Synthesis, Characterization and Antimicrobial Activity of Some Hydroxypyrazolines

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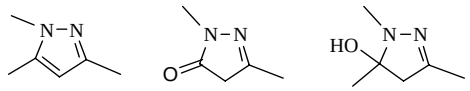
*New hydroxypyrazolines were obtained starting from some hydrazides. 4-(4-X-Phenyl-sulfonyl)benzoic acid hydrazides **1a-c** were treated with 1,1,1-trifluoroacetylacetone in order to obtain new corresponding pyrazole compounds. However, formation of desired pyrazoles from these hydrazides failed and instead new hydroxypirazolines intermediates **2a-c** were obtained. Also, by reaction of 2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)acetohydrazide **6b** with 1,1,1-trifluoroacetylacetone was obtained the new hydroxypirazoline **7b** and not corresponding pyrazole. The new hydrazide **6b** was obtained by reaction of ethyl 2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)acetate **5b** with hydrazine hydrate. The new ester **5b** was obtained by alkylation of corresponding 1,2,4-triazol-3-thione with ethyl bromoacetate in basic media. By reaction of hydrazide **1a** with ethyl (4,4,4-trifluoroacetyl)acetate did not occur expected pyrazolone and the acylhydrazone intermediate **3a**. The structures of these compounds were elucidated by elemental analysis and IR, UV, ^{1}H-NMR, ^{13}C-NMR, MS spectra. The hydroxypyrazoles obtained were tested for their antimicrobial activity.*

Keywords: pyrazole, hydroxypyrazoline, hydrazide, antimicrobial activity

Pyrazole derivatives are important pentaatomic heterocyclic compounds containing nitrogen and have occupied a significant position in the design and synthesis of new biologically active agents.

In this class, pyrazole derivatives of type **I** and **II** are cited in the literature for their biological activity such as analgesic, anti-inflammatory [1-7], antimicrobial [7-10], anticancer [1,11], antitumoral, etc.

Also, hydroxypyrazoline derivatives of type **III** display antimycobacterial, antibacterial, anti-inflammatory properties [12-15].



I **II** **III**

On the other hand, diarylsulfone derivatives have antibacterial [16,17], anti-inflammatory [18], anticancer [19] activities.

Furthermore, the introduction of fluorine atoms or fluorine-containing groups into heterocyclic rings has made possible the discovery of new bioactive products [20,21]. In particular, pyrazoles containing fluoroalkyl groups are of considerable interest due to their pharmaceutical properties [20].

Based on these literature data we decided to introduce in the same structural model three pharmacophore centers (pyrazole nucleus, diphenylsulfone moiety and a trifluoromethyl radical) in order to obtain new biologically active pyrazole derivatives.

For the preparation of pyrazole and their derivatives, hydrazides are versatile synthons in reaction with different 1,3-dicarbonyl derivatives which are important synthetic building blocks for synthesis of heterocyclic compounds.

In the present paper, synthesis, characterization and antimicrobial activities of some hydroxypyrazolines carrying diphenylsulfone moiety is described, thus continuing our previous research in the field of pentaatomic heterocyclic compounds [22-25].

Experimental part

Melting points were determined on a Böetius apparatus and were not corrected. The IR spectra were carried out on a Vertex 70 Bruker spectrometer using KBr (ν, cm^{-1}). The IR bands are given as w – weak, m – medium, s – strong, vs – very strong. The NMR spectra were recorded on a Varian Gemini 300BB spectrometer (^{1}H-NMR at 300 MHz and ^{13}C-NMR at 75 MHz) using DMSO-d_6 or $CDCl_3$ as the solvent and TMS as the internal standard. Chemical shifts are expressed in δ ppm and coupling constants *J* in Hz. Spin multiplets are given as: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet) and b (broad). The mass spectra ESI-MS were recorded with a triple quadrupole mass spectrometer Varian 1200 L/MS/MS, coupled with a high performance liquid chromatograph with Varian ProStar 240 SDM ternar pump. The sample solution (2μg/mL in chloroform/methanol 2/1, v/v) was introduced in the ESI interface by direct infusion, after a tenth dilution with methanol, at a flow rate of 20νL/min. The instrument was operated in positive or negative ions mode. The UV spectra were carried out, in methanolic solutions (~ $2.5 \cdot 10^{-5}$ mol/L), on a SPECORD 40 Analytik Jena spectrophotometer.

Synthesis of new compounds

The 4-(4-X-phenylsulfonyl)benzoic acid hydrazides **1a-c** (X=H,Cl,Br) were synthesized following the method described in literature [26].

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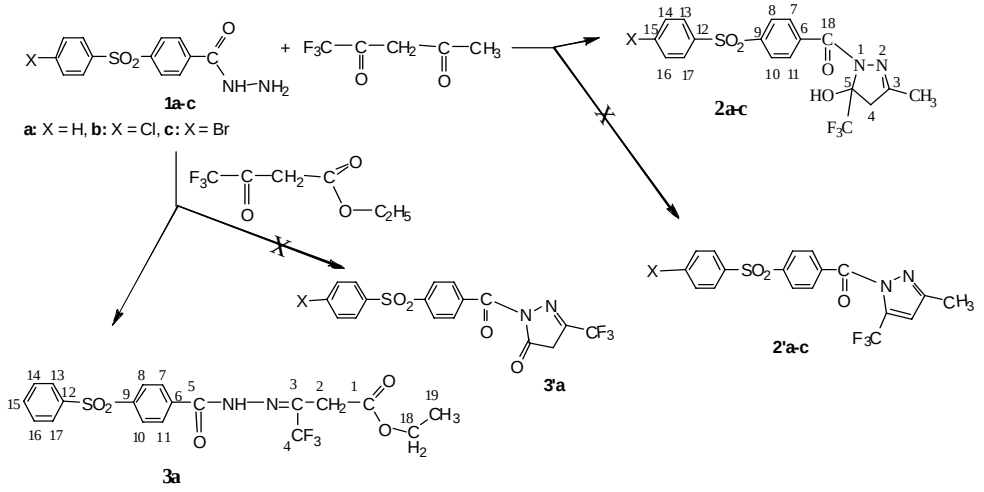
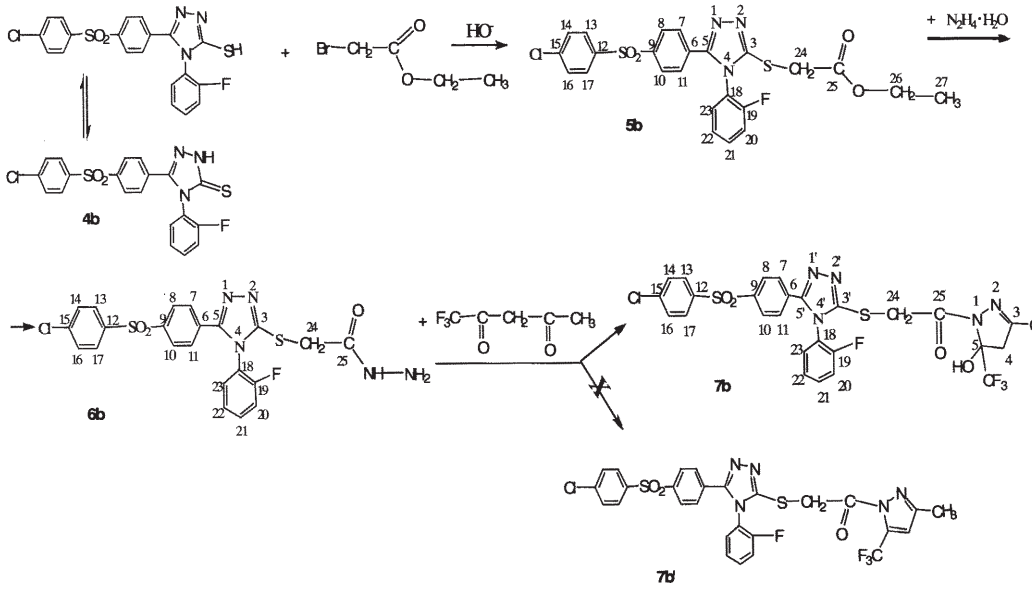


Scheme 1. Synthesis of hydroxypyrazolines **2a-c** and hydrazone derivative **3a**



Scheme 2. Synthesis of hydroxypyrazoline **7b** and derivatives **5b** and **6b**

By reaction of hidrazides **1a-c** with 1,1,1-trifluoroacetylacetone, in ethanol or dioxane, at reflux, were obtained hydroxypyrazolines **2a-c** and not the expected pyrazoles **2'a-c**.

By reaction of same hydrazide **1a** with ethyl (4,4,4-trifluoroacetyl)acetate, in dioxane, was obtained acylhydrazone intermediate **3a** and not pyrazolone **3a'** derivative.

In order to study nucleophilicity of hydrazides in reaction with trifluoroacetylacetone, was synthesized a new hydrazide **7b** starting from 5-(4-(4-clorophenyl-sulfonyl) phenyl)-4-(2-fluorophenyl)-2*H*-1,2,4-triazol-3(4*H*)-thione **4b** previously synthesized [27].

In a first step, by reaction of 1,2,4-triazole **4b** with ethyl bromoacetate, in sodium ethoxide medium, was obtained the new 1,2,4-triazole S-alkylated, named ethyl 2-(5-(4-(4-chloro-phenylsulfonyl)phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)acetate **5b**.

In the second step, by treatment of ester **5b** with hydrazine hydrate, the new hydrazide, 2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)acetohydrazide **6b**, was obtained.

By condensation of this hydrazide **6b** with 1,1,1-trifluoroacetylacetone was also obtained the corresponding 5-hydroxypyrazoline **7b** and not the pyrazole derivative **7b'**.

*Preparation of (4-(4-chlorophenylsulfonyl)phenyl)(5-hydroxy-3-methyl-5-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)methanone **2a-c** and 7b*

A mixture of hydrazide **1a-c** or **6b** (1mmol) and 1,1,1-trifluoroacetylacetone (1.5 mmol) was heated under reflux, in dioxane (10 mL), for about 10 h. The solvent was removed *in vacuo* and the residue was poured into ice-water. The obtained precipitated was filtered off, washed with water, dried and the product obtained was recrystallized from ethanol/H_2O (1:2, v/v).

*(5-hydroxy-3-methyl-5-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)(4-(phenylsulfonyl)-phenyl)-methanone **2a***

m.p.= 148-150°C; yield = 76%

IR (KBr cm^{-1}): 3374m, 3098w, 3073w, 2984w, 2857w, 2934w, 1654s, 1599m, 1589m, 1569m, 1492m, 1435s, 1316s, 1293m, 1177s, 1155vs

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 8.01 (d; 8.8; 2H; H-8;H-10); 7.97 (d; 8.8; 2H; H-7;H-11); 7.95 (d, 8.0; 2H; H-13;H-17); 7.60 (bt, 7.1; H-15); 7.53 (bt; 8.0; 2H; H-14;H-16); 6.43 (bs; 1H; OH); 3.32 (d; 19.5; 1H; H-4a); 3.15 (d; 19.5; 1H; H-4b); 2.03 (s; 3H; CH_3)

^{13}C-NMR ($CDCl_3$, δ ppm): 169.06 (C-18); 156.06 (C-3); 144.50 (C-9); 141.00 (C-12); 137.54 (C-6); 133.70 (C-15); 130.98 (C-7;C-11); 129.55 (C-14;C-16); 127.98 (C-13;C-17); 127.28 (C-8;C-10); 124.08 (q; *J* = 287.1 Hz; CF_3); 93.12 (q; *J* = 34.4 Hz; C-5); 46.87 (C-4); 15.82 (CH_3);

UV (methanol;λ_{max}/nm, log ε): 223.5 (4.17); 240.5 (4.27)

Elemental analysis: Found: C: 52.48; H:3.61; N:6.86 %; Calcd. for $C_{18}H_{15}F_3N_2O_4S$ (412.38 g/mol): C:52.43; H:3.67; N:6.79 %.

ESI-MS, *m/z*: 413 $[M+H]^+$

ESI-MS, *m/z*: 411 $[M-H]^-$

(4-(4-chlorophenylsulfonyl)phenyl)(5-hydroxy-3-methyl-5-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)methanone 2b

m.p.= 139-142°C; yield = 93%

IR (KBr cm^{-1}): 3316m, 3092w, 3073w, 2955w, 2854w, 2921w, 1657s, 1581m, 1493m, 1476m, 1435s, 1322vs, 1282m, 1183s, 1156vs, 768s

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.91 (s; 4H; H-7;H-8; H-10;H-11); 7.83 (d, 8,5; 2H; H-13;H-17); 7.73 (d; 8.5; 2H; H-14;H-16); 6.35 (bs; 1H; OH); 3.26 (d; 19.2; 1H; H-4a); 3.09 (bd; 19.2; 1H; H-4b); 1.97 (bs; 3H; CH_3)

^{13}C-NMR ($CDCl_3$, δ, ppm): 168.93 (C-18); 156.11 (C-3); 144.08 (C-9); 140.51 (C-12); 139.57 (C-15); 137.82 (C-6); 131.10 (C-7;C-11); 129.90 (C-14;C-16); 129.45 (C-13;C-17); 127.28 (C-8;C-10); 124.09 (q; *J* = 267,4 Hz; CF_3); 93.15 (q; *J* = 34.1 Hz; C-5); 46.89 (C-4); 15.82 (CH_3);

UV (methanol;λ_{max}/nm, log ε): 224.7 (4.19); 248.5 (4.35)

Elemental analysis: Found: C: 48.31; H:3.22; N:6.35 %; Calcd. for $C_{18}H_{14}ClF_3N_2O_4S$ (446.83 g/mol): C:48.38; H:3.16; N:6.27 %

ESI-MS, *m/z* (%): 445 $[M-H]^-$ (^{35}Cl); 447 $[M-H]^-$ (^{37}Cl)

*(4-(4-bromophenylsulfonyl)phenyl)(5-hydroxy-3-methyl-5-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)methanone **2c***

m.p.= 129-131°C; yield = 89%

IR (KBr cm^{-1}): 3319m, 3089m, 3071w, 2953w, 2856w, 2921w, 1656s, 1598m, 1573s, 1492m, 1471m, 1435s, 1321vs, 1292m, 1182s, 1159vs, 574m

^{1}H-NMR ($CDCl_3$, δ, ppm, *J* Hz): 7.91 (s; 4H; H-7;H-8;H-10;H-11); 7.75 (d, 8.5; 2H; H-13;H-17); 7.60 (d; 8.5; 2H; H-14;H-16); 6.38 (bs; 1H; 5-OH); 3.26 (d; 19.5; 1H; H-4a); 3.09 (d; 19.5; 1H; H-4b); 1.97 (bs; 3H; CH_3)

^{13}C-NMR ($CDCl_3$, δ ppm): 168.85 (C-18); 156.03 (C-3); 143.94 (C-9); 140.03 (C-12); 137.76 (C-6); 133.82 (C-14;C-16); 131.04 (C-7;C-11); 129.42 (C-13;C-17); 129.00 (C-15); 127.20 (C-8;C-10); 124.01 (q; *J* = 286.9 Hz; CF_3); 92.28 (q; *J* = 32.0 Hz; C-5); 46.81 (C-4); 15.75 (CH_3);

UV (methanol; λ_{max}/nm, log ε):228.2 (4.24); 251.1 (4.34)

Elemental analysis: Found: C: 44.10; H:2.94; N:5.64 %; Calcd. for $C_{18}H_{14}BrF_3N_2O_4S$ (491.28 g/mol): C:44.01; H:2.87; N:5.70 %

ESI-MS, *m/z*: 489 $[M-H]^-$ (^{79}Br); 491 $[M-H]^-$ (^{81}Br)

*Preparation of ethyl 4,4,4-trifluoro-3-(2-(4-(phenylsulfonyl)benzoyl)hydrazono)butanoate **3a***

A mixture of hydrazide **1a** (1 mmol) and ethyl 4,4,4-trifluoromethylacetylacetoacetate (1.5 mmol) in dioxane (5 mL) was heated, under reflux for about 10 h. The solvent was removed *in vacuo* and the residue was poured into ice-water. The precipitate obtained was separated by filtration, washed with water, dried and recrystallized $CHCl_3$/petroleum ether (1:2, v/v).

m.p.= 77-79°C; yield = 68%

IR (KBr cm^{-1}): 3203m, 3092s, 3062m, 3004w, 2982w, 2854w, 2937w, 2820w, 1734s, 1687s, 1599m, 1572m, 1478m, 1448m, 1310s, 1296s, 1159vs

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.90-8.02 (m; 6H; H-7;H-8;H-10;H-11;H-13;H-17); 7.45-7.60 (m; 3H; H-14;H-15;H-16); 4.21 (q; 7,1; 2H; -OCH_2); 3.75 (s, 2H; 2-CH_2); 1.28 (t; 7,.1; 3H; -CH_3);

^{13}C-NMR ($CDCl_3$+DMSO-d_6, δ ppm): 166.79 (C-1); 164.49 (C-5); 144.08 (C-9); 140.55 (C-12); 137.8 (q; *J* = 31.2 Hz; C-3); 136.55 (C-6); 133.40 (C-15); 129.22 (C-14;C-16); 128.62 (C-13;C-17); 128.04 (C-8;C-10); 127.45 (C-7;C-11); 120.97 (q; *J* = 274.8 Hz; 4-CF_3); 61.90 (C-18); 31.81 (C-2); 13.74 (C-19);

UV (methanol;λ_{max}/nm, log ε): 246.7 (4.48);

Elemental analysis: Found: C: 51.52; H:3.82; N:6.40 %; Calcd. for $C_{19}H_{17}F_3N_2O_5S$ (442.41 g/mol): C:51.58; H:3.87; N:6.33 %

ESI-MS, *m/z*: 443 $[M+H]^+$;

ESI-MS, *m/z*: 441 $[M-H]^-$

*Preparation of ethyl 2-(5-(4-(4-chlorophenylsulfonyl) phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)acetate **5b***

To a solution of triazole **4b** (3 mmol) in sodium ethoxide (0.069 g metallic sodium and 30 mL absolute ethanol), ethyl bromoacetate (3 mmol) was added. The reaction mixture was stirred at room temperature for 10 h, and then was poured into ice-water. The obtained precipitate was filtered off, washed with water, dried and recrystallized from ethanol.

m.p.= 177-179°C; yield = 87%

IR (KBr cm^{-1}): 3085m, 3061w, 3048, 2983m, 2840w, 2939m, 1727s, 1600m, 1583m, 1507s, 1465m, 1317s, 1281m, 1264m, 1159vs, 771s

^{1}H-NMR ($CDCl_3$, δ ppm, *J* Hz): 7.85 (d; 8.6; 2H; H-8;H-10); 7.83 (d, 8.6; 2H; H-13;H-17); 7.65 (d; 8.6; 2H; H-7;H-11); 7.60 (m; 1H; H-21); 7.48 (d; 8.6; 2H; H-14;H-16); 7.20-7.45 (m; 2H; H-22;H-23); 7.28 (t; 8.4; 1H; H-20); 4.21 (q; 7.1; 2H; H-26); 4.19 (d; 16.1; 1H; H-24a); 4.07 (d; 16.1; 1H; H-24b); 1.27 (t; 7.1; 3H; H-27)

^{13}C-NMR ($CDCl_3$, δ ppm): 168.02 (C-25); 157.93 (d; *J* = 254.5 Hz; C-19); 153.67 (C-3); 153.23 (C-5); 142.40 (C-9); 140.40 (C-12); 139.48 (C-15); 131.29 (C-6); 129.84 (C-14;C-16); 129.37 (C-13;C-17); 129.31 (d; *J* = 12.5 Hz; C-21); 128.30 (C-7;C-11); 133.01 (d; *J* = 7.5 Hz; C-23); 128.08 (C-8;C-10); 125.85 (d; *J* = 3.75 Hz; C-22); 121.45 (d; *J* = 13.5 Hz; C-18); 118.48 (d; *J* = 18.9 Hz; C-20); 62.29 (C-26); 34.75 (C-24); 14.15 (C-27)

UV (methanol; λ_{max}/nm, log ε): 223.8 (4.10); 246.7 (4.18); 279.3 (4.21);

Elemental analysis: Found: C: 54.12; H:3.54; N:7.96 %; Calcd. for $C_{24}H_{19}ClFN_3O_4S_2$ (532.01 g/mol): C:54.18; H:3.60; N:7.90 %

ESI-MS, *m/z* (%): 532 $[M+H]^+$ (^{35}Cl); 534 $[M+H]^+$ (^{37}Cl)

*Preparation of 2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)acetohydrazide **6b***

A mixture of ester **5b** (2 mmol) and hydrazine hydrate (8 mmol) in 35 mL ethanol was refluxed for 7 h. The precipitate obtained was filtered off and washed with cold ethanol. The product obtained was recrystallized from ethanol.

m.p.= 200-203°C; yield = 75%

IR (KBr cm^{-1}): 3326s, 3250m, 3083w, 3061w, 2931w, 2840w, 1682s, 1613m, 1598m, 1582m, 1506s, 1472m, 1323s, 1285m, 1231s, 1160vs, 771s

^{1}H-NMR (DMSO-d_6, δ ppm, *J* Hz): 9.38 (s; NH); 7.99 (d, 8.8; 2H; H-13;H-17); 7.97 (d; 8.5; 2H; H-8;H-10); 7.69 (d; 8.5; 2H; H-7;H-11); 7.62 (d; 8.8; 2H; H-14;H-16); 7.60-7.80 (m; 2H; H-21;H-23); 7.42-7.60 (m; 2H; H-20,H-22); 4.40 (bs; NH_2); 3.96 (s; H-24)

^{13}C-NMR (DMSO-d_6, δ ppm): 165.77 (C-25) 156.19 (d; *J* = 250.8 Hz; C-19); 153.10 (C-3); 153.01 (C-5); 141.80 (C-

9); 139.26 (C-12); 139.18 (C-15); 133.42 (d; J = 8.0 Hz; C-23); 131.01 (C-6); 130.09 (C-7;C-11); 129.60 (C-8;C-10); 129.50 (d; J = 12.5 Hz; C-21); 128.34 (C-14;C-16); 128.12 (C-13;C-17); 126.21 (d; J = 3.4 Hz; C-22); 120.80 (d; J = 12.6 Hz; C-18); 117.34 (d; J = 19.2 Hz; C-20); 34.25 (C-24);

UV (methanol; λ_{max}/nm, log ε): 224.7 (4.17); 244.9 (4.22); 279.3 (4.20);

Elemental analysis: Found: C:51.08; H:3.36; N:13.46 %; Calcd. for $C_{22}H_{17}ClFN_5O_3S_2$ (517.98 g/mol): C:51.01; H:3.31; N:13.52 %

ESI-MS, *m/z* (%): 518 $[M+H]^+$ (^{35}Cl); 520 $[M+H]^+$ (^{37}Cl)

2-(5-(4-(4-chlorophenylsulfonyl)phenyl)-4-(2-fluorophenyl)-4H-1,2,4-triazol-3-ylthio)-1-(5-hydroxy-3-methyl-5-(trifluoromethyl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone ***7b***

m.p.= 98-100°C; yield = 68%

IR (KBr cm^{-1}): 3318m, 3088w, 3072w, 2955w, 2854w, 2921w, 1682s, 1597m, 1581m, 1508s, 1436s, 1324s, 1283m, 1183s, 1161vs, 770s

^{1}H-NMR ($CDCl_3$, δ ppm, J Hz): 7.89 (d, 8.5; 2H; H-13;H-17); 7.84 (d; 8.5; 2H; H-8;H-10); 7.60 (d; 8.5; 2H; H-7;H-11); 7.48 (d; 8.5; 2H; H-14;H-16); 7.20 (dd; 9.3;1.1; 1H; H-20); 7.21-7.48 (m; 3H; H-21;H-22;H-23); 6.38 (bs; 1H; OH); 4.71 (d; 16.1; 1H; H-24a); 4.42 (d; 16.1; 1H; H-24b); 3.26 (d; 19.0; 1H; H-4a); 3.14 (bd; 19.0; 1H; H-4b); 2.30 (bs; 3H; CH_3)

^{13}C-NMR ($CDCl_3$, δ ppm): 168.12 (C-25); 158.70 (C-3'); 157,01 (d; J = 253,5 Hz; C-19); 156.72 (C-3); 156.02 (C-5'); 142.40 (C-9); 140.44 (C-12); 139.50 (C-15); 134,00 (d; J = 8.3 Hz; C-21); 132.50 (d; J = 12.3 Hz; C-23); 131.34 (C-6); 130.15 (J = 12,6 Hz; C-18); 129.87 (C-14;C-16); 129.34 (C-8;C-10); 128.35 (C-13;C-17); 128.11 (C-7;C-11); 125.80 (d; J = 3.4 Hz; C-22); 123.18 (q; J = 265.0 Hz; CF_3); 117,81 (d; J = 18,8 Hz; C-20); 92.20 (q; J = 34.4 Hz; C-5); 47.03 (C-4); 36.85 (C-24); 15.80 (CH_3);

UV (methanol; λ_{max}/nm, log ε): 226.4 (4.35); 278.4 (4.19)

Elemental analysis: Found: C:49.65; H:3.00; N:10.66 %; Calcd. for $C_{27}H_{20}ClF_4N_5O_4S_2$ (654.06 g/mol): C:49.58; H:3.08; N:10.71 %

ESI-MS, *m/z* (%): 654 $[M+H]^+$ (^{35}Cl); 656 $[M+H]^+$ (^{37}Cl);

ESI-MS, *m/z* (%): 652 $[M-H]^+$ (^{35}Cl); 654 $[M-H]^+$ (^{37}Cl)

Antibacterial activity testing

The antibacterial activity of hydroxypyrazolines **2a-c** and **7b** was tested against the Gram-positive bacteria: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus ATCC* 13061, the Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Enterobacter cloacae* ATCC 49141, *Acinetobacter baumannii* ATCC 19606, *Pseudomonas aeruginosa* ATCC 27853 and the yeasts: *Candida albicans* ATCC 90028, *Candida parapsilosis* ATCC 22019, *Candida glabrata* ATCC 15126 and *Candida tropicalis* ATCC 13803.

The *in vitro* testing of the antimicrobial activity of compounds was performed using the broth microdilution method, in order to detect the minimum inhibitory concentrations (MIC).

The stock solution (2048 mg/mL) of each compound was made using dimethyl sulfoxide as solvent. This solvent showed no antibacterial activity against the tested bacterial and yeasts strains.

Series of two-fold dilutions (from 1/2 to 1:1024) of compounds were performed in cation-adjusted Mueller-Hinton broth when were tested against the bacterial strains and in Sabouraud broth when were tested against the yeast strains.

As control, *S. aureus* ATCC 25923, *E. coli* ATCC 25922 and P. *aeruginosa* ATCC 27853 were tested against amikacin, and *C. parapsilosis* ATCC 22019 against fluconazole by the broth microdilution method.

Protocol used for testing of antimicrobial activity of hydroxypyrazolines **2a-c** and **7b** was described in the previous work [23].

Results and discussions

Chemistry

Reaction of hydrazine/hydrazide derivatives with 1,3-dicarbonyl compounds was studied intensively, different products being obtained, depending on the nature of substituents and of reaction conditions [11,28-32].

In order to obtain pyrazoles, we used hydrazides with withdrawing substituent (arylsulfonylphenyl) and a dicarbonyl compound (trifluoroacetylacetone) containing also strongly withdrawing CF_3 group. Instead of pyrazoles, 5-hydroxypyrazolines intermediates **2a-c** were isolated in all cases. Although we tried to obtain corresponding pyrazoles by dehydration of hydroxypyrazolines in the presence of P_2O_5 [33], the reaction did not occur. By prolonging of the reaction time to 50 h or by using of different solvents were also obtained hydroxypyrazolines.

Because the trifluoroacetylacetone is an asymmetric dicarbonyl compound, it would have been expected to obtain two isomeric hydroxypyrazolines (**A** and **B,** fig. 1).

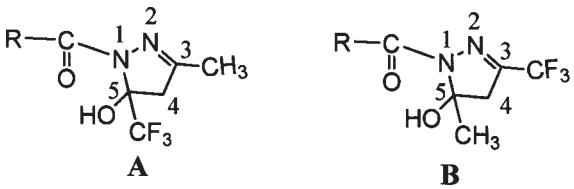


Fig. 1. Structure of isomeric hydroxypyrazolines **A** and **B**

The compound that we isolated was isomer **A** probably due to the trifluoromethyl substituent from position 5 and the fragment R from position 1, both with strong withdrawing effect, thus stabilizing the OH group from 5 position and possibly hindering the elimination of water and the subsequent aromatization of five-membered ring [34].

In order to study nucleophilicity of hydrazides, we obtained a new compound, hydrazide **6b**, in which the SCH_2 radical linked to *hydrazide functional group*, -$CONHNH_2$, which has not the same withdrawing effect comparatively with phenyl radical from hydrazides **1a-c**. In this case the compound obtained was also a hydroxypyrazoline, but the reaction yield was lower.

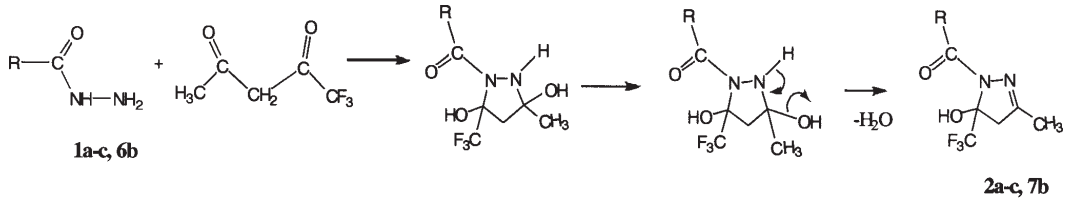
The probable mechanism of formation of pyrazolines **2a-c** and **7b** is presented in scheme 3.

In case of reaction of hydrazide **2a** with ethyl trifluoroacetylacetate, cyclization did not take place, the only product isolated was hydrazone derivative **3a**.

The structures of compounds **2, 3** and **5-7** were established by their IR, ^{1}H-NMR, ^{13}C-NMR and MS spectra.

In the IR spectra of hydroxypyrazolines **2a-c** and **7b** is present an absorption band characteristic to stretching vibration of hydroxyl group which appears in 3316-3374 cm^{-1} region. The νC=O absorption band from these compounds appeared in 1654-1682 cm^{-1} region.

Their structure was further supported by ^{1}H-NMR spectral data which exhibited a singlet signal around 6.35-6.43 ppm assigned for the proton of hydroxyl group. Also, these hydroxypyrazolines show the ^{1}H-NMR chemical shifts of



Scheme 3. The probable mechanism of formation of pyrazolines **2a-c** and **7b**

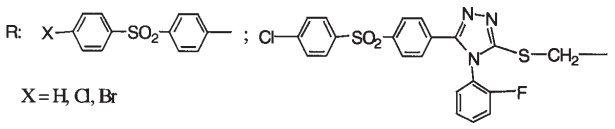


Table 1

THE MIC (μg/mL) OF THE NEWLY HYDROXYPYRAZOLINES **2a-c** AND **7b** TESTED AGAINST SOME BACTERIA AND YEASTS

Nr. Comp.	*Gram-positive bacteria*		*Gram-negative bacteria*				*Yeasts*			
	S. aureus ATCC 25923	*B. cereus* ATCC 13061	*E. coli* ATCC 25922	*E. cloacae* ATCC 49141	*A. baumannii* ATCC 19606	*P. aeruginosa* ATCC 27853	*C. albicans* ATCC 90028	*C. parapsilosis* ATCC 22019	*C. glabrata* ATCC 15126	*C. tropicalis* ATCC 13803
2a	128	128	256	256	64	256	128	128	128	128
2b	128	64	256	256	64	128	128	128	64	128
2c	128	32	256	256	64	128	256	512	128	256
7b	128	32	128	128	64	256	64	128	64	64
Control (Amikacin)	2	-	2	-	-	2	-	-	-	-
Control (Fluconazole)	-	-	-	-	-	-	-	2	-	-

the diastereotopic methylene protons (H-4a and H-4b) as a characteristic AB system and as doublet at δ ~ 3.26-3.32 ppm and another doublet at δ ~ 3.09-3.15 ppm, respectively with a geminal coupling constant J = 19.0-19.5 ppm [33,35,36]. The methyl protons resonated as a singlet at δ ~ 2 ppm [35].

The ^{13}C-NMR spectra showed typical chemical shifts of 5-hydroxy-4,5-dihydro-1*H*-pyrazole rings at δ ~156 ppm (C-3), ~ 47 ppm (C-4) and ~ 93 ppm (C-5, J = ~ 34 Hz). Another proof which shows that the reaction between hydrazides **1a-c**, **6b** and trifluoroacetylacetone occurred, is the quartet signal of CF_3 group that resonated at ~ 124 ppm with J_{C-F} = 265.0-287.1 Hz [33,35,36]. Also, the signal of C=O and CH_3 groups are presented at ~ 169 ppm and ~ 16 ppm, respectively [35,36].

The structure of compound **3a** obtained by reaction between hydrazide **2a** and ethyl trifluoroacetylacetate was elucidated with spectral data obtained. The NMR data indicated that hydrazonic intermediate **3a** was obtained and not pyrazolone **3'a**. Thus, the ^{1}H-NMR spectra showed clearly the protons signals of -O-CH_2-CH_3 groups which resonated at 4.21 ppm (-CH_2-, quartet; J=7.1 Hz) and at 1.28 ppm (-CH_3, triplet, J=7.1 Hz), respectively. The signal carbons of same groups appeared in ^{13}C-NMR at 61.90 ppm (for -OCH_2-) and 13.74 ppm (for -CH_3) [32].

The spectral data obtained for intermediates **5b** and **6b** are in accordance with their structure.

In the IR spectra, the νC=O absorption band appeared at 1727 cm^{-1} (for **5b**) and 1682 cm^{-1} (for **6b**), respectively, while in the ^{13}C-NMR, the signal carbon of same C = O group appeared at 168.02 ppm (**5b)** and 165.77 ppm (**6b**). Other important signals present in the ^{1}H-NMR spectrum of **5b** are those characteristic of protons and carbons from OCH_2-CH_3 groups which resonate at 4.21 ppm (quartet; J = 7.1 ppm, OCH_2-CH_3) and 1.27 ppm (triplet; J = 7.1 ppm; OCH_2-CH_3). Also, in ^{13}C-NMR spectra these carbons signals appeared at 62.29 ppm for OCH_2-CH_3 and 14.15 ppm for OCH_2-CH_3. In NMR spectrum of hydrazide **6b**, as expected, ethyl group signals disappeared and two new singlet signals characteristic to NH (9.38 ppm) and NH_2 (4.40 ppm) appeared.

In compounds **5b** and **7b** the diastereotopic methylene protons of S-CH_2 group appeared in ^{1}H-NMR spectra as a doublet at 4.19 ppm (for **5b**)/ 4.71 ppm (for **7b**) and another doublet at 4.07 ppm (for **5b**)/4.42 ppm (for **7b**). Unexpectedly, in hydrazide **6b** the same SCH_2 group appeared as singlet at 3.96 ppm. In ^{13}C-NMR spectra of **5b-7b** the carbon signal of SCH_2 group appeared in region 34.25-36.85 ppm.

Also, based on spectral data, it was demonstrated that the alkylation reaction of triazole **4b** with ethyl bromoacetate took place at the sulfur atom and not nitrogen atom. Absence in the IR spectrum of derivative **5b** of νC = S absorption band and in ^{13}C-NMR spectra of the C=S signal indicated that was obtained the S-alkylate derivative and not the N-alkylated. The heterocyclic carbon C-3' signal appeared at 153.67 ppm, more shielded than the C=S [37,38].

Another proof that confirms the structure of all compounds is mass spectra. The obtained molecular ion peak confirmed the molecular weight of proposed compound.

Antimicrobial activity

The values of the MIC (μg/mL) obtained by testing of hydroxypyrazolines **2a-c** and **7b** against 6 bacteria and 4 yeasts are presented in table 1.

From the results obtained can be observed that the hydroxypyrazolines **2c** and **7b** had the strongest inhibitory action of strains studied against Gram-positive bacteria *B. cereus*.

A better antimicrobial activity with MIC = 64 μg/mL had hydroxypyrazoline **2b** against *B. cereus* and *C. glabrata* and hydroxypyrazoline **7b** against C. *albicans*, *C. tropicalis* and *C. grablata* yeasts. All compounds studied had the same value of 64 μg/mL against *A. baumanni*. Other values of MIC obtained were between 128-512 μg/mL which indicates a weaker inhibitory action of strains.

A brief evaluation of structure-biological activity relationship allows to conclude that the most active compound on almost all strains studied is hydroxypyrazoline **7b**, probably due to the presence in its molecule of a triazole nucleus (with a 2-fluorophenyl fragment linked at nitrogen heterocyclic atom from position 4) linked by thioacetyl group of pyrazoline.

Conclusions

This paper presents research undertaken to obtain a new pyrazole class by reaction of the some hydrazides with 1,3,-dicarbonyl compounds. Reaction of hydrazides **1a-c** and **6b** with trifluoroacetylacetone occurred with obtaining of hydroxypyrazoline derivatives **2a-c** and **7b** and not of expected pyrazole. By reaction of hydrazide **2a** with 4,4,4-ethyl trifluoroacetylacetate, cyclization did not occur and hydrazone derivative **3a** was obtained.

The structure of the obtained compounds was confirmed by elemental analysis and different spectral methods.

By testing *in vitro* of new hydroxypyrazolines **2a-c** and **7b** for their antimicrobial activity it was noticed that hydroxypyrazoline **7b** showed the best antimicrobial activity against majority of strains.

These results obtained suggest the need of further investigation in synthesis of new similar structures in order to identify new biological agents.

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