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Synthesis and antibacterial activity of furo[3,2-*b*]pyrrole derivatives

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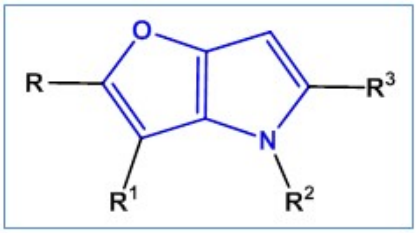
Abstract

8-Ethoxyfuro[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]triazine was synthesized by reaction of appropriate triazinone with $POCl_3$ and subsequent treating of 8-chloro derivative with sodium ethoxide in ethanol. Furo[3,2-*b*]pyrrole-5-carboxylates were hydrolysed to form acids, which underwent one-pot decarboxylation with TFA and formylation of the *in situ* formed furo[3,2-*b*]pyrrole with triethyl orthoformate to give 5-carbaldehydes**.** Hydrazinolysis of bis-esters led to bis-carbohydrazides which subsequently cyclized in acetic acid under microwave irradiation to form either pyrazine or acetamide derivatives with unusual chirality. Prepared compounds were evaluated for their antibacterial activity on *Escherichia coli* and *Micrococcus luteus*.



Keywords: Furo[3,2-*b*]pyrrole, cyclisation, formylation, antibacterial activity, microwave irradiation, chirality

Introduction

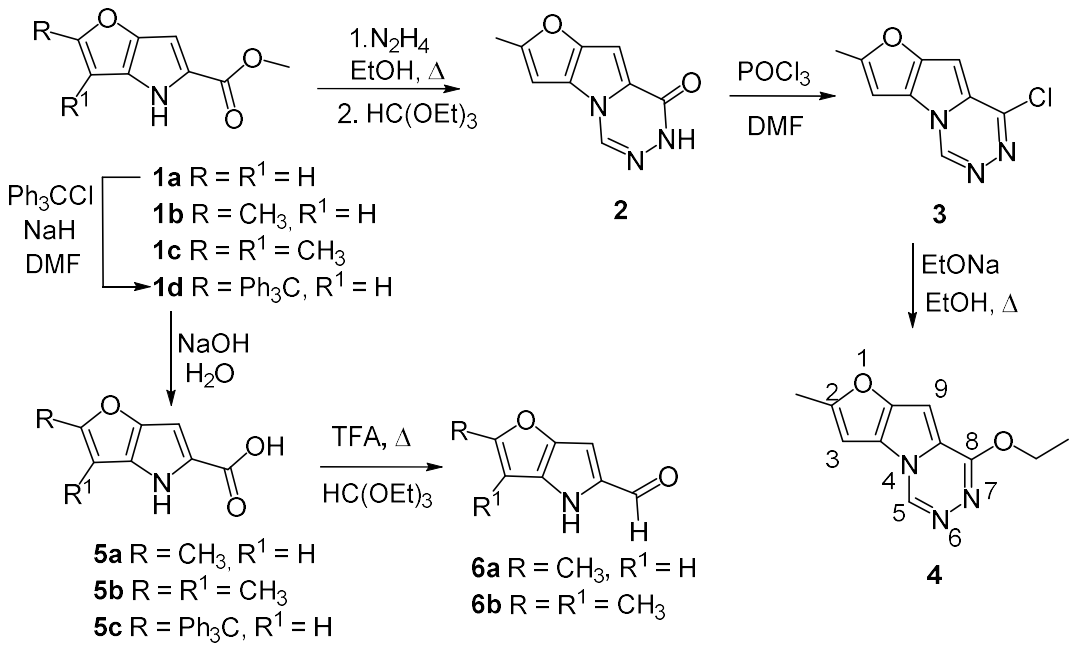
Furo[3,2-*b*]pyrroles are isosteres of the indole ring system in which the benzene ring is replaced by a furan ring. Interest in pyrrolo-fused heteroaromatic compounds such as furo-, thieno- and seleno-pyrroles stems mainly from the array of interesting biological activities[1-3] or their use as fluorescent dyes.[4]

More complex furo- and thieno-pyrroles, such as 6,7-dihydrofuro- and 6,7-dihydrothieno[2′,3′:4,5]pyrrolo-[1,2-*a*]pyrazin-8(5*H*)-ones,[5] furo[2′,3′:4,5]pyrrolo[2,1-*c*][1,4]oxazines,[6] furo[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]-triazolo[3,4-*f*][1,2,4]triazines[7] or thieno[3,2-*b*]pyrrolo[3,2-*d*]pyridazinones were synthesized and evaluated for their anticancer activity.[8] Moreover 2,3,5,7-tetrabromobenzofuro[3,2-*b*]pyrrole has been isolated from *Pseudoalteromonas* species and showed antimicrobial activity against methicillin-resistant *Staphylococcus aureus*.[9] Among fused furo[3,2-*b*]pyrrole derived heterocycles, the synthesis of furo[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]triazin-8(7*H*)-ones has been studied extensively.[7,10,11] However the preparation of appropriate 8-substituted derivatives (in the triazine ring) is limited to 8-hydrazino[7,10] or 8-methylsulfanyl[12] compounds.

As part of our current studies on the development of new methods in heterocyclic synthesis,[13,14] we report an efficient synthesis of furo[3,2-*b*]pyrrole derivatives fused with six-membered rings and evaluation of the synthesized compounds as to their antibacterial activity.

Results and Discussion

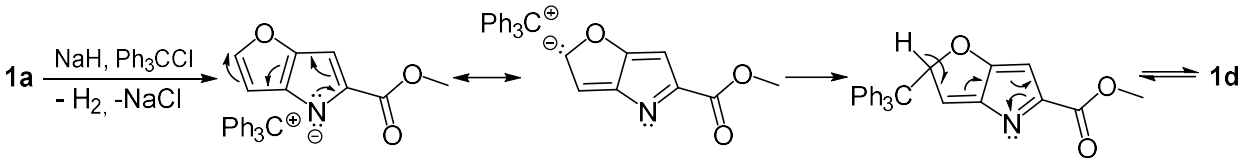
We have recently reported[15] the synthesis of furo[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]triazin-8(7*H*)-ones **2** from substituted carboxylates **1.** Herein we report the synthesis of furo[[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]triazine derivatives **3** and **4**, 7-amino-2-methylfuro[[2′,3′:4,5]pyrrolo[1,2-*a*]pyrazine-6,8(5*H*,7*H*)-diones **9** and acetamides **10**, as well as the synthesis of furo[3,2-*b*]pyrrole derived acids and aldehydes, in order to study their antibacterial activity.



Scheme 1. Synthesis of furo[3,2-*b*]pyrrole derivatives **3**-**6**.

A convenient synthetic route to transform a triazinone into a substituted triazine ring consists in treating the triazinone **2** with phosphorus oxychloride for 4h to give the 8-chloro substituted triazine **3**, which was used for the next step without further purification. Finally, use of sodium ethoxide resulted in the substitution of compound **3** and the title 8-ethoxytriazine **4** was obtained in 57% yield after 48h of reflux (Scheme 1). The ^{1}H NMR spectrum of **4** shows the singlets of H-5, H-3 and H-9 at 7.93, 7.57, and 7.32 ppm, respectively. Protons of the ethoxy group resonate as a quartet at 4.16 ppm and triplet at 1.97 ppm. The ^{13}C NMR spectra of **4** display the C-5 and C-8 carbons at 155.9 and 148.3 ppm, respectively.

The synthetic availability of the starting carboxylates **1** was also investigated with the intention to synthesize furo[3,2-*b*]pyrrole derivatives for screening of their antibacterial activity. Thus, 2-triphenylmethyl-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate **1d** was synthesized in high yield (89%) by reaction of **1a** with triphenylmethyl chloride in dimethylformamide and sodium hydride, according to the method used for the [2,3-*b*]-isomer[16] (Scheme 1). Compound **1d** displays in its ^{1}H NMR spectrum a singlet due to the NH group at 11.66 ppm, and singlets of H-6 and H-3 at 6.74 and 6.06 ppm, respectively. The ^{13}C NMR spectrum of **1d** shows the signal of ester carboxyl carbon at 165.2 ppm. The proposed mechanism involves formation of pyrrole N-anion in the initial step of reaction, but the direct bond interaction between bulky tritylium ion and pyrrole N-anion would be thermodynamicaly unstable due to steric hindrance caused by the adjacent -COOMe group and the furane ring. The ion pair can be formed initially, but thanks to the suitable mesomerism the relatively stable tritylium ion can move towards the most electron-rich C-2 carbon, which is also stericaly more favorable (Scheme 2).



Scheme 2. Mechanism explaining the synthesis of methyl 2-trityl-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate **1d**.

Carboxylates **1** were converted into the appropriate 4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acids **5a-5c** in 66-80% yields by hydrolysis of **1b-1d** in aqueous NaOH for 1.5-4h (Scheme 1). Acids **5a**-**5c** display in their ^{1}H NMR spectra a broad singlet at 12.8-12.13 ppm of carboxylic hydrogen and a singlet at 11.29-11.44 ppm due to the NH group. The H-6 protons of **5a** and **5c** resonate as singlets at 6.52-6.65 ppm or, in case of **5b**, as doublet at 6.58 ppm (*J* 1.8Hz). The IR spectra of **5a**-**5c** exhibit absorption bands of the NH group at 3373-3190 cm^{-1} and the carbonyl group at 1630-1640 cm^{-1}. The ^{13}C NMR spectra of **5a**-**5c** display the carboxyl carbons at 163.1 ppm.

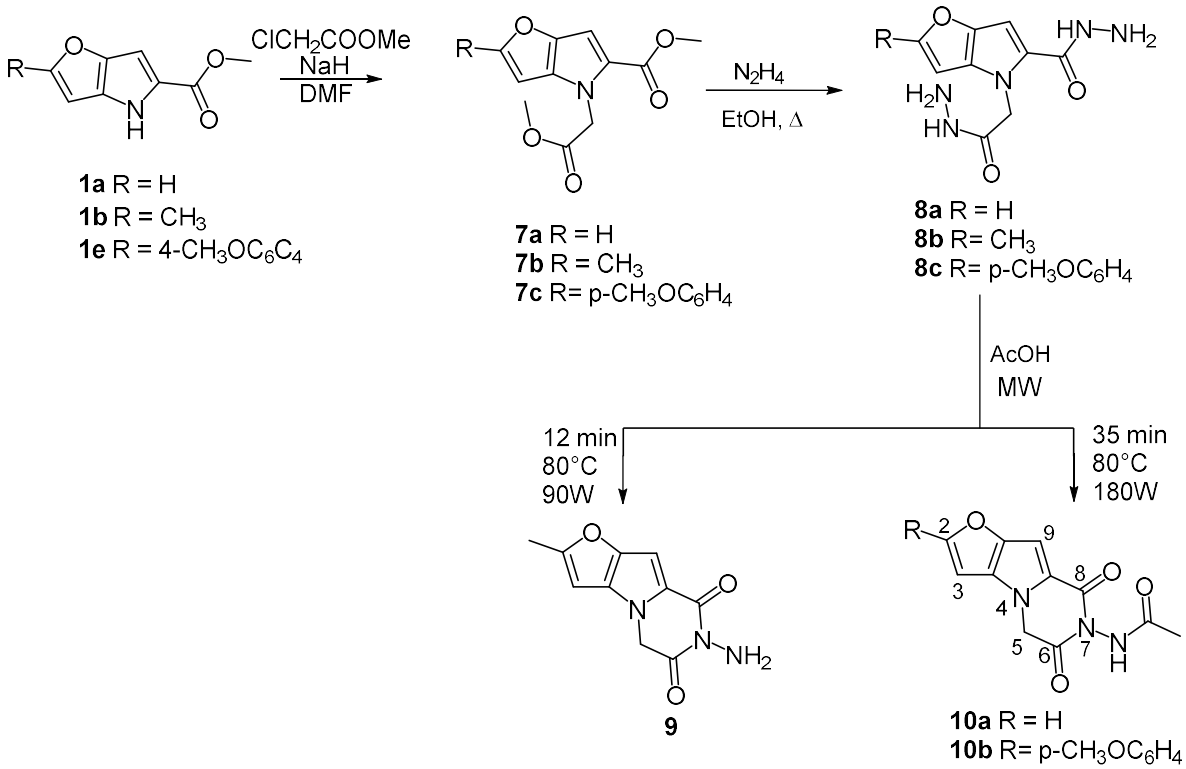
Furo[3,2-*b*]pyrrole derivatives can be formylated under Vilsmeier-Haack reaction conditions[17] and 2-formylated products are obtained preferably. When the C-2 position is occupied, the formylation at C-5 or N-4 can take place, while the C-6 position is the least reactive. The synthesis of 4*H*-furo[3,2-*b*]pyrrole-5-carbaldehydes **6** was taken place by method of Umezawa,[4] which consists in the decarboxylation of acids **5** with trifluoroacetic acid and formylation of the *in situ* formed 4*H*-furo[3,2-*b*]pyrrole with triethyl orthoformate. The resulting aldehydes **6a** and **6b** were obtained in 55 and 58% yields, respectively. Compounds **6a, 6b** display in their ^{1}H NMR spectra the singlets at 11.68-11.75 ppm due to NH group and at 9.32-9.31 ppm of the formyl group. The H-6 protons resonate as singlets at 6.76-6.82 ppm. IR spectra of **6** exhibit a formyl absorption band at 1618-1626 cm^{-1}. The ^{13}C NMR spectra of **6a,b** show the formyl carbon at 179.2 and 179.1 ppm, respectively.

Alkylation of methyl 4*H*-furo[3,2-*b*]pyrrole-5-carboxylates **1a, 1b** and **1e** with methyl chloroacetate in dimethylformamide in the presence of sodium hydride at room temperature overnight provided methyl 4-(2-methoxy-2-oxoethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carboxylates **7a-7c** in 61-67% yields (Scheme 3). The structures of

compounds **7** were established by ^{1}H NMR, ^{13}C NMR and IR spectroscopy. ^{1}H NMR spectra show doublets of H-6 protons at 7.11- 6.78 ppm region (*J* 0.6 Hz), H-3 protons resonate either as doublets of doublets (**7a**) at 6.83 ppm (*J* 2.1, 0.9 Hz) or as singlets (**7b**, **7c**) at 6.89 and 6.43 ppm, respectively. The CH_2 protons appear as singlets at 5.15-5.21 ppm. ^{13}C NMR spectra show ester carboxyl carbon signals of at 168.6-169.6 ppm and 161.5-162.1 ppm. The characteristic bands observed at 1747-1754 and 1685 cm^{-1} in IR spectra of **7b** and **7c** correspond to the ester carbonyl groups.

The presence of 2-methoxy-2-oxoethyl and methyl carboxylate groups in α-position enables the cyclisation with hydrazine to afford ring with either *N*-aminoimide or *N,N'*-diacylhydrazine structural units. Monge and co-workers[18] have reported the reaction of methyl 2-(2-methoxy-2-oxoethyl)-1*H*-indole-3-carboxylate with 40% hydrazine hydrate without any solvent for 36h to obtain 2,3,5,6-tetrahydro[1,2]diazepino[5,4-*b*]indole-1,4-dione, while higher concentration of hydrazine led to the bis-hydrazide. On the other hand Voieduvskyi and co-workers[19] have synthesized a 2-amino-1,3-dioxo-1,2,3,4-tetrahydropyrrolo[1,2-*a*]pyrazine derivative by reaction of an appropriate 1-(2-methoxy-2-oxoethyl)pyrrole-2-carboxylate with 95% hydrazine hydrate in ethanol at 60 °C for 1h.

In order to synthesize new tricyclic 5-5-6 fused furo[3,2-*b*]pyrrole derivatives, we have realized the cyclisation of **7** either by the method of Monge[18] with 40% hydrazine overnight or by heating in ethanol[19] for 48h. In both cases the cyclisation has not taken place and only bis-hydrazides **8a-8c** were formed in 65-70% yields. Compounds **8a**-**8c** show in their ^{1}H NMR spectra two singlets at 9.23-9.34 and 9.09-9.15 ppm regions due to NH groups. Broad singlets of NH_2 groups appear at 4.28-4.29 and 4.18-4.21 ppm, respectively and singlets of CH_2 group at 4.98-5.02 ppm. IR spectra of **8a**-**8c** exhibit absorption bands of ester carbonyl groups at 1675-1644 cm^{-1}. The ^{13}C NMR spectra of **8a**-**8c** display the hydrazide carbonyl carbons at 167.2 and 162.5 ppm.



1a R = H
1b R = CH_3
1e R = 4-$CH_3OC_6C_4$

7a R = H
7b R = CH_3
7c R= p-$CH_3OC_6H_4$

8a R = H
8b R= CH_3
8c R= p-$CH_3OC_6H_4$

9

10a R = H
10b R= p-$CH_3OC_6H_4$

Scheme 3. Synthesis of bis-hydrazides **8** and fused pyrazinediones **9** and **10**.

The cyclisation of bis-hydrazides to pyridazine ring can be achieved by heating in acid media.[20] Conventional heating at 80-90° C was not effective, because the starting derivative **7** was observed on TLC after 3 days of

heating, therefore we heated the bis-hydrazides **8** in acetic acid by microwave irradiation. When compounds **8a** or **8c** were irradiated in microwave oven at 180W and 80° C for 35 min, acetamides **10a, 10b** were synthesized in 85 and 86% yields, respectively; irradiation of **8b** for a shorter period (12 min) at 90 W and 80° C led to the pyrazine **9** in 87 % yield (Scheme 3).

The structures of compounds **9** and **10** were established by ^{1}H NMR, ^{13}C NMR and IR spectroscopy. While ^{1}H NMR spectrum of **9** show no signal of NH group and one singlet signal of NH_2 group at 5.31 ppm, in the spectra of **10a, 10b** there is a singlet for one NH group at 10.41 ppm and no signals of NH_2 groups were observed. The most distinct signals of **10** in the ^{13}C NMR spectra were the three carbonyl group signals at 168.6, 164.8 and 156.1 ppm. That of C-5 appears at 48.7 ppm, and the methyl carbon at 20.8 ppm. The characteristic bands observed at 1729-1669 cm^{-1} in the IR spectra correspond to the C=O groups. By an analysis of ^{1}H-NMR spectrum of product **10b** we observed an unexpected chemical shift doubling (5.43 and 5.32 δ) and splitting (19.1 Hz) of the methylene hydrogens at C-5 (Figure 1).

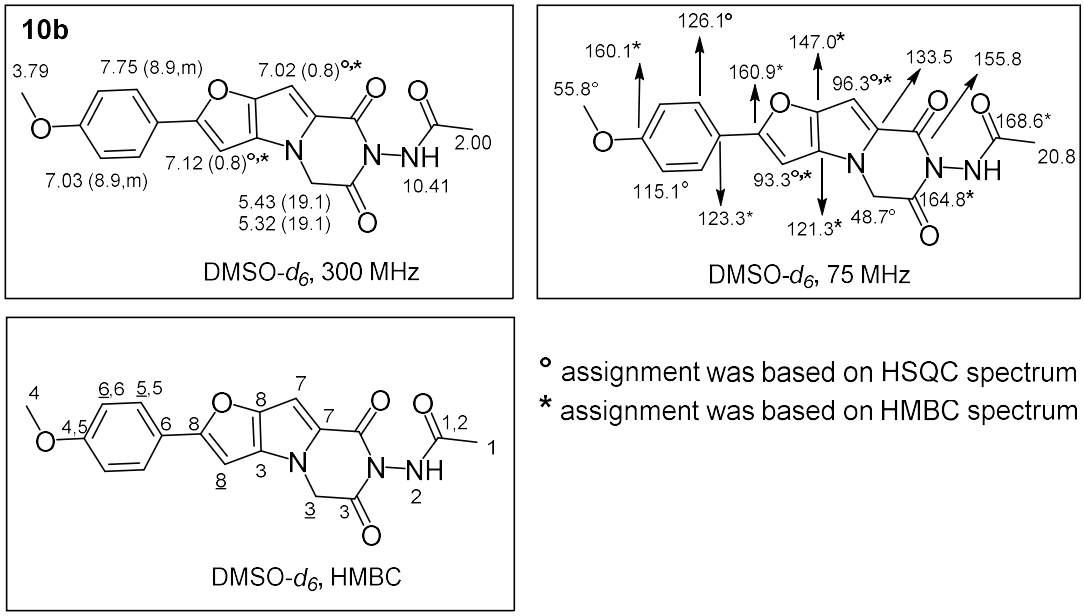


Figure 1. Graphical diagram of ^{1}H- and ^{13}C-NMR spectral characteristics for particular H and C atoms. The exact assignments are based on analysis of 1D and 2D NMR (HSQC and HMBC) spectra. The numbers in the bottom picture represent observed HMBC interactions between hydrogens and carbons up to a distance of three bonds.

The observed AB type of multiplicity for methylene group in ^{1}H-NMR spectrum of **10a, 10b** is a consequence of diastereotopicity of methylene hydrogens in the chiral molecules **10**. The chirality of **10** is either due to the presence of a stereochemically unstable amidic nitrogen stereogenic unit and / or by an axial chirality based on the electrostatic repulsion between two oxygens of dihydropyrazinedione and oxygen from a partially enolised amidic carbonyl group that cannot freely rotate around the hydrazidic N-N bond in **10**. Considering the above effects two enantiomers of **10b** can be drawn (Figure 2). According to our knowledge, this is the first time to explain this kind of the chirality issue on unsymmetrical triacylhydrazide compounds, although some similar structures have been described.[21-23] Unfortunately no X-ray structure analysis or chirality study was performed on these type of compounds.

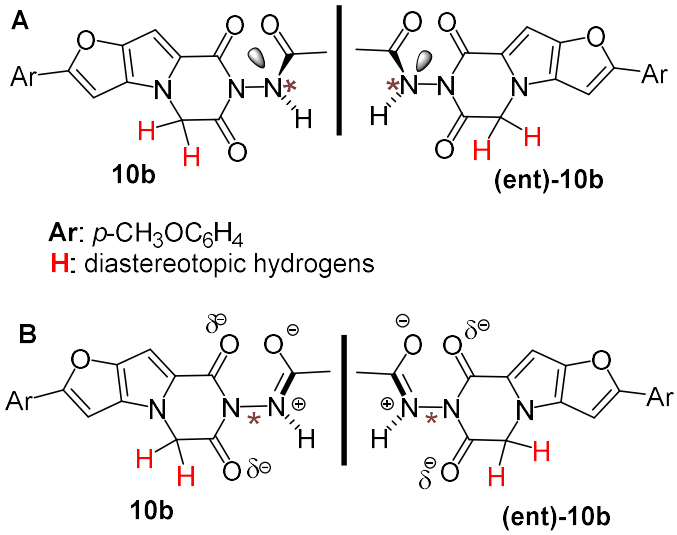


Figure 2. The structures of the two enantiomers of **10b** are shown as an object and its non-superimposable mirror image. The asterisk means either the central (**A**) or the axial (**B**) stereogenic unit in **10b** responsible for its chirality. The same is valid for a compound **10a** (not shown here).

Antibacterial activity

Compounds **1e, 5a-5c, 6a, 7b, 7c** and **8a-8c** were screened for their antibacterial activity against G^- bacterial taxon *Escherichia coli*, CCM 7929 and G^+ bacterial taxon *Micrococcus luteus*, CCM 732. The antibacterial activity of all tested structures were compared with standard 6-aminopenicillanic acid (6-APA). The results are presented in Table 1.

Table 1. Antibacterial activity of standard 6-APA and furo[3,2-*b*]pyrroles **1e**-**8c** on a G^- bacterium *Escherichia coli* CCM 7929 and a G^+ bacterium *Micrococcus luteus* CCM 732

Compound	MIC (mM)	
	Escherichia coli	*Micrococcus luteus*
6-APA	3.84	5.12
1e	3.84	5.12
5a	5.12	>20.48
5b	20.48	>20.48
5c	**0.16**	**>0.1**
6a	20.48	>20.48
7b	16.38	>20.48
7c	10.24	15.36
8a	5.12	5.12
8b	10.24	>20.48
8c	**2.56**	**1.92**

The antibacterial activity of the tested compounds, expressed as MIC parameter, is over a very wide range (0.1-20.48 mM). Two compounds, **1e** and **8a**, are comparable with the activity of selected standard 6-APA

against both bacterial species. Two compounds expressed a higher antibacterial activity than the standard, particularly compound **8c** with MIC value 2.56 mM on *Escherichia coli* and 1.92 mM on *Micrococcus luteus* and mainly compound **5c** with activity in micromolar range (MIC = 0.16 mM) on *Escherichia coli* and MIC value under 0.1 mM on *Micrococcus luteus*. Compounds **5a, 5b, 6a** and **7b** expressed antibacterial activity over the testing range with MIC value >20.48 mM on *Micrococcus luteus*.

Because oxygen heteroatom of furane ring in furo[3,2-*b*]pyrrole core can serve as an acceptor of hydrogen bond (AHb), there is necessary for better activity to offer functional groups - hydrogen bond donors (DHb), because antibacterial effect is the result of either the receptor occupation or enzyme inhibition mechanism. This is the probable reason for the lower MIC value for compounds **6a** and **8b**.

The promising antibacterial activity of compound **5c** on both bacterial species could be explained by the presence of bulky lipophilic triphenylmethyl substituent, representing the lipophilic tail of the molecule, opposite to the carboxy group on the other side of the molecule. This construction leads to compounds with "detergent" properties and contributes to the passage of the bacterial membrane barrier of such compounds. Especially compound **5c** should be a lead structure for the next generation of compounds with increased antibacterial potency.

Conclusions

New furo[3,2-*b*]pyrrole derivatives and furo[3,2-*b*]pyrrolo-fused triazines, their carboxylic acids and aldehydes, were synthesized. Hydrazinolysis of bis-esters **7** led to bis-carbohydrazides **8** which subsequently cyclized in acetic acid under microwave irradiation to form either pyrazine **9** or the acetamides **10**. The synthesized compounds were evaluated for their antibacterial activity on *Escherichia coli* and *Micrococcus luteus*. Micromolar range of activity on *Escherichia coli* was achieved in the case of 2-tritylfuro[3,2-*b*]pyrrole-5-carboxylic acid.

Experimental Section

General. Melting points of products were determined on a Kofler hot plate apparatus. ^{1}H NMR/^{13}C NMR spectra were obtained on a 300 MHz/75 MHz spectrometer Varian Gemini 2000 in DMSO-d_6 with tetramethylsilane as the internal standard. The infrared spectra were taken on Agilent Cary 630 FTIR spectrometer with diamond ATR. Elemental analyses were performed on a Flash EA 2000 CHNS/O-OEA analyser. MS spectra were measured at Agilent Technologies 1200 Series apparatus. All solvents were distilled and dried appropriately prior to use. Microwave-assisted reactions were performed in an Initiator Biotage microwave synthesizer in a sealed vessel and the reaction mixture was continuously stirred by magnetic stirring. The course of reactions was monitored by TLC in ethyl acetate –hexane. Methyl 4*H*-furo[3,2-*b*]pyrrole-5-carboxylates **1a-1c** were synthesized following the published procedures.[10,24] Other chemicals were purchased from the suppliers as the highest purity grade. Bacteriological thermostat BT 120 (Czech Republic) was used for the cultivation of samples. All bacterial species were purchased from the Czech Collection of Microorganisms - CCM (Brno, Czech Republic). Microplates were purchased from VWR, Inc. (Vienna, Austria).

Methyl 2-(triphenylmethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate (**1d**). A solution of ester **1a** (0.83 g, 5 mmol) in DMF (10 mL) was added slowly to NaH (0.24 g, 6 mmol) in DMF (15 mL). The mixture was stirred at 20 °C until evolution of hydrogen ceased, then triphenylmethylchloride (1.39 g, 5 mmol) was added and stirring was continued at 25 °C overnight. The solution was poured into ice water (100 mL) and the precipitate was

crystallized from methanol to give **1d** as a white solid, yield 89%, mp 262-265 °C. ^{1}H NMR (DMSO-d_6) δ 11.66 (s, 1H, NH); 7.36-7.28 (m, 9H, H-3', H-4', H-5'); 7.07 (dd, *J* 8.4, 1.8 Hz, 6H, H-2', H-6'); 6.74 (s, 1H, H-6); 6.06 (d, 1H, *J* 0.6 Hz, H-3); 3.77 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.2, 161.6, 146.9, 144.1, 129.7, 129.2, 127.9, 126.9, 122.6, 100.5, 96.2, 61.5, 51.2. Anal. Calcd. for $C_{27}H_{21}NO_3$ (407.47) C 79.59, H 5.19, N 3.44. Found: C 79.32, H 5.16, N 3.42 %.

8-Chloro-2-methylfuro[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]triazine (**3**). A mixture of triazinone **2**[15] (1 g, 5.7 mmol) and $POCl_3$ (5 mL) was refluxed and stirred for 4h. After cooling, the mixture was poured into ice and neutralized with ammonia (5 mL). The solid precipitate was washed with water and dried. The crude product was used without isolation for the next reaction step.

8-Ethoxy-2-methylfuro[2′,3′:4,5]pyrrolo[1,2-*d*][1,2,4]triazine (**4**)**.** A solution of chlorotriazine **3** (0.58g, 3.1 mmol) in absolute ethanol (5 mL) was slowly added to sodium ethoxide (0.02g, 8 mmol of Na) in ethanol (5 mL) at 0°C. The reaction mixture was stirred at room temperature for 3h and then heated to reflux for 48h. After cooling the solvent was evaporated, the crude solid product was filtered off, washed with water (15 mL) and crystallized from ethanol to give **4** as yellow solid. Yield 57 %, mp 217-220 °C. ^{1}H NMR (DMSO-d_6) δ 7.93 (s, 1H, H-5); 7.57 (s, 1H, H-6); 7.33 (s, 1H, H-3); 4.16 (q, 2H, CH_2); 2.40 (s, 3H, CH_3); 1.19 (t, 3H, *J* 6.9Hz, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 155.9, 148.3, 147.2, 145.6, 127.7, 118.8, 96.5, 92.2, 56.5, 18.9, 15.1. Anal. Calcd. for $C_{11}H_{11}N_3O_2$ (217.23) C 60.82, H 5.10, N 19.34. Found: C 61.16, H 5.18, N 19.78 %.

2-Substituted 4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acids (**5a-5c**)**.**

To a solution of **1** (2.8 g, 15 mmol) in ethanol (10 mL) was added NaOH (0.9 g, 22 mmol) in water (10mL) and the mixture was refluxed for 1.5-4h. After cooling, the mixture was acidified with concentrated hydrochloric acid. The resulting solid product was filtered off, washed with water and recrystallized from ethanol to give **5a-5c** as grey solids.

2-Methyl-4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acid (**5a**)**.** Yield 66%, mp 192-194 °C. IR (KBr) ν/cm^{-1} 3373 (NH), 1640 (C=O). ^{1}H NMR (DMSO-d_6) δ 12.13 (brs, 1H, OH); 11.29 (s, 1H, NH); 6.58 (s, 1H, H-6); 6.21(s, 1H, H-3); 2.34 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.1, 158.8, 146.3, 130.4, 123.0, 96.2, 96.0, 15.1. Anal. Calcd. for $C_8H_7NO_3$ (165.15) C 58.18, H 4.27, N 8.48. Found: C 57.91, H 4.29, N 8.27 %.

2,3-Dimethyl-4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acid (**5b**). Yield 70%, mp 162-164 °C. IR (KBr) ν/cm^{-1} 3200 (NH), 1630 (C=O). ^{1}H NMR (DMSO-d_6) δ 12.08 (brs, 1H, OH); 11.38 (s, 1H, NH); 6.52 (d, *J* 1.8 Hz, 1H, H-6); 2.26 (s, 3H, CH_3); 2.01(s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.2, 154.1, 144.9, 132.0, 122.8, 104.3, 95.9, 12.9, 8.3. Anal. Calcd. for $C_9H_9NO_3$ (179.18) C 60.33, H 5.06, N 7.82. Found C 69.88, H 5.11, N 7.15%.

2-Triphenylmethyl-4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acid (**5c**). Yield 80%, mp 138-140 °C. IR (KBr) ν/cm^{-1} 3190 (NH), 1640 (C=O). ^{1}H NMR (DMSO-d_6) δ 11.44 (s, 1H, NH); 7.33 (t, 3H, *J* 7.5, 3.6 Hz, H-4'); 7.27 (t, *J* 7.2, 3.6 Hz, 6H, H-3', H-5'); 7.04 (d, *J* 3.9 Hz, 6H, H-2', H-6'); 6.65 (s, 1H, H-6); 6.01(s, 1H, H-3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 165.1, 163.1, 147.4, 144.7, 130.2, 129.0, 128.4, 127.3, 124.3, 101.1, 96.4, 61.9. Anal. Calcd. for $C_{26}H_{19}NO_3$ (393.44) C 79.37, H 4.87, N 3.56. Found: C 78.96, H 4.94, N 3.48%.

Substituted 4*H*-furo[3,2-*b*]pyrrole-5-carbaldehydes (**6a, 6b**).

Appropriate 4*H*-furo[3,2-*b*]pyrrole-5-carboxylic acid **5** (6 mmol) was dissolved in trifluoroacetic acid (8 mL) and stirred at 50°C for 10 min. Triethyl orthoformate (1 mL) was added into the reaction mixture, and stirring continued for further 10 min. After cooling, the reaction mixture was poured into saturated aqueous $NaHCO_3$. The formed precipitate was filtered off, washed with water and purified by column chromatography on SiO_2 with *n*-hexane/ethyl acetate (75:25 to 60:40) to obtain compounds **6** as yellow solids.

2-Methyl-4*H*-furo[3,2-*b*]pyrrole-5-carbaldehyde (**6a**). Yield 55%, mp 162-164 °C. IR (KBr) ν/cm^{-1} 3198 (NH), 1618 (C=O). ^{1}H NMR (DMSO-d_6) δ 11.68 (s, 1H, NH); 9.32 (s, 1H, H-C=O); 6.82 (s, 1H, H-6); 6.29 (s, 1H, H-3); 2.37

(s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 179.3, 161.9, 147.1, 134.2, 133.6, 101.8, 96.2, 15.3. Anal. Calcd. for $C_8H_7NO_2$ (149.15) C 64.42, H 4.73, N 9.39. Found: C 64.81, H 4.69, N 8.94 %.

2,3-Dimethyl-4*H*-furo[3.2-*b*]pyrrole-5-carbaldehyde (**6b**). Yield 58%, mp 132-134 °C. IR (KBr) ν/cm^{-1} 3205 (NH), 1626 (C=O). ^1H NMR (DMSO-d_6) δ 11.75 (s, 1H, NH); 9.31 (s, 1H, H-C=O); 6.77 (s, 1H, H-6); 2.29 (s, 3H, CH_3); 2.02 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 179.1, 157.4, 145.7, 135.8, 133.5, 104.3, 101.7, 13.1, 8.1. Anal. Calcd. for $C_9H_9NO_2$ (163.18) C 66.25, H 5.56, N 8.58. Found: C 66.03, H 5.59, N 8.21%.

Methyl 4-(2-methoxy-2-oxoethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carboxylates (**7a-7c**).

A solution of 2-substituted methyl 4*H*-furo[3,2-*b*]pyrrolo-5-carboxylate **1** (0.66g, 4.0 mmol) in dimethyl formamide (15 mL) was added to a suspension of NaH (0.5 g, 21 mmol) in dimethyl formamide (10 mL). After stirring at room temperature for 5 min, methyl chloroacetate 0.8 mL (9 mmol) was added dropwise. The mixture was stirred at room temperature overnight, then poured into ice water (20 mL) and HCl was added to reach pH 2. The mixture was extracted in diethylether (2 x 20 mL), the organic layer was dried with Na_2SO_4 and the solvent was evaporated and and the solid products were crystallized from ethanol to obtain **7a-7c** as yellow solids**.**

Methyl 4-(2-methoxy-2-oxoethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate (**7a**). Yield 61%, mp 72-74 °C. ^1H NMR (DMSO-d_6) δ 7.83 (d, *J* 2.4 Hz, 1H, H-2); 6.89 (d, *J* 0.9 Hz, 1H, H-6); 6.83 (dd, *J* 2.4, 0.9, 1H, H-3); 5.20 (s, 2H, CH_2); 3.73 (s, 6H, 2 x CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6, 161.5, 149.5, 144.7, 133.7, 122.8, 99.2, 98.5, 60.8, 51.1, 48.7. Anal. Calcd. for $C_{11}H_{11}NO_5$ (237.21) C 55.70, H 4.67, N 5.90. Found: C 55.31, H 4.56, N 5.48 %.

Methyl 4-(2-methoxy-2-oxoethyl)-2-methyl-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate (**7b**). Yield 55%, mp 95-97 °C. IR (KBr) ν/cm^{-1} 1754, 1685 (C=O). ^1H NMR (300 MHz, DMSO-d_6) δ 6.78 (d, 1H, *J* 0.6 Hz, H-6); 6.43 (s, 1H, H-3); 5.15 (s, 2H, CH_2); 3.69 (s, 3H, CH_3); 3.65 (s, 3H, CH_3); 2.36 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.6, 162.1, 159.7, 144.0, 135.3, 121.4, 98.9, 95.9, 52.4, 51.4, 48.9, 15.2. Anal. Calcd. for $C_{12}H_{13}NO_5$ (251.24) C 57.37, H 5.22, N 5.58. Found: C 56.99, H 5.29, N 5.69 %.

Methyl 4-(2-methoxy-2-oxoethyl)-2-(4-methoxyphenyl)-4*H*-furo[3,2-*b*]pyrrole-5-carboxylate (**7c**). Yield 67 %, mp 173-175 °C. IR (KBr) ν/cm^{-1} 1747, 1685 (C=O). ^1H NMR (300 MHz, DMSO-d_6) δ 7.67(d, 2H, *J* 4.5 Hz, H-2', H-6'); 7.11 (s, 1H, H-6); 7.02 (d, 2H, *J* 4.2 Hz, H-3', H-5'); 6.89 (s, 1H, H-3); 5.21 (s, 2H, CH_2); 3.78 (s, 3H, CH_3); 3.72 (s, 3H, CH_3); 3.67(s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 169.6, 161.9, 159.9, 159.8, 144.5, 135.8, 125.8, 123.6, 122.6, 115.0, 99.0, 93.2, 55.7, 52.5, 51.6, 49.0. Anal. Calcd. for $C_{18}H_{17}NO_6$ (343.34) C 62.97, H 4.99, N 4.08. Found: C 63.22, H 5.08, N 4.53 %.

4-(2-Hydrazino-2-oxoethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carbohydrazides (**8a-8c**).

Compounds **7a-c** (10 mmol) were refluxed in ethanol (30 mL) with hydrazine hydrate (20 mmol) for 48h. After cooling the solid compounds were filtered off and crystallized from ethanol to give **8a-8c** as yellow solids.

4-(2-Hydrazino-2-oxoethyl)-4*H*-furo[3,2-*b*]pyrrole-5-carbohydrazide (**8a**)**.** Yield 70%, mp 194-196 °C; ^1H NMR (300 MHz, DMSO-d_6) δ 9.34 (s, 1H, NH); 9.12 (s, 1H, NH); 7.65 (d, 1H, *J* 2.4 Hz, H-2); 6.78 (s, 1H, H-6); 6.64 (dd, 1H, *J* 2.1, 0.6 Hz, H-3); 5.02 (s, 2H, CH_2); 4.28 (brs, 2H, NH_2); 4.18 (brs, 2H, NH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.2, 162.1, 147.2, 144.9, 131.2, 125.9, 99.4, 93.6, 48.0. Anal. Calcd. for $C_9H_{11}N_5O_3$ (237.22) C 45.57, H 4.67, N 29.52. Found: C 45.98, H 4.66, N 28.87 %.

4-(2-Hydrazino-2-oxoethyl)-2-methyl-4*H*-furo[3,2-*b*]pyrrole-5-carbohydrazide (**8b**). Yield 70%, mp 188-190 °C; IR (KBr) ν/cm^{-1} 3296, 3195, 2919, 2851 (NH), 1655, 1602 (C=O). ^1H NMR (300 MHz, DMSO-d_6) δ 9.23 (s, 1H, NH); 9.09 (s, 1H, NH); 6.72 (s, 1H, H-6); 6.26 (s, 1H, H-3); 4.98 (s, 2H, CH_2); 4.21(brs, 4H, 2 x NH_2); 2.33 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.7, 162.8, 157.1, 144.2, 132.7, 124.5, 96.1, 94.1, 48.5, 15.1. Anal. Calcd. for $C_{10}H_{13}N_5O_3$ (251.25) C 47.81, H 5.22, N 27.88. Found: C 47.59, H 5.19, N 28.04 %

4-(2-Hydrazino-2-oxoethyl)-2-(4-methoxyphenyl)-4*H*-furo[3,2-*b*]pyrrole-5-carbohydrazide (**8c**). Yield 65%, mp 263-266 °C; IR (KBr) ν/cm^{-1} 3275, 3159, 2918, 2850 (NH), 1675, 1644 (C=O). ^1H NMR (300 MHz, DMSO-d_6) δ 9.34 (s, 1H, NH); 9.15 (s, 1H, NH); 7.65 (d, 2H, *J* 4.2 Hz, H-2', H-6'); 6.99 (d, 2H, *J* 4.4 Hz, H-3', H-5'); 6.96 (s, 1H, H-6);

6.81 (s, 1H, H-3); 5.05 (s, 2H, CH_2); 4.28 (brs, 2H, NH_2); 4.20 (brs, 2H, NH_2); 3.77(s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 167.7, 162.5, 159.3, 157.6, 144.9, 133.4, 125.8, 125.3, 124.3, 114.9, 94.2, 93.6, 55.7, 48.5. MS (ES): *m/z* 342.0 (MH^-). Anal. Calcd. for $C_{16}H_{17}N_5O_4$ (343.34) C 55.97, H 4.99, N 20.40. Found: C 56.28, H 4.94, N 19.97 %.

7-Amino-2-methylfuro[2′,3′:4,5]pyrrolo[1,2-*a*]pyrazine-6,8(5*H*,7*H*)-dione (**9**). Compound **8b** (0.5g, 2 mmol) was dissolved in acetic acid (5 mL) and irradiated in a microwave oven at 80 °C and 90W for 12 min. After cooling, the solid product was filtered off, washed with water (20 mL) and crystallized from ethanol to give **9** as orange solid. Yield 87%, mp 221-224 °C; IR (KBr) ν/cm^{-1} 3337 (NH), 1665 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 6.83 (s, 1H, H-6); 6.41 (s, 1H, H-3); 5.31 (s, 2H, NH); 5.13 (s, 2H, CH_2); 2.39 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 163.9, 160.4, 156.3, 146.5, 132.1, 120.6, 96.0, 95.0, 48.3, 15.2. Anal. Calcd. for $C_{10}H_9N_3O_3$ (219.20) C 54.79, H 4.14, N 19.17. Found: C 54.38, H 4.17, N 19.65 %.

***N*-(6,8-Dioxo-5,6-dihydrofuro[2′,3′:4,5]pyrrolo[1,2-*a*]pyrazin-7(8*H*)-yl)acetamides** (**10a, 10b**). Compounds **8** (2 mmol) were dissolved in acetic acid (5 mL) and irradiated in a microwave oven at 80 °C and 180W for 35 min. After cooling the solid products were filtered off, washed with water (20 mL) and crystallized from ethanol to give **10a, 10b** as red solids.

***N*-(6,8-Dioxo-5,6-dihydrofuro[2′,3′:4,5]pyrrolo[1,2-*a*]pyrazin-7(8*H*)-yl)acetamide** (**10a**)**.** Yield 86%, mp 278-280 °C; IR (KBr) ν/cm^{-1} 3191 (NH), 1729, 1669 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (s, 1H, NH); 7.91(d, 1H, *J* 2.4 Hz, H-2); 6.99 (d, 1H, *J* 0.9 Hz, H-9); 6.79 (dd, 1H, *J* 2.1, 0.6 Hz, H-3); 5.44 (d, 1H, *J* 19.6 Hz, H-5$_\alpha$); 5.34 (d, 1H, *J* 19.6 Hz, H-5$_\beta$); 1.99 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6, 164.9, 156.1, 151.2, 147.7, 131.2, 122.0, 99.9, 96.2, 48.7, 20.8. Anal. Calcd. for $C_{11}H_9N_3O_4$ (247.21) C 53.44, H 3.67, N 17.00. Found: C 52.98, H 3.71, N 17.32 %.

***N*-[2-(4-methoxyphenyl)-6,8-dioxo-5,6-dihydrofuro[2′,3′:4,5]pyrrolo[1,2-*a*]pyrazin-7(8*H*)-yl]acetamide** (**10b**). Yield 85%, mp 286-288 °C; IR (KBr) ν/cm^{-1} 3180 (NH), 1729, 1670, 1607 (C=O). ^{1}H NMR (300 MHz, DMSO-d_6) δ 10.41 (s, 1H, NH); 7.75 (d, 2H, *J* 8.9 Hz, H-2', H-6'); 7.12 (d, 1H, *J* 0.8 Hz, H-3); 7.03 (d, 3H, *J* 8.7, Hz, H-9, H-3', H-5'); 5.43 (d, 1H, *J* 19.1 Hz, H-5$_\alpha$); 5.32 (d, 1H, *J* 19.1 Hz, H-5$_\beta$); 3.79 (s, 3H, CH_3); 2.00 (s, 3H, CH_3). ^{13}C NMR (75 MHz, DMSO-d_6) δ 168.6, 164.8, 160.9, 160.1, 155.8, 147.0, 133.4, 126.1, 123.3, 121.3, 115.1, 96.3, 93.3, 55.8, 48.7, 20.8. Anal. Calcd. for $C_{18}H_{15}N_3O_5$ (353.33) C 61.19, H 4.28, N 11.89. Found: C 60.94, H 4.16, N 11.26 %.

Antibacterial determination of Minimal Inhibition Concentration (MIC) parameters. On determination of MIC parameters there were used sterile microplates (type P), where the suspension of bacterial species in nutrient broth medium with dissolved tested compound has been achieved by convenient dilution method using automatic multichannel pipets. The concentration from the column 1 to column 12 was in the decreasing order: 20.48 mM; 10.24 mM; 5.12 mM; 2.56 mM; 1.28 mM; 0.64 mM; 0.32 mM; 0.16 mM; 0.08 mM; 0.04 mM; 0.02 mM and 0.01 mM. The inoculum concentration of bacterial species suspension in nutrient broth medium was before filling set by McFarland Densitometer DEN-1 (UK) on the value 0.1. The first two rows **A** and **B** were occupied by the standard 6-aminopenicillanic acid (6-APA) on each microplate and the tested compounds were in the rows **C-G**. After 24h of cultivation at 37 °C in the bacteriological thermostat 40 µl of 0.03% solution of Thiazolyl Blue (MTT) in water was added to each well and incubated again for 1h under the same conditions. Bacterial proliferation led to the production of bacterial mitochondrial dehydrogenase, which turned yellow colored solution of MTT to intensely blue colored formazan product. MIC parameter was identified visually as the last not colored well in the row. All experiments were carried out in triplicate.

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Supplementary Material

^{1}H and ^{13}C NMR, and IR spectra of compounds **1e**-**10b.**

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