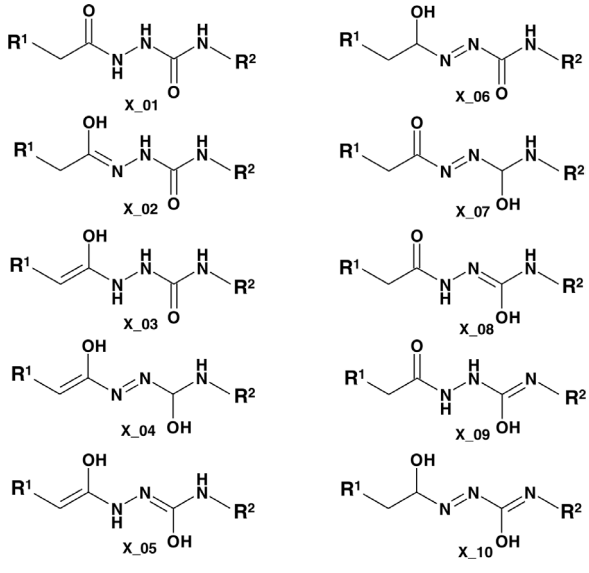


Fig. 1. Possible tautomeric forms of the keto-semicarbazide chain in compounds **1**–**8**.

carbazide chains. In order to find the relative stabilities of the tautomeric forms of molecules **1**–**18**, energy calculations for each of the 10 possible tautomeric forms of model compound **2** and compound **7**, antibacterially the most active in the investigated series, were performed in the gas phase at the RHF/SCF/6-31G** level of theory. The results of these calculations are presented in Table 2. As can be seen from Table 2, the energetically most stable form existing in the gas phase is X_01 (X = **2**, **7**; di-keto) for both investigated compounds **2** and **7**. The population of the remaining

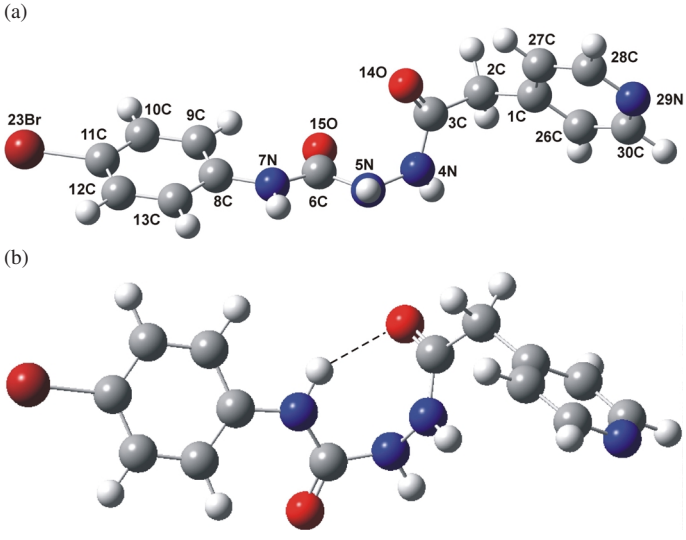


Fig. 2 (color online). A view of the molecule **7** with the atomic labelling adopted (a) in the low-energy extended conformation, and (b) cyclic conformation of the ketosemicarbazide chain with N–H···O intramolecular hydrogen bond (dashed line), calculated at the RHF/SCF/6-31G** level.

Table 2. The stabilization energy (ΔE) for tautomeric forms of **2** and **7**.

	ΔE (kcal mol^{-1})	
Tautomeric form	X = **2**	X = **7**
X_01	0	0
X_02	16.34	12.85
X_03	28.00	22.69
X_04	55.38	52.42
X_05	42.44	36.93
X_06	35.96	33.17
X_07	52.36	49.75
X_08	19.37	18.17
X_09	21.28	19.25
X_10	50.36	46.74

Table 3. Bond lengths and angles (Å, deg) for **7** calculated at the RHF/SCF/6-31G** level.

2C–1C	1.52	1C–2C–3C	111.7
3C–2C	1.52	2C–3C–4N	115.2
4N–3C	1.37	2C–3C–14O	122.9
5N–4N	1.37	4N–3C–14O	121.8
6C–5N	1.39	3C–4N–5N	119.2
7N–6C	1.36	4N–5N–6C	116.2
8C–7N	1.41	5N–6C–7N	112.1
14O–3C	1.19	5N–6C–15O	121.9
15O–6C	1.19	7N–6C–15O	125.9
		6C–7N–8C	127.9

tautomeric forms *in vacuo* estimated using a non-degenerated Boltzmann distribution is below the threshold of the detectability of conventional analytical methods. The energy differences between X_01 and the other remaining forms change in the relatively wide range of 16.34 – 55.38 kcal mol^{-1} for **2** and 12.85 – 52.42 kcal mol^{-1} for **7** and do not depend on the type of the substituents R^1 and R^2. The results of the theoretical calculations are in good agreement with the experimental data obtained from the solid-state structure of the structurally related (*R*)-(+)-1-[(1-methylpyrrol-2-yl)acetyl]-4-(1-phenylethyl)-semicarbazide [11], in which the semicarbazide chain exists in the same di-keto tautomeric form in the solid state. The IR spectra revealed the absence of hydroxyl groups but the presence of an NH absorption in the region 3268 – 3311 cm^{-1}. Additionally, the ν (C=O) band was observed in the region 1683 – 1651 cm^{-1}. The ^{1}H NMR spectra revealed the absence of the signal of OH protons, but exhibited a hydrazine NH proton signal in the region 8.00 – 10.46 ppm which disappeared on addition of deuterium oxide. This is in agreement with the results of quantum chemical calculations. A presentation of the low-energy conformation of molecule **7** as calculated at the RHF/SCF/6-31G** level, with the semicarbazide spacer in the di-keto tautomeric form, is shown in Fig. 2. The ketosemicarbazide chain adopts a *gauche-gauche-trans-gauche-trans-trans-cis* conformation with torsion angles ω_1 = 26C–1C–2C–3C = 120.4°, ω_2 = 1C–2C–3C–4N = −95.3°, ω_3 = 2C–3C–4N–5N = −170.7°, ω_4 = 3C–4N–5N–6C = 70.8°, ω_5 = 4N–5N–6C–7N = −167.2°, ω_6 = 5N–6C–7N–8C = −176.6°, and ω_7 = 6C–7N–8C–9C = −11.3°. Bond lengths and angles of **7** are listed in Table 3. The 3C–14O and 6C–15O bond lengths of 1.20 and 1.19 Å, respectively, are in good agreement with that of a C=O double bond. In the central semicarbazide moiety the bond lengths 5N–4N = 1.37 Å, 6C–5N = 1.39 Å and 7N–6C = 1.36 Å are typical for a conjugated π electron system.

Table 4. Potential-derived (ESP) and Mulliken atomic charges on the atoms in the semicarbazide part of molecules **2** and **7** at the RHF/SCF/6-31G** level.

	2		**7**	
Atom	ESP	Mulliken	ESP	Mulliken
2C	−0.20	−0.34	−0.28	−0.32
3C	+0.82	+0.75	+0.81	+0.76
4N	−0.43	−0.52	−0.47	−0.51
5N	−0.69	−0.53	−0.64	−0.53
6C	+1.23	+1.04	+1.18	+1.04
7N	−0.96	−0.84	−0.91	−0.85
14O	−0.66	−0.61	−0.62	−0.59
15O	−0.70	−0.62	−0.68	−0.62
23Br	–	–	−0.18	−0.04

It is worthwhile to note that the possibility of forming cyclic structures of the semicarbazide *via* the 7N–H···14O intramolecular hydrogen bond exists in the energetically lowest X_01 tautomeric form. The theoretical calculations at the RHF/SCF/6-31G** level for this kind of cyclic structure of **7** showed that the ketosemicarbazide chain adopts a *gauche-cis-trans-gauche-cis-trans-cis* conformation with torsion angles $\omega_1 = 78.6°$, $\omega_2 = 22.01°$, $\omega_3 = -176.3°$, $\omega_4 = -90.6°$, $\omega_5 = 38.1°$, $\omega_6 = 179.0°$, $\omega_7 = 23.7°$ and an H···14O distance of 2.22 Å and a 7N–H···14O angle of 145.5° typical for intramolecular hydrogen bonds (Fig. 2b). However, its energy is higher than that of the non-cyclic low-energy conformation of **7** by about 2.11 kcal mol^{-1}, resulting in a 3 % contribution of the cyclic conformation to the equilibrium of conformers of X_01 in the gas phase as estimated using a non-generated Boltzman distribution. The relatively large contribution of the cyclic form may be one reason for the amorphous (non-crystalline) state of the investigated compounds. The N–H···O and O–H···O intramolecular hydrogen bonds may exist in one or the other of the analyzed tautomeric forms. However, the relatively wide energy range between the lowest-energy tautomeric form and other remaining forms (12 – 50 kcal mol^{-1}) causes us to believe that intramolecular hydrogen bonds associated with an energy of (4 – 5 kcal mol^{-1}) should not affect much the tautomeric equilibrium.

Potential-derived (ESP) and Mulliken atomic charges on the atoms in the ketosemicarbazide part of molecules **2** and **7** calculated at the RHF/SCF/6-31G** level of theory (Table 4) are very similar and independent on the type of substituent on both sides of the semicarbazide spacer. Large positive and large negative charges are observed at the carbon and heteroatoms, respectively. This effect causes a strong polarization of the bonds in the semicarbazide chain, but the distribution of the partial bond dipoles results in a relatively small dipole moment of 0.81 D for **2** and 1.44 D for **7**. Similar values of dipole moments in the range of 1.03 – 1.89 D were calculated for molecules of other compounds in the series **1** – **8**.

All of the tested compounds were evaluated *in vitro* for their antibacterial activity using the agar dilution method, which is used as a standard procedure for preliminary checking and helps to select compounds showing promising activity. According to our results none of the tested compounds, except compound **7**, inhibited the growth of the used reference strains of Gram-positive or Gram-negative bacteria completely even at 1000 μg mL^{-1} concentration. Only partial inhibitory effects (about 60 – 80 % inhibition) were observed for two reference species of *Staphylococcus* spp. (*Staphylococcus epidermidis* ATCC 12228, *Staphylococccus aureus* ATCC 6538) with high concentrations of compound **8** (500 or 1000 μg mL^{-1}) or for *Staphylococcus epidermidis* ATCC 12228 and *Bacillus subtilis* ATCC 6633 with compounds **5** and **6** at 1000 μg mL^{-1}. Compound **7** showed an inhibitory effect on Gram-positive bacteria with MIC values ranging from 31.25 – 250 μg mL^{-1} (Table 5). Using the broth microdilution method, it was found that compound **7** inhibited the growth of Gram-positive bacteria with MIC values from 15.63 to 125 μg mL^{-1} (Table 5). The difference among MIC values determined by two kinds of methods (agar dilution procedure and broth microdilution test) is in accordance with results communicated by other authors [12, 13]. It should be noted that in our experiments the activity of gentamicin, widely used for the treatment of serious infections caused by Gram-negative and Gram-positive bacteria, for *Staphylococcus* species was very high (MIC = 0.12 μg mL^{-1}). According to our previous data [6], only a few of the tested compounds possessed selective and concentration-dependent antibacterial activity against the reference strains of Gram-positive (beside *Staphylococcus* spp.) or Gram-negative bacteria which was revealed as complete or partial reduction (20 – 90 %) of the growth depending on the strains and the concentration of the compound.

In conclusion, we have described the synthesis of 1,4-disubstituted semicarbazide and 4,4′-bis[1-substituted semicarbazide]diphenylmethane derivatives and investigated their biological activity. The theoretical calculations at the RHF/SCF/6-31G** level provided the geometrical, conformational and electronic

Table 5. Influence of compound **7** on the growth of Gram-positive reference strains of bacteria[a].

MIC (μg mL^{-1})											
Sa 6538		Sa 25923		Se 12228		Bs 6633		Bc 10876		Ml 10240	
adm	bdm	adm	bdm	adm	bdm	adm	bdm	adm	bdm	adm	bdm
250	62.5	125	31.25	125	62.5	31.25	15.63	250	125	250	15.63

[a] adm – agar dilution method; bdm – broth microdilution method; Sa – *Staphylococcus aureus*; Se – *Staphylococcus epidermidis*; Bs – *Bacillus subtilis*; Bc – *Bacillus cereus*; Ml – *Micrococcus luteus*.

parameters of molecules **1** – **8**, which can be correlated with their biological activity. Compound **7** described in the present paper could be regarded as a leading structure with increased antibacterial activity against Gram-positive bacteria, including staphylococci (coagulase-positive *S. aureus* and coagulase-negative *S. epidermidis*).

Experimental Section

All chemicals were purchased from Merck Co. or Alfa-Aesar (Gdańsk, Poland) and used withouth further purification. Melting points were determined in a Fisher-Johns block and were not corrected. IR spectra were recorded from KBr discs using a Specord IR-75 spectrophotometer. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200F instrument in [D$_6$]DMSO with TMS as internal standard. The purity of the obtained compounds was checked by TLC on aluminum oxide 60 F_{254} plates (Merck) in a $CHCl_3$-C_2H_5OH (10 : 1 and 10 : 2) solvent system with UV or iodine visualization.

The hydrazides used for the reactions were obtained by published methods [10]. Compounds **1** – **6** and **9** – **14** were obtained and characterized earlier [6, 14 – 16].

*Preparation of 1,4-disubstituted semicarbazides **7**, **8**, **13**, **15** – **17***

A mixture of the appropriate hydrazide (10 or 20 mmol) and isocyanate (10 mmol) in 10 mL of diethyl ether was kept for 48 h at r. t. Then the product was filtered off, washed with diethyl ether and crystallized from ethanol.

*Compound **7***

M. p. 127 – 129 °C. – Yield 82 %. – IR (KBr) ν = 3268, 3027, 2928, 1683 (C=O), 1431 cm^{-1}. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.58 (s, 2H, CH_2), 7.25 – 7.82 (m, 4H, Ar-H), 8.40 – 8.81 (m, 4H, Ar-H), 9.94, 9.78, 10.27 (3× s, 3H, 3NH). – ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 18.92 (CH_2), 117.05, 117.38, 123.16, 123.27, 127.80, 129.29, 129.61, 135.51, 135.84, 143.25, 147.99, 151.47, 154.11 (all C-Ar), 166.70 (C=O), 167.84 (C=O). – $C_{14}H_{13}N_4O_2Br$ (349.2): calcd. C 48.15, H 3.75, N 16.05; found C 48.08, H 3.84, N 16.18.

*Compound **8***

M. p. 240 – 241 °C. – Yield 79 %. – IR (KBr) ν = 3271, 3033, 2931, 1681 (C=O), 1428 cm^{-1}. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.22 (s, 3H, CH_3), 3.39 (s, 2H, CH_2), 7.04 – 7.40 (m, 4H, Ar-H), 8.41 – 8.66 (m, 4H, Ar-H), 8.86, 9.28, 9.98 (3× s, 3H, 3NH). – ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 17.16 (CH_3), 54.85 (CH_2), 116.20, 123.17, 123.53, 123.96, 126.31, 129.66, 136.95, 137.08, 143.02, 147.86 (all C-Ar), 167.64 (C=O), 179.80 (C=O). – $C_{15}H_{16}N_4O_2$ (284.3): calcd. C 63.36, H 5.67, N 19.71; found C 63.42, H 5.73, N 19.68.

*Compound **13***

M. p. 260 – 262 °C. – Yield 71 %. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.71 (s, 4H, 2CH_2), 3.79 (s, 2H, CH_2), 6.90 – 7.90 (m, 14H, Ar-H), 8.05, 8.78, 9.93 (3s, 6H, 6NH). – ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 33.05, 39.12, 115.72, 117.36, 123.63, 125.02, 125.27, 127.46, 128.30, 133.78, 135.37, 136.09, 153.92, 167.73. – $C_{27}H_{26}N_6O_4S_2$ (562.6): calcd. C 57.63, H 4.66, N 14.93; found C 57.71, H 4.57, N 14.86.

*Compound **15***

M. p. 244 – 246 °C. – Yield 68 %. – IR (KBr) ν = 3311, 3037, 2923, 1651 (C=O), 1497 cm^{-1}. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.63 (t, J = 6.1, 4H, 2CH_2), 3.78 (s, 2H, CH_2), 4.20 (t, J = 6.1, 4H, 2CH_2), 6.65 – 7.39 (m, 18H, Ar-H), 8.05, 8.58, 9.79 (3× s, 6H, 6NH). – $C_{33}H_{34}N_6O_6$ (610.7): calcd. C 64.90, H 5.61, N 13.76; found C 65.12, H 5.84, N 13.84.

*Compound **16***

M. p. 211 – 212 °C. – Yield 67 %. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 2.50 (s, 6H, 2 CH_3), 3.73 (s, 2H, CH_2), 6.80 – 7.62 (m, 12H, Ar-H), 8.00, 8.75, 9.79 (3× s, 6H, 6NH). – ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 11.85 (CH_3), 39.00 (CH_2), 107.70, 112.35, 115.73, 117.25, 127.45, 128.28, 130.44, 133.81, 136.05, 136.14, 139.48, 154.33, 155.50 (all Ar-C), 161.77 (C=O). – $C_{27}H_{26}N_6O_6$ (530.5): calcd. C 61.12, H 4.94, N 15.84; found C 61.23, H 4.84, N 15.78.

*Compound **17***

M. p. 256 – 258 °C. – Yield 70 %. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.85 (s, 2H, CH_2), 4.27 (s, 1H, CH), 7.07 – 7.46 (m, 10H, Ar-H), 8.83, 9.75, 10.06 (3× s, 3H, 3NH). –

^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 38.88 (CH_2), 117.13, 117.54, 117.73, 127.44, 133.97, 135.81, 135.90, 142.80, 153.72, 154.83, 157.85, 158.88, 159.49, 160.29 (all Ar-C), 165.57 (C=O), 166.54 (C=O). – $C_{17}H_{18}N_6O_4$ (370.4): calcd. C 55.13, H 3.75, N 22.69; found C 55.09, H 3.84, N 22.58.

Preparation of 4,4′-bis[1-(isoquinolin-3-yl)semicarbazide] diphenylmethane ***18***

Isoquinoline carboxylic acid hydrazide (20 mmol) was dissolved in absolute ethanol (10 mL) and 4,4′-methylenediphenylisocyanate (10 mmol) was added. The solution was warmed for 3 h on a water bath, allowed to cool to r. t. and then filtered to give a colorless solid.

M. p. 250 – 251 °C. – Yield 71 %. – ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 3.86 (s, 2H), 7.08 – 9.42 (m, 20H), 9.50 (2× s, 2H), 9.99 (2× s, 2H), 10.46 (2× s, 2H). – ^{13}C NMR (300 MHz, [D$_6$]DMSO): δ = 39.06 (CH_2), 116.92, 117.21, 119.01, 126.46, 126.64, 127.45, 127.55, 128.02, 130.08, 133.74, 133.85, 136.07, 141.64, 150.33 (all Ar-C), 162.73 (C=O). – $C_{35}H_{28}N_8O_4$ (624.6): calcd. C 67.29, H 4.52, N 17.94; found C 67.31, H 4.44, N 17.88.

Theoretical calculations

Energies, geometrical parameters (bond lengths, angles and torsion angles) and charge distribution on the atoms for structures **1** – **8** were calculated with NWCHEM 5.0 [17, 18] at the RHF/SCF/6-31G** level of theory. The structures were fully optimized without any symmetry constraints, and the initial geometries were built *de novo* using the AM1 semi-empirical SCF-MO method [19] implemented in the program package HYPERCHEM (version 4.5) [20]. The program ECCE [21, 22] was used for graphical visualization of the molecular structure of compound **7**.

Antimicrobial activity

All synthesized compounds were screened for *in-vitro* antibacterial activity against 10 reference strains of aerobic bacteria, including Gram-positive (*Staphylococcus aureus* ATCC 6538, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240) and Gram-negative microoorganisms (*Escherichia coli* ATCC 25922, *Proteus mirabilis* ATCC 12453, *Klebsiella pneumoniae* ATCC 13883, *Pseudomonas aeruginosa* ATCC 9027). In the first step, the agar dillution method was used with Mueller-Hinton medium containing from 31.25 to 1000 μg mL^{-1} of the tested compounds per plate. All stock solutions of the tested compounds were dissolved in dimethyl sulfoxide (DMSO) mixed with distilled water (1 : 1). It was found that DMSO at the final concentration had no influence on the growth of the tested microorganisms. Bacterial suspensions were prepared in sterile saline (0.85 % NaCl) with an optical density of McFarland standard 0.5 (150×10^6 CFU mL^{-1}; CFU = colony forming units), and then diluted (1 : 100). 20 μL of each suspension was put onto Mueller-Hinton agar containing the tested compounds. The plates were incubated at 37 °C for 18 h. The MIC (minimal inhibitory concentration) was defined as the lowest concentration preventing visible growth of the tested bacteria.

In the next step, the susceptibility of reference strains to compound **7** was assayed spectrophotometrically by the broth microdilution method; the MIC was again defined as the lowest concentration of a compound at which there is no visible growth of the tested bacteria. After incubation (37 °C for 18 h), the optical density (OD_{600}) was measured for the bacterial culture in the broth medium, and the MIC values were determined by comparison with the growth of a control (compound-free) medium. Gentamycin, a broad-spectrum aminoglicoside, was used as a antibacterial reference compound. The same volume of DMSO without any test compound was used as a control, and no inhibition was observed.

[1] M. Pitucha, L. Mazur, U. Kosikowska, A. Pachuta-Stec, A. Malm, Ł. Popiołek, Z. Rzączyńska, *Heteroatom Chem.* **2010**, *21*, 215 – 221.
[2] M. Pitucha, M. Dobosz, K. Stępniak, I. Dybała, A. E. Kozioł, *Pol. J. Chem.* **2007**, *81*, 2103 – 2114.
[3] M. Dobosz, M. Pitucha, I. Dybała, A. E. Kozioł, *Coll. Czech. Chem. Commun.* **2003**, *68*, 792 – 800.
[4] P. Yogeeswari, D. Sriran, V. Saraswat, J. V. Ragavendran, M. M. Kumar, S. Murugesan, R. Thirumurugan, J. P. Stables, *Eur. J. Pharma. Sci.* **2003**, *20*, 341 – 346.
[5] O. V. Fedorova, G. G. Mordovskoi, G. L. Rusinov, I. G. Ovchinnikova, M. N. Zueva, M. A. Kravchenko, O. N. Chupakhin, *Pharm. Chem. J.* **1998**, *32*, 64 – 66.
[6] M. Pitucha, B. Polak, R. Świeboda, U. Kosikowska, A. Malm, *Z. Naturforsch.* **2009**, *64b*, 570 – 576.
[7] S. V. Andurkar, C. Beguin, J. P. Stables, H. Kohn, *J. Med. Chem.* **2001**, *44*, 1475 – 1478.
[8] G. S. Nichol, W. Clegg, *Acta Crystallogr.* **2005**, *C61*, o383 – o385.
[9] M. A. Trikoupis, P. Gerbaux, D. J. Lavorato, R. Flammang, J. K. Terlouw, *Intern. J. Mass Spectrom.* **2002**, *217*, 1 – 22.
[10] M. Wujec, M. Pitucha, M. Dobosz, U. Kosikowska, A. Malm, *Acta Pharm.* **2004**, *54*, 251 – 260.
[11] M. Pitucha, Z. Rzączyńska, L. Mazur, *X-ray Structure Analysis Online* **2009**, *25*, 67 – 68.
[12] M. Karlsson, C. Fellstrom, A. Gunnarsson, A. Landen, A. Franklin, *J. Clin. Microbiol.* **2003**, *41*, 2596 – 2604.
[13] J. Rohde, M. Kessler, C. G. Baums, G. Amtsberg, *Veterinary Microbiology* **2004**, *102*, 25 – 32.

[14] M. Dobosz, M. Pitucha, M. Wujec, *Acta Pol. Pharm.* **1996**, *53*, 31 – 38.
[15] M. Pitucha, A. Chodkowska, M. Maciejewski, E. Jagiełło-Wójtowicz, A. Pachuta-Stec, *Monatsh. Chem.* **2010**, *141*, 199 – 203.
[16] M. Pitucha, M. Wujec, M. Dobosz, *Annals of the Polish Chemical Society* **2005**, *1*, 205 – 207.
[17] E. J. Bylaska, W. A. de Jong, K. Kowalski, T. P. Straatsma, M. Valiev, D. Wang, E. Apra, T. L. Windus, S. Hirata, M. T. Hackler, Y. Zhao, P. D. Fan, R. J. Harrison, M. Dupuis, D. M. A. Smith, J. Nieplocha, V. Tipparaju, M. Krishnan, A. A. Auer, M. Nooijen, E. Brown, G. Cisneros, G. I. Fann, H. Fruchtl, J. Garza, K. Hirao, R. Kendall, J. A. Nichols, K. Tsemekhman, K. Wolinski, J. Anchell, D. Bernholdt, P. Borowski, T. Clark, D. Clerc, H. Dachsel, M. Deegan, K. Dyall, D. Elwood, E. Glendening, M. Gutowski, A. Hess, J. Jaffe, B. Johnson, J. Ju, R. Kobayashi, R. Kutteh, Z. Lin, R. Littlefield, X. Long, B. Meng, T. Nakajima, S. Niu, L. Pollack, M. Rosing, G. Sandrone, M. Stave, H. Taylor, G. Thomas, J. van Lenthe, A. Wong, Z. Zhang, NWCHEM (version 5.0), A Computational Chemistry Package for Parallel Computers, Pacific Northwest National Laboratory, Richland, Washington (USA) **2006**.
[18] R. A. Kendall, E. Apra, D. E. Bernhold, E. J. Bylaska, M. Dupois, G. I. Fann, R. J. Harrison, J. Ju, J. A. Nichols, J. Nieplocha, T. P. Straatsma, T. L. Windus, A. T. Wong, *Comp. Phys. Commun.* **2000**, *128*, 260 – 283.
[19] M. J. S. Dewar, E. G. Zoebish, E. F. Healy, J. P. Stewart, *J. Am. Chem. Soc.* **1985**, *107*, 3902 – 3909.
[20] HYPERCHEM (rel. 4.5), Hypercube Inc., Waterloo, Ontario (Canada) **1988**.
[21] G. Black, B. Didier, T. Elsethagen, D. Feller, D. Gracio, M. Hackler, S. Havre, D. Jones, E. Jurrus, T. Keller, C. Lansing, S. Matsumoto, B. Palmer, M. Peterson, K. Schuchardt, E. Stephan, H. Taylor, G. Thomas, E. Vorpagel, T. Windus, C. Winters, ECCE (version 3.2), A Problem Solving Environment for Computional Chemistry, Pacific Northwest National Laboratory, Richland, Washington (USA) **2004**.
[22] K. L. Schuchardt, B. T. Didier, G. D. Black, *Concurrency and Computation: Practic and Experience* **2002**, *14*, 1221 – 1239.