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RESEARCH ARTICLE

Synthesis, antiproliferative and antimicrobial activity of new Mannich bases bearing 1,2,4-triazole moiety

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Abstract

This study presents the synthesis, antiproliferative and antimicrobial evaluation of a new series of Mannich base derivatives containing 1,2,4-triazole system. New compounds were prepared by the reaction of 4,5-disubstituted 1,2,4-triazole-3-thiones with formaldehyde and various amines. The structures of the prepared compounds were confirmed by means of ^{1}H NMR, ^{13}C NMR and elemental analyses. Twelve compounds were evaluated for their *in vitro* antiproliferative activities against six chosen cancer cell lines. All synthesized compounds were screened for their *in vitro* antimicrobial activity by using the agar dilution technique. For 17 potentially active compounds, their antibacterial activity was confirmed on the basis of MIC (minimal inhibitory concentration) by broth microdilution method using the reference Gram-positive and Gram-negative bacterial strains.

Keywords

1,2,4-Triazole derivatives, antimicrobial activity, antiproliferative activity, Mannich base derivatives

History

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Introduction

The search for novel antibacterial and antitumor agents devoid of side effects continues to be an active area of research in medicinal chemistry. Among all heterocyclic systems the therapeutic importance of 1,2,4-triazoles and their derivatives is well documented and they have been reported to possess various biological activities such as analgesic[1], antifungal[2,3], antibacterial[4,5], antiviral[6], antiphlogistic[7,8], and antitubercular[9]. Therefore, 4,5-substituted 1,2,4-triazoles seems to be suitable candidates for further chemical modifications and might be of interest as pharmacologically active compounds.

Our interest was directed to Mannich bases which in recent years have gained importance due to their application in pharmaceutical chemistry and comprehensive bioactivities like antibacterial[10–13], antifungal[14,15], anticancer[16], analgesic[17] and anticonvulsant[18] properties. They are also used in polymer industry as paints and surface active reagents[19].

Mannich reaction is a three-component condensation reaction involving an active hydrogen containing compound, formaldehyde and a secondary amine. The scope of Mannich reaction has been expanded in recent years to include a wide variety of amines, ammonia equivalents, imines and acetylated imines[20]. The aminomethylation of aromatic substrates by Mannich reaction is of considerable importance for the synthesis and modification of biological active compounds[21].

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Literature survey also reveals that piperazine or morpholine ring is significant for antimicrobial activity[22,23]. Mannich bases of 1,2,4-triazole containing *N*-methylpiperazine moiety are known as antimicrobial agents[24]. Some *in vitro* cytotoxic activitiy of Mannich bases on Maurine P388 lyphocytic leukemia have also been reported[25,26].

Prompted by these observations and in an attempt to continue our studies on the synthesis of biologically active nitrogen and sulfur containing heterocycles, we herein report the synthesis, spectral data and evaluation of biological activities of new Mannich bases containing 1,2,4-triazole moiety.

Experimental

Chemistry

General

All reagents were purchased from Sigma-Aldrich (Munich, Germany) and Merck Co. (Darmstadt, Germany) and used without further purification. Melting points were determined in Fisher-Johns blocks (Fisher Scientific, Schwerte, Germany) and presented without any corrections. The ^{1}H NMR spectra were recorded on a Bruker Avance 300 apparatus (Bruker BioSpin GmbH, Rheinstetten/Karlsruhe, Germany) in DMSO-d_6 with TMS as internal standard. The ^{13}C NMR spectra were recorded on a Bruker Avance 300 apparatus. Chemical shifts are given in ppm (δ-scale). Multiplicities of NMR signals are represented as singlet (s), doublet (d), doublet of doublet (dd), triplet (t), quartet (q) and multiplet (m). The purity of obtained compounds was

checked by TLC on aluminium oxide 60 F254 plates (Merck Co. Whitehouse Station, NJ), in a $CHCl_3/C_2H_5OH$ (10:1, v/v) solvent system. The spots were detected by exposure to a UV lamp at 254 nm. Elemental analyses of the obtained compounds were performed for C, H, N, S on AMZ 851 CHX analyser (PG, Gdańsk, Poland). The maximum percentage differences between calculated and found values for each element were within the error and amounted to ±0.4%.

Preparation of thiosemicarbazide derivatives

A mixture of appropriate hydrazide: cyclopropopanecarbohydrazide, cyclopentanecarbohydrazide, cyclohexanecarbohydrazide or nicotinic hydrazide (10 mmol) and 10 mmol appropriate isothiocyanate was heated in an oil bath at 50–90 °C for 8–12 h. The product was washed with diethyl ether to remove unreacted isothiocyanate. Then it was filtered off, dried and crystallized from ethanol.

_N_-(4-chlorophenyl)-2-(cyclopropylcarbonyl)hydrazinecarbothioamide (1a)

CAS Registry Number: 685113-56-8. Yield: 80%; m. p. 170 °C–172 °C (dec.). Temperature of reaction: 70 °C for 12 h. ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.63–0.66 (m, 2H, CH_2-cyclopropyl), 0.75–0.81 (m, 2H, CH_2-cyclopropyl), 1.42–1.50 (m, 1H, CH-cyclopropyl), 7.32–7.58 (m, 4H, Ar-H), 9.17 (s, 1H, NH), 9.77 (s, 1H, NH), 10.11 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 10.76 ($2 \times 2 \times CH_2$-cyclopropyl), 16.93 (CH-cyclopropyl), 121.95, 128.20, 129.37 ($5C_{ar}$), 139.93 (C_{ar}), 176.35 (C=O), 179.38 (C=S). Analysis for $C_{11}H_{12}ClN_3OS$ (269.75); Calculated: C, 49.98; H, 4.48; N, 15.58; S, 11.89; Found: C, 49.96; H, 4.45; N, 15.62; S, 11.86%.

2-(Cyclopentylcarbonyl)-_N_-(2,4-dichlorophenyl)hydrazinecarbothioamide (1b)

CAS Registry Number: 891381-19-4. Yield: 91%; m. p. 162 °C–164 °C (dec.). Temperature of reaction: 90 °C for 12 h. ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.50–1.83 (m, 8H, CH_2-cyclopentyl), 2.63–2.73 (m, 1H, CH-cyclopentyl), 7.39–7.72 (m, 3H, Ar-H), 9.37 (s, 1H, NH), 9.79 (s, 1H, NH), 9.94 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 26.12, 32.26 ($4 \times CH_2$-cyclopentyl), 46.12 (CH-cyclopentyl), 125.30, 128.73, 129.00, 129.96, 130.95, 134.63 ($6C_{ar}$), 176.59 (C=O), 180.86 (C=S). Analysis for $C_{13}H_{15}Cl_2N_3OS$ (332.25); Calculated: C, 46.99; H, 4.55; N, 12.65; S, 9.65; Found: C, 46.92; H, 4.57; N, 12.61; S, 9.62%.

_N_-(4-chlorophenyl)-2-(cyclohexylcarbonyl)hydrazinecarbothioamide (1c)

CAS Registry Number: 891077-72-8. Yield: 90%; m. p. 172 °C–174 °C (dec.). Temperature of reaction: 90 °C for 12 h. ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.04–2.28 (m, 10H, cyklohexyl), 2.63 (m, 1H, CH), 7.35–7.57 (m, 4H, Ar-H), 9.07, 9.63, 9.84 (3s, 3H, 3NH). ^{13}C NMR: δ (ppm) = 25.15, 25.92, 27.93 ($5 \times CH_2$-cyclohexyl), 42.43 (CH-cyclohexyl), 121.95, 128.20, 129.37, 139.93 ($6C_{ar}$), 176.11 (C=O), 179.38 (C=S). Analysis for $C_{14}H_{18}ClN_3OS$ (311.83); Calculated: C, 53.92; H, 5.82; N, 13.48; S, 10.28; Found: C, 53.89; H, 5.88; N, 13.45; S, 10.25%.

_N_-phenyl-2-(pyridin-3-ylcarbonyl)hydrazinecarbothioamide (1d)

CAS Registry Number: 54584-49-5. Yield: 92%; m. p. 120 °C–122 °C (dec.). Temperature of reaction: 50 °C for 8 h. ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.13–7.15 (m, 1H, Ar-H), 7.28–7.32 (m, 2H, Ar-H), 7.34–7.38 (m, 2H, Ar-H), 7.57–7.59 (m, 1H, Ar-H); 8.21–8.23 (m, 1H, Ar-H), 8.69–8.71 (m, 1H, Ar-H), 8.90–8.92 (m, 1H, Ar-H), 9.34, 9.71, 10.09 (3s, 3H, 3NH). ^{13}C NMR: δ (ppm) = 121.54, 122.68, 124.47, 132.64, 137.58, 149.45, 150.91 ($11C_{ar}$), 168.05 (C=O), 179.39 (C=S). Analysis for $C_{13}H_{12}N_4OS$ (272.32); Calculated: C, 57.34; H, 4.44; N, 20.57; S, 11.77; Found: C, 57.41; H, 4.40; N, 20.61; S, 11.81%.

Preparation of 1,2,4-triazole derivatives

A mixture of appropriate thiosemicarbazide **1a**–**1d** (10 mmol) and 20–40 ml of 2% aqueous solution of sodium hydroxide was refluxed for 2 h. Then, the solution was neutralized with diluted hydrochloric acid and the formed precipitate was filtered off and crystallized from ethanol.

4-(4-Chlorophenyl)-5-cyclopropyl-2,4-dihydro-3_H_-1,2,4-triazole-3-thione (2a)

CAS Registry Number: 1038358-95-0. Yield: 37%; m. p. 172 °C–174 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.78–0.92 (m, 4H, CH_2-cyclopropyl), 1.46–1.55 (m, 1H, CH-cyclopropyl), 7.52–7.54 (dd, 2H, Ar-H, J = 6 Hz), 7.65–7.67 (dd, 2H, Ar-H, J = 6 Hz), 13.65 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 6.67 (CH-cyclopropyl), 7.31 ($2 \times CH_2$-cyclopropyl), 129.93, 130.84, 133.20, 134.52 ($6C_{ar}$), 154.04 (C-5 triazole), 168.01 (C=S). Analysis for $C_{11}H_{10}ClN_3S$ (251.73); Calculated: C, 52.48; H, 4.00; N, 16.69; S, 12.74; Found: C, 52.47; H, 4.05; N, 16.57; S, 12.72%.

5-Cyclopentyl-4-(2,4-dichlorophenyl)-2,4-dihydro-3_H_-1,2,4-triazole-3-thione (2b)

Yield: 89%; m. p. 203 °C–205 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.44–1.78 (m, 8H, CH_2-cyclopentyl), 2.63–2.73 (m, 1H, CH-cyclopentyl), 7.67–7.73 (m, 2H, Ar-H), 7.96–7.99 (m, 1H, Ar-H), 13.83 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 26.12, 34.79 ($4 \times CH_2$-cyclopentyl), 38.72 (CH-cyclopentyl), 129.46, 130.82, 131.69, 135.53, 136.44, 137.07 ($6C_{ar}$), 156.90 (C-5 triazole), 167.17 (C=S). Analysis for $C_{13}H_{13}Cl_2N_3S$ (314.23); Calculated: C, 49.69; H, 4.17; N, 13.37; S, 10.20; Found: C, 49.72; H, 4.21; N, 13.42; S, 10.08%.

4-(4-Chlorophenyl)-5-cyclohexyl-2,4-dihydro-3_H_-1,2,4-triazole-3-thione (2c)

Yield: 93%; m. p. 212 °C–214 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.04–1.80 (m, 10H, cykloheksyl), 2.84 (m, 1H, CH), 7.52–7.55 (dd, 2H, Ar-H, J = 9 Hz), 7.69–7.72 (dd, 2H, Ar-H, J = 9 Hz), 11.04 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 25.15, 29.92, 29.47 ($5 \times CH_2$-cyclohexyl), 34.20 (CH-cyclohexyl), 129.74, 130.25, 133.45, 135.65 ($6C_{ar}$), 157.97 (C-5 triazole), 167.24 (C=S). Analysis for $C_{14}H_{16}ClN_3S$ (293.81); Calculated: C, 57.23; H, 5.49; N, 14.30; S, 10.91; Found: C, 57.29; H, 5.53; N, 14.27; S, 10.87%.

4-Phenyl-5-(pyridin-3-yl)-2,4-dihydro-3_H_-1,2,4-triazole-3-thione (2d)

CAS Registry Number: 57600-03-0. Yield: 89%; m. p. 222–223 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 7.16–7.18 (m, 1H, Ar-H), 7.34–7.38 (m, 2H, Ar-H), 7.46–7.50 (m, 2H, Ar-H), 7.57–7.59 (m, 1H, Ar-H), 8.28 (m, 1H, Ar-H), 8.69–8.71 (m, 1H, Ar-H), 8.72–8.74 (m, 1H, Ar-H), 10.98 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 126.04, 128.71, 129.36, 131.48, 134.49, 136.86, 146.07, 146.57 ($11C_{ar}$), 157.02 (C-5 triazole), 168.22 (C=S). Analysis for $C_{13}H_{10}N_4S$ (254.31); Calculated: C, 61.40; H, 3.96; N, 22.03; S, 12.61; Found: C, 61.51; H, 3.98; N, 21.98; S, 12.67%.

Preparation of Mannich base derivatives

To a solution of corresponding compounds **2a**–**2d** (10 mmol) in 10 ml of 96% ethanol, appropriate amine (10 mmol) and formaldehyde (37%, 0.2 ml) were added. The mixture was stirred under reflux at room temperature for 1h. Next, distilled water was added and the precipitate formed was filtered off and crystallized from ethanol.

4-(4-Chlorophenyl)-5-cyclopropyl-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3a)

Yield: 65%; m. p. 108 °C–110 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.88–0.95 (m, 4H, 2 × CH_2-cyclopropyl), 1.53–1.62 (m, 1H, CH-cyclopropyl), 1.66–1.70 (m, 4H, 2 × CH_2-pyrrolidine), 2.77–2.81 (m, 4H, 2 × CH_2-pyrrolidine), 5.10 (s, 2H, CH_2), 7.55–7.57 (dd, 2H, Ar-H, J = 6 Hz), 7.68–7.70 (dd, 2H, Ar-H, J = 6 Hz). ^{13}C NMR: δ (ppm) = 6.50 (CH-cyclopropyl), 7.53 (2 × CH_2-cyclopropyl), 29.31, 50.19 (4 × CH_2-pyrrolidine), 65.21 (–CH_2–), 129.37, 130.87, 133.64, 134.64 (6C_{ar}), 152.70 (C-5 triazole), 168.84 (C=S). Analysis for $C_{16}H_{19}ClN_4S$ (334.87); Calculated: C, 64.18; H, 6.40; N, 18.71; S, 10.71; Found: C, 64.15; H, 6.45; N, 18.67; S, 10.65%.

4-(4-Chlorophenyl)-5-cyclopropyl-2-(piperidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3b)

Yield: 93%; m. p. 132 °C–134 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.85–0.95 (m, 4H, 2 × CH_2-cyclopropyl), 1.35–1.38 (m, 2H, CH_2-piperidine), 1.49–1.54 (m, 4H, 2 × CH_2-piperidine), 1.55–1.62 (m, 1H, CH-cyclopropyl), 2.66–2.70 (m, 4H, 2 × CH_2-piperidine), 4.98 (s, 2H, CH_2), 7.56–7.58 (dd, 2H, Ar-H, J = 6 Hz), 7.67–7.69 (dd, 2H, Ar-H, J = 6 Hz). ^{13}C NMR: δ (ppm) = 10.50 (CH-cyclopropyl), 10.71 (2 × CH_2-cyclopropyl), 23.42, 24.57, 53.90 (5 × CH_2-piperidine), 63.51 (–CH_2–), 129.72, 130.29, 133.71, 135.24 (6C_{ar}), 154.66 (C-5 triazole), 170.69 (C=S). Analysis for $C_{17}H_{21}ClN_4S$ (348.89); Calculated: C, 58.52; H, 6.07; N, 16.06; S, 9.19; Found: C, 58.53; H, 6.09; N, 16.11; S, 9.22%.

4-(4-Chlorophenyl)-5-cyclopropyl-2-(piperazin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3c)

Yield: 15%; m. p. 222 °C–224 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.83–0.95 (m, 2H, CH_2-cyclopropyl), 1.03–1.13 (m, 2H, CH_2-cyclopropyl), 1.51–1.60 (m, 1H, CH-cyclopropyl), 2.68–2.82 (m, 8H, CH_2-piperazine), 5.12 (s, 2H, CH_2), 7.54–7.58 (dd, 2H, Ar-H, J = 12 Hz), 7.64–7.68 (dd, 2H, Ar-H, J = 12 Hz), 8.78 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 10.50 (CH-cyclopropyl), 10.71 (2 × CH_2-cyclopropyl), 45.59, 53.21 (4 × CH_2-piperazine), 63.51 (–CH_2–), 129.72, 130.29, 133.71, 135.24 (6C_{ar}), 154.66 (C-5 triazole), 170.69 (C=S). Analysis for $C_{16}H_{20}ClN_5S$ (349.88); Calculated: C, 54.92; H, 5.76; N, 20.02; S, 9.16; Found: C, 54.95; H, 5.79; N, 20.05; S, 9.14%.

4-(4-Chlorophenyl)-5-cyclopropyl-2-{[4-(2-hydroxyethyl) piperazin-1-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3d)

Yield: 73%; m. p. 154 °C–156 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.74–0.97 (m, 4H, 2 × CH_2-cyclopropyl), 1.54–1.63 (m, 1H, CH-cyclopropyl), 2.32–2.40 (m, 4H, 2 × CH_2-piperazine), 2.70–2.76 (m, 4H, 2 × CH_2-piperazine), 2.89–2.91 (t, 2H, CH_2), 3.46–3.48 (t, 2H, CH_2), 4.99 (s, 2H, CH_2), 7.34–7.71 (m, 4H, Ar-H), 8.89 (s, 1H, OH). ^{13}C NMR: δ (ppm) = 6.48 (CH-cyclopropyl), 7.50 (2 × CH_2-cyclopropyl), 52.43, 53.58 (4 × CH_2-piperazine), 50.21 (–CH_2–), 59.25 (–CH_2–), 63.51 (–CH_2–), 120.32, 125.98, 129.13, 129.94, 130.85, 139.06 (6C_{ar}), 154.66 (C-5 triazole), 170.69 (C=S). Analysis for $C_{18}H_{24}ClN_5OS$ (393.93); Calculated: C, 54.88; H, 6.14; N, 17.78; S, 8.14; Found: C, 54.92; H, 6.17; N, 17.80; S, 8.10%.

4-(4-Chlorophenyl)-5-cyclopropyl-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (3e)

CAS Registry Number: 1286639-67-5. Yield: 65%; m. p. 108 °C–110 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 0.85–0.98 (m, 4H, 2 × CH_2-cyclopropyl), 1.54–1.63 (m, 1H, CH-cyclopropyl), 2.86–2.89 (t, 4H, 2 × CH_2-piperazine), 3.12–3.15 (t, 4H, 2 × CH_2-piperazine), 5.09 (s, 2H, CH_2), 6.76–6.81 (m, 1H, Ar-H), 6.93–6.95 (m 2H, Ar-H), 7.19–7.24 (m 2H, Ar-H), 7.58–7.61 (dd, 2H, Ar-H, J = 9 Hz), 7.67–7.70 (dd, 2H, Ar-H, J = 9 Hz). ^{13}C NMR: δ (ppm) = 10.50 (CH-cyclopropyl), 10.71 (2 × CH_2-cyclopropyl), 50.36, 51.43 (4 × CH_2-piperazine), 63.51 (–CH_2–), 116.77, 120.31 (2C_{ar}), 129.35, 129.72, 130.29, 133.71, 135.24 (8C_{ar}), 150.96 (C_{ar}), 154.66 (C-5 triazole), 170.69 (C=S). Analysis for $C_{22}H_{24}ClN_5S$ (425.98); Calculated: C, 62.03; H, 5.68; N, 16.44; S, 7.53; Found: C, 62.08; H, 5.61; N, 16.41; S, 7.56%.

5-Cyclopentyl-4-(2,4-dichlorophenyl)-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (4a)

CAS Registry Number: 929980-59-6. Yield: 68%; m. p. 72 °C–74 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.49–1.59 (m, 2H, CH_2-cyclopentyl), 1.69–1.71 (m, 4H, 2 × CH_2-pyrrolidine), 1.72–1.79 (m, 6H, 3 × CH_2-cyclopentyl), 2.68–2.75 (m, 1H, CH-cyclopentyl), 2.76–2.81 (m, 4H, 2 × CH_2-pyrrolidine), 5.14 (s, 2H, CH_2), 7.40–7.76 (m, 2H, Ar-H), 8.00–8.02 (m, 1H, Ar-H). ^{13}C NMR: δ (ppm) = 24.01, 25.31, 25.38, 30.47 (4 × CH_2-cyclopentyl), 30.94 (CH_2-pyrrolidine), 35.70 (CH-cyclopentyl), 39.99, 40.27 (2 × CH_2-pyrrolidine), 50.09 (CH_2-pyrrolidine), 65.18 (–CH_2–), 129.41, 130.60, 131.43, 133.19, 134.02, 136.27 (6C_{ar}), 154.40 (C-5 triazole), 167.12 (C=S). Analysis for $C_{18}H_{22}Cl_2N_4S$ (397.36); Calculated: C, 54.41; H, 5.58; N, 14.10; S, 8.07; Found: C, 54.45; H, 5.59; N, 14.13; S, 8.04%.

5-Cyclopentyl-4-(2,4-dichlorophenyl)-2-(piperidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (4b)

Yield: 92%; m. p. 88 °C–90 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.32–1.38 (m, 2H, CH_2-cyclopentyl), 1.47–1.54 (m, 6H, 3 × CH_2-piperidine), 1.63–1.79 (m, 6H, 3 × CH_2-cyclopentyl), 2.63–2.70 (m, 4H, 2 × CH_2-piperidine), 2.72–2.79 (m, 1H, CH-cyclopentyl), 5.05 (s, 2H, CH_2), 7.67–7.76 (m, 2H, Ar-H), 7.99–8.01 (m, 1H, Ar-H). ^{13}C NMR: δ (ppm) = 24.00, 25.29, 25.36, 25.99 (4 × CH_2-cyclopentyl), 30.94 (CH_2-piperidine), 35.68 (CH-cyclopentyl), 40.25, 51.61 (4 × CH_2-piperidine), 69.99 (–CH_2–), 129.40, 130.60, 131.43, 133.19, 134.04, 136.25 (6C_{ar}), 154.21 (C-5 triazole), 168.95 (C=S). Analysis for $C_{19}H_{24}Cl_2N_4S$ (411.39); Calculated: C, 55.47; H, 5.88; N, 13.62; S, 7.79; Found: C, 55.49; H, 5.91; N, 13.60; S, 7.75%.

5-Cyclopentyl-4-(2,4-dichlorophenyl)-2-(piperazin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (4c)

Yield: 82%; m. p. 244 °C–246 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.23–1.29 (m, 2H, CH_2-cyclopentyl), 1.48–1.56 (m, 6H, 3 × CH_2-cyclopentyl), 2.05–2.13 (m, 1H, CH-cyclopentyl), 2.70–2.78 (m, 8H, 4 × CH_2-piperazine), 5.09 (s, 2H, CH_2), 7.66–7.75 (m, 2H, Ar-H), 7.78–8.06 (m, 1H, Ar-H), 9.17 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 26.12, 34.79 (4 × CH_2-cyclopentyl), 39.66 (CH-cyclopentyl), 45.59, 53.21 (4 × CH_2-piperazine), 63.51 (–CH_2–), 129.56, 131.01, 131.62, 135.43, 135.98, 136.22 (6C_{ar}), 154.02 (C-5 triazole), 169.21 (C=S). Analysis for $C_{18}H_{23}Cl_2N_5S$ (412.38); Calculated: C, 52.43; H, 5.62; N, 16.98; S, 7.78; Found: C, 52.46; H, 5.63; N, 16.96; S, 7.74%.

5-Cyclopentyl-4-(2,4-dichlorophenyl)-2-{[4-(2-hydroxyethyl)-piperazin-1-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (4d)

Yield: 61%; m. p. 92 °C–94 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 1.47–1.57 (m, 2H, CH_2-cyclopentyl), 1.60–1.80 (m, 6H, 3 × CH_2-cyclopentyl), 2.33–2.37 (t, 2H, CH_2), 2.69–2.74 (m, 4H, 2 × CH_2-piperazine), 2.77–2.81 (m, 1H, CH-cyclopentyl), 3.44–3.50 (m, 4H, 2 × CH_2-piperazine), 3.97 (s, 1H, OH), 4.38–4.42 (t, 2H, CH_2), 5.06 (s, 2H, CH_2), 7.40–7.77 (m, 2H, Ar-H), 8.00–8.01 (m, 1H, Ar-H). ^{13}C NMR: δ (ppm) = 26.12, 34.79 (4 × CH_2-cyclopentyl), 39.66 (CH-cyclopentyl), 52.43, 53.58 (4 × CH_2-piperazine), 58.02 (–CH_2–), 59.25 (–CH_2–), 63.51 (–CH_2–), 129.56, 131.01, 131.62, 135.43, 135.98, 136.22 (6C_{ar}), 154.02 (C5-triazole), 169.21 (C=S). Analysis for $C_{20}H_{27}Cl_2N_5OS$

(456.43); Calculated: C, 52.63; H, 5.96; N, 15.34; S, 7.03; Found: C, 52.65; H, 5.98; N, 15.37; S, 7.01%.

5-Cyclopentyl-4-(2,4-dichlorophenyl)-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (4e)

Yield: 87%; m. p. 67 °C–69 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.49–1.56 (m, 2H, CH_2-cyclopentyl), 1.63–.179 (m, 6H, 3 × CH_2-cyclopentyl), 2.59–2.62 (t, 4H, 2 × CH_2-piperazine), 2.84–2.94 (m, 1H, CH-cyclopentyl), 4.13–4.16 (t, 4H, 2 × CH_2-piperazine), 5.16 (s, 2H, CH_2), 6.78–6.81 (m, 1H, Ar-H), 6.94–6.96 (m, 2H, Ar-H), 7.18–7.25 (m, 2H, Ar-H), 7.66–7.78 (m, 2H, Ar-H), 8.00–8.02 (m, 1H, Ar-H). ^{13}C NMR: δ (ppm) = 26.12, 34.79 (4 × CH_2-cyclopentyl), 39.66 (CH-cyclopentyl), 50.36, 51.43 (4 × CH_2-piperazine), 63.51 (–CH_2–), 116.77, 120.31 (3C$_{ar}$), 129.35, 129.56, 131.01, 131.62, 135.43, 135.98, 136.22 (8C$_{ar}$), 150.96 (C$_{ar}$), 154.02 (C-5 triazole), 169.21 (C=S). Analysis for $C_{24}H_{27}Cl_2N_5S$ (488.47); Calculated: C, 59.01; H, 5.57; N, 14.34; S, 6.56; Found: C, 59.06; H, 5.61; N, 14.37; S, 6.61%.

4-(4-Chlorophenyl)-5-cyclohexyl-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5a)

Yield: 73%; m. p. 91 °C–93 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.16–1.62 (m, 10H, 5 × CH_2-cyclohexyl), 1.70–1.92 (m, 4H, 2 × CH_2-pyrrolidine), 2.38–2.46 (m, 1H, CH-cyclohexyl), 2.69–2.78 (m, 4H, 2 × CH_2-pyrrolidine), 5.16 (s, 2H, CH_2), 7.48–7.50 (dd, 2H, Ar-H, J = 6 Hz), 7.66–7.68 (dd, 2H, Ar-H, J = 6 Hz). ^{13}C NMR: δ (ppm) = 24.92 (2 × CH_2-pyrrolidine), 25.15, 25.92, 29.47 (5 × CH_2-cyclohexyl), 34.92 (CH-cyclohexyl), 53.65 (2 × CH_2-pyrrolidine), 62.10 (–CH_2–), 129.72, 130.29, 133.71, 135.24 (6C$_{ar}$), 157.28 (C-5 triazole), 170.69 (C=S). Analysis for $C_{20}H_{27}ClN_4S$ (376.94); Calculated: C, 60.54; H, 6.68; N, 14.86; S, 8.51; Found: C, 60.58; H, 6.71; N, 14.82; S, 8.55%.

4-(4-Chlorophenyl)-5-cyclohexyl-2-(piperidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5b)

Yield: 78%; m. p. 87 °C–89 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.11–1.56 (m, 10H, 5 × CH_2-cyclohexyl), 1.59–1.64 (m, 4H, 2 × CH_2-piperidine), 2.06–2.18 (m 4H, 2 × CH_2-piperidine), 2.39–2.44 (m, 1H, CH-cyclohexyl), 2.56–2.62 (m, 2H, CH_2-piperidine), 5.35 (s, 2H, CH_2), 7.37–7.40 (dd, 2H, Ar-H, J = 9 Hz), 7.44–7.47 (dd, 2H, Ar-H, J = 9 Hz). ^{13}C NMR: δ (ppm) = 24.57, 25.10 (4 × CH_2-piperidine), 25.15, 26.92, 29,47 (5 × CH_2-cycohexyl), 34.92 (CH-cyclohexyl), 53.90 (CH_2-piperidine), 63.51 (–CH_2–), 129.72, 130,29, 133.71, 135.24 (6C$_{ar}$), 157.28 (C-5 triazole), 170.69 (C=S). Analysis for $C_{20}H_{27}ClN_4S$ (390.97); Calculated: C, 61.44; H, 6.96; N, 14.33; S, 8.20; Found: C, 61.38; H, 6.99; N, 14.30; S, 8.17%.

4-(4-Chlorophenyl)-5-cyclohexyl-2-(piperazin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5c)

Yield: 92%; m. p. 182 °C–184 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.05–1.75 (m, 10H, 5 × CH_2-cyclohexyl), 2.38–2.45 (m, 4H, 2 × CH_2-piperazine), 2.68–2.74 (m, 4H, 2 × CH_2-piperazine), 2.77–2.83 (m, 1H, CH-cyclohexyl), 5.03 (s, 2H, CH_2), 7.52–7.54 (dd, 2H, Ar-H, J = 6 Hz), 7.67–7.69 (dd, 2H, Ar-H, J = 6 Hz), 8.71 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 25.45, 25.62, 30.41 (5 × CH_2-cyclohexyl), 34.52 (CH-cyclohexyl), 40.26; 40.54 (4 × CH_2-piperazine), 50.32 (–CH_2–), 129.72, 130.15, 133.02, 135.24 (6C$_{ar}$), 157.28 (C-5 triazole), 170.69 (C=S). Analysis for $C_{19}H_{26}ClN_5S$ (391.96); Calculated: C, 58.22; H, 6.69; N, 17.87; S, 8.18; Found: C, 58.29; H, 6.72; N, 17.85; S, 8.15%.

4-(4-Chlorophenyl)-5-cyclohexyl-2-{[4-(2-hydroxyethyl)piperazin-1-yl]methyl}-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5d)

Yield: 82%; m.p. 153 °C–155 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.22–1.48 (m, 10H, 5 × CH_2-cyclohexyl), 2.48–2.58 (m, 4H, 2 × CH_2-piperazine), 2.67–2.82 (m, 4H, 2 × CH_2-piperazine), 2.84–2.89 (m, 1H, CH-cyclohexyl), 2.95 (t, 2H, CH_2), 3.54 (t, 2H, CH_2), 3.77 (s, 1H, OH), 4.78 (s, 2H, CH_2), 7.24–7.48 (m, 4H, Ar-H). ^{13}C NMR: δ (ppm) = 25.15, 25.92, 29.47 (5 × CH_2-cyclohexyl), 34.92 (CH-cyclohexyl), 52.43, 53.58 (4 × CH_2-piperazine), 58.02 (–CH_2–), 59.25 (–CH_2–), 63.51 (–CH_2–), 129.72; 130.29, 133.71, 135.24 (6C$_{ar}$), 157.28 (C-5 triazole), 170.69 (C=S). Analysis for $C_{21}H_{30}ClN_5OS$ (436.01); Calculated: C, 57.85; H, 6.94; N, 16.06; S, 7.35; Found: C, 57.87; H, 6.97; N, 16.11; S, 7.32%.

4-(4-Chlorophenyl)-5-cyclohexyl-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (5e)

Yield: 79%; m. p. 68 °C–70 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.13–1.76 (m, 10H, 5 × CH_2-cyclohexyl), 2.39–2.51 (m, 1H, CH-cyclohexyl), 2.68–2.72 (m, 4H, 2 × CH_2-cyclohexyl), 3.13–3.17 (m, 4H, 2 × CH_2-cyclohexyl), 5.14 (s, 2H, CH_2), 6.76–6.81 (m, 2H, Ar-H), 6.93–6.96 (m, 2H, Ar-H), 7.19–7.25 (m, 3H, Ar-H), 7.53–7.56 (m, 1H, Ar-H), 7.66–7.70 (m, 1H, Ar-H), 8.72 (s, 1H, CH). ^{13}C NMR: δ (ppm) = 25.15, 25.92, 29.47 (5 × CH_2-cyclohexyl), 34.92 (CH-cyclohexyl), 50.36, 51.43 (4 × CH_2-piperazine), 63.51 (–CH_2–), 116, 120.31 (3C$_{ar}$), 129.35, 129.72, 130.29, 130.29, 133.71, 135.24 (8C$_{ar}$), 150.96 (C$_{ar}$), 157.28 (C-5 triazole), 170.69 (C=S). Analysis for $C_{25}H_{30}ClN_5S$ (468.06); Calculated: C, 64.15; H, 6.46; N, 14.96; S, 6.85; Found: C, 64.19; H, 6.48; N, 14.99; S, 6.88%.

4-Phenyl-2-(pyrrolidin-1-ylmethyl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6a)

CAS Registry Number: 142529-43-9. Yield: 23%; m. p. 134 °C–136 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.67–1.76 (m, 4H, 2 × CH_2-pyrrolidine), 2.88–2.92 (t, 4H, 2 × CH_2-pyrrolidine), 5.29 (s, 2H, CH_2), 5.29 (s, 2H, CH_2), 7.44–7.54 (m, 6H, Ar-H), 7.69–7.73 (m, 1H, Ar-H), 8.52–8.63 (m, 2H, Ar-H). ^{13}C NMR: δ (ppm) = 23.96, 50.26 (4 × CH_2-pyrrolidine), 65.79 (–CH_2–), 122.45, 124.00, 129.28, 129.95, 130.21, 135.15, 136.51, 147.56, 149.24 (9C$_{ar}$), 150.28, 151.66 (2C$_{ar}$), 154.80 (C-5 triazole), 170.04 (C=S). Analysis for $C_{18}H_{19}N_5S$ (337.44); Calculated: C, 64.07; H, 5.68; N, 20.75; S, 9.50; Found: C, 64.11; H, 5.64; N, 20.81; S, 9.45%.

4-phenyl-2-(piperidin-1-ylmethyl)-5-(pyridin-3-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6b)

CAS Registry Number: 142529-46-2. Yield: 89%; m. p. 150 °C–152 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.30–1.54 (m, 6H, 3 × CH_2-piperidine), 2.68–2.79 (m, 4H, 2 × CH_2-piperidine), 5.12 (s, 2H, CH_2), 7.30–8.63 (m, 9H, Ar-H). ^{13}C NMR: δ (ppm) = 23.42, 24.57, 53.90 (5 × CH_2-piperidine), 63.51 (–CH_2–), 126.15, 128.65, 128.83, 129.36, 132.06, 134.57, 136.59 (9C$_{ar}$), 146.42, 149.92 (2C$_{ar}$), 154.70 (C-5 triazole), 168.26 (C=S). Analysis for $C_{19}H_{21}N_5S$ (351.47); Calculated: C, 64.93; H, 6.02; N, 19.93; S, 9.12; Found: C, 64.98; H, 6.07; N, 19.88; S, 9.08%.

4-Phenyl-2-(piperazin-1-ylmethyl)-5-(pyridin-3-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6c)

Yield: 78%; m. p. 242 °C–244 °C (dec.). ^{1}H NMR (DMSO-d$_6$): δ (ppm) = 1.04–1.14 (m, 2H, CH_2-piperazine), 1.25–1.31 (m, 2H, CH_2-piperazine), 3.20–3.30 (m, 4H, 2 × CH_2-piperazine), 5.24 (s, 2H, CH_2), 7.46–8.56 (m, 9H, Ar-H), 9.23 (s, 1H, NH). ^{13}C NMR: δ (ppm) = 45.59, 53.21 (4 × CH_2-piperazine), 63.51 (–CH_2–), 126.15, 128.65, 128.83, 129.36, 132.06, 136.59 (9C$_{ar}$), 146.42, 146.92 (2C$_{ar}$), 154.70 (C-5 triazole), 168.26 (C=S). Analysis for $C_{18}H_{20}N_6S$ (352.46); Calculated: C, 61.34; H, 5.72; N, 23.84; S, 9.10; Found: C, 61.27; H, 5.78; N, 23.80; S, 9.06%.

2-{[4-(2-Hydroxyethyl)piperazin-1-yl]methyl}-4-phenyl-5-(pyridin-3-yl)-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6d)

Yield: 70%; m. p. 168 °C–170 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.89–2.95 (m, 4H, 2 × CH_2-piperazine), 3.21 (t, 2H, CH_2), 3.36–3.43 (m, 4H, 2 × CH_2-piperazine), 3.55 (t, 2H, CH_2), 4.46 (s, 2H, CH_2), 7.17–7.60 (m, 5H, Ar-H), 8.27–8.77 (m, 4H, Ar-H), 8.87 (s, 1H, OH). ^{13}C NMR: δ (ppm) = 52.43, 53.58 (4 × CH_2-piperazine), 58.02 (–CH_2–), 59.25 (–CH_2–), 63.51 (–CH_2–), 126.15, 128.65, 128.83, 129.36, 132.06, 134.57, 136.59 (9C_{ar}), 146.42, 146.92 (2C_{ar}), 154.70 (C-5 triazole), 168.26 (C=S). Analysis for $C_{20}H_{24}N_6OS$ (396.50); Calculated: C, 60.58; H, 6.10; N, 21.20; S, 8.09; Found: C, 60.62; H, 6.05; N, 21.24; S, 8.01%.

4-Phenyl-5-(pyridin-3-yl)-2-[(4-phenylpiperazin-1-yl)methyl]-2,4-dihydro-3*H*-1,2,4-triazole-3-thione (6e)

CAS Registry Number: 915144-29-5. Yield: 95%; m. p. 154 °C–156 °C (dec.). ^{1}H NMR (DMSO-d_6): δ (ppm) = 2.62–2.65 (t, 2H, CH_2-piperazine), 3.00–3.04 (t, 2H, CH_2-piperazine), 3.15–3.19 (t, 2H, CH_2-piperazine), 3.46–3.48 (t, 2H, CH_2-piperazine), 5.32 (s, 2H, CH_2), 6.79–6.84 (m, 2H, Ar-H), 6.96–7.07 (m, 4H, Ar-H), 7.22–7.27 (m, 4H, Ar-H), 7.42–7.63 (m, 2H, Ar-H), 7.73–7.77 (m, 1H, Ar-H), 8.57–8.65 (m, 1H, Ar-H). ^{13}C NMR: δ (ppm) = 50.36, 51.43 (4 × CH_2-piperazine), 63.51 (–CH_2–), 116.77 (2C_{ar}), 120.31 (C_{ar}), 126.15, 128.65, 128.83, 129.35, 129.36, 132.06, 134.57, 136.59 (11C_{ar}), 146.42, 146.92 (2C_{ar}), 150.96 (C_{ar}), 154.70 (C-5 triazole), 168.26 (C=S). Analysis for $C_{24}H_{24}N_6S$ (428.55); Calculated: C, 67.26; H, 5.64; N, 19.61; S, 7.48; Found: C, 67.34; H, 5.61; N, 19.64; S, 7.44%.

Antiproliferative activity

Materials and methods

The experiment was conducted on reference cancer cell lines: A549 (*Adenocarcinomic human alveolar basal epithelial cells*), HeLa (*Human cervical cancer cells*), TOV-112D (*Human ovarian cancer cell line*), T47D (*Human ductal breast epithelial tumor cell line*) and L929 (*Murine aneuploid fibrosarcoma cell line*) and GMK (*Green monkey kidney cell line*) as a normal cell lines. The control group was the chosen cell line untreated with compounds. Cell lines were obtained from European Collection of Cell Cultures (ECAACC). Cell cultures were grown at 37 °C in a humidified atmosphere consisting 5% CO_2 in air. Cultures were maintained at density of 2–5 × 10^6 cell/ml in exponential growth serum free conditions containing RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS, Sigma), 100 U/ml of penicillin (Sigma), 100 µg/ml streptomycin (Sigma), and routinely passaged twice a week. Cell viability was assessed by the ability to exclude trypan blue dye (0.5% v/v, Sigma). Next cultures were incubated in the presence of tested compounds (**3a**, **3b**, **3c**, **3e**, **4a**, **4b**, **4d**, **5c**, **5e**, **6a**, **6b**, **6e**) at the concentrations of 50, 100 and 150 µM during 24, 48 and 72 h. The investigated 12 compounds were dissolved in dimethylsulfoxide (DMSO, Sigma) and then diluted in cell culture media. The antiproliferative activity of novel compounds was assessed by 5-bromo-2′-deoxyuridine test (BrdU, Sigma) on El_x808_{iu} ELISA reader. In these method, 5-bromo-2′-deoxyuridine, a thymidine analog, replaces [3H] thymidine. BrdU is incorporated into newly synthesized DNA strands of actively proliferating cells. Following partial denaturation of double stranded DNA, BrdU is detected immunochemically allowing the assessment of the population of cells, which are synthesizing DNA. All results were done in triplicates.

Microbiology

Materials and methods

All the derivatives were screened for their *in vitro* antibacterial activity using agar plate method (with 1000 µg/ml concentration of compounds) and next the compounds with potential antibacterial activity were tested using microdilution technique to estimate minimal inhibitory concentration (MIC), as was described earlier [27]. Eleven reference strains of aerobic bacteria from American Type Culture Collection were used. There were representing Gram-positive bacteria *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 25923, *S. aureus* ATCC 43300, *S. epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 10876, *Micrococcus luteus* ATCC 10240 and representing Gram-negative bacteria *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Proteus mirabilis* ATCC 12453 and *Pseudomonas aeruginosa* ATCC 27853. All the bacterial cultures were adjusted to 0.5 McFarland standards 150 × 10^6 CFU/ml (CFUs – colony forming units) in sterile 0.85% NaCl. All stock solutions of the tested compounds were prepared in DMSO (dimethyl sulfoxide). The medium with DMSO at the final concentration and without the tested compounds served as negative control – no microbial growth inhibition was observed. Cefuroxime (second generation of cephalosporins) was used as a positive control.

Using Mueller–Hinton agar plate method with 1000 µg/ml concentration of each derivative, 10 µl of each bacterial suspension was put onto the prepared solid media and antibacterial activity was detected after incubation (37 °C for 18 h) on the basis of the microbial growth inhibition. MIC of compounds, which inhibited the growth of bacteria on agar medium, was tested through the Mueller–Hinton broth microdilution method as recommended by Clinical Laboratory Standards Institute[28]. MIC is usually defined as the lowest concentration of the compound at which there was no visible growth of microorganisms. The 96-well microplates and Mueller–Hinton broth with a series of two-fold dilution of the tested compound in the range of final concentrations from 7.82 to 1000 µg/ml were used. After incubation (at 35 °C for 18 h), spectrophotometric measurements of optical density (OD_{600}) of the bacterial cultures with the tested compounds were performed in order to determine MIC. OD_{600} of bacterial cultures in the medium without the tested compounds and the blank wells with two-fold dilution of each of the tested compounds added to broth without bacterial suspension were used as controls. In our study, the bioactivity of tested compounds against bacteria was defined as mild (MIC > 500 µg/ml), moderate (MIC in the range >125–500 µg/ml), good (MIC in the range ≥31.25–125 µg/ml), according to O'Donnell et al.[29].

Results and discussion

Chemistry

The hydrazides of cyclopropionic acid, cyclopentanoic acid, cyclohexanoic acid and nicotinic acid were initial substrates for the synthesis of new Mannich base derivatives **3a–3e**, **4a–4e**, **5a–5e**, **6a–6e**. In the first step, thiosemicarbazide derivatives **1a–1d** were obtain by the reaction of corresponding carboxylic acid hydrazide with approptiate isothiocyanate. The substracts were heated in an oil bath, temperatures were selected experimentally (t = 50–90 °C). Subsequently, the reaction of thiosemicarbazide derivatives **1a–1d** with 2% aqueous solution of sodium hydroxide lead to the formation of 4,5-disubstituted 1,2,4-triazole-3-thione derivatives **2a–2d**. The treatment of 1,2,4-triazole derivatives with formaldehyde and corresponding secondary amine in ethanol afforded new Mannich base derivatives **3a–3e**, **4a–4e**, **5a–5e**, **6a–6e** in moderate to good yields.

Compounds **1a**, **1b**, **1c**, **2a**, **3e**, **4a**, **6a**, **6b** are registered in CAS (Chemical Abstracts Service) database but without references and methods of synthesis. Compounds **1d**[30], **2d**[31], **6e**[32] were synthesized earlier.

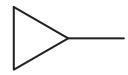
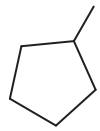
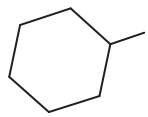
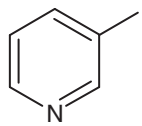
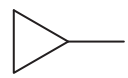
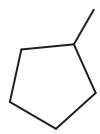
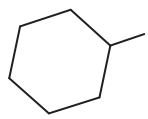
All the newly obtained compounds are air stable solids and soluble in DMSO at ambient temperature. The purity of the synthesized compounds was checked by elemental analyses and thin layer chromatography. The structures of prepared derivatives were determined on the basis of ^{1}H NMR and ^{13}C NMR spectra and all of the synthesized compounds have satisfactory analyses for their proposed structures. ^{1}H NMR spectral results for all compounds together with hydrogen assignments and ^{13}C NMR spectra results are presented in Experimental Section.

^{1}H NMR spectra of the thiosemicarbazide derivatives **1a–d** show three proton signals typical for the NH group in the δ 9.07–10.11 ppm range, whereas in the ^{1}H NMR spectra of the 1,2,4-triazole compounds **2a–d** the singlet peak due to the proton of NH group appeared in the region of δ 11.04–13.65 ppm, which confirmed the successful formation of the desired products. All the ^{1}H NMR spectra of Mannich base derivatives derivatives **3a–e**, **4a–e**, **5a–e**, **6a–e** confirmed adequate *N*-substitution of 1,2,4-triazole-3-thiones. The singlet signal for CH_2 group was observed in the δ 4.46-5.35 ppm range (compounds **3a–e**, **4a–e**, **5a–e**, **6a–e**), for NH group (compounds **3c**, **4c**, **5c**, **6c**) δ 8.71–9.23 ppm, and for OH group in the region of 3.77–3.97 and 8.87–8.89 ppm (compounds **3d**, **4d**, **5d**, **6d**). All other aliphatic and aromatic protons for obtained compounds were observed at expected regions.

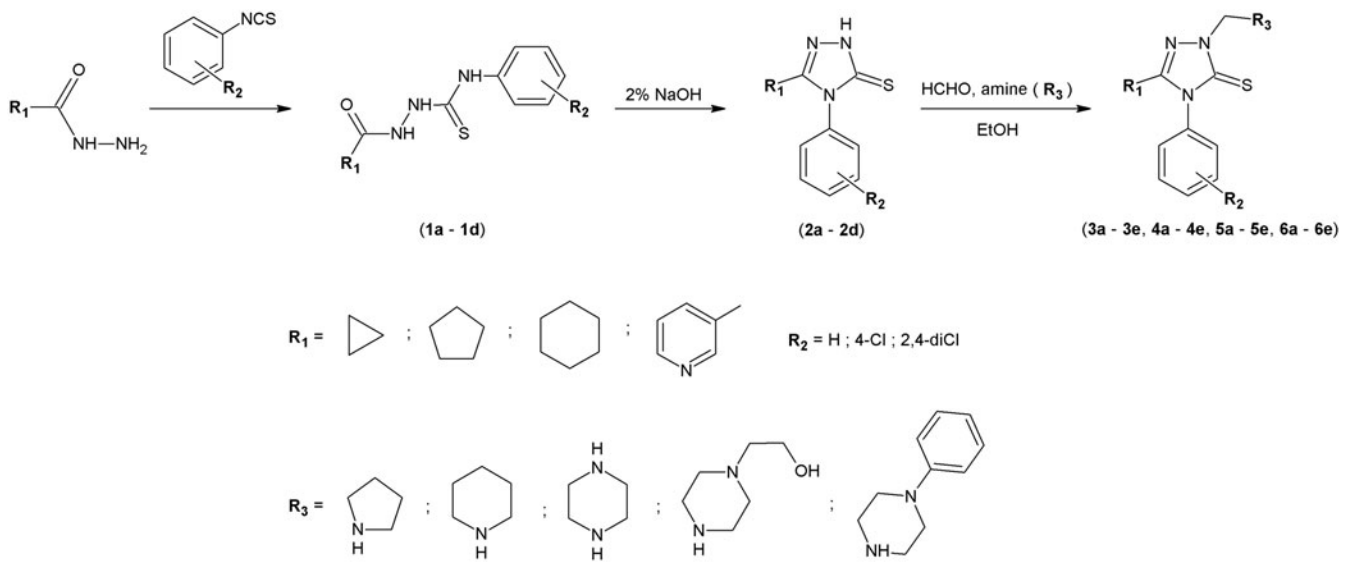
In the ^{13}C NMR spectra of thiosemicarbazide derivatives **1a–d** the carbon of C=S group had a typical signal at about 176 ppm and for C=O group at about 168–180 ppm. Similarly, in the 1,2,4-triazole derivatives **2a–d**, the presence of the C=S group was also confirmed by a signal at about 167–168 ppm and for the C-5 in 1,2,3-triazole system in the range of 154–157 ppm. The **2a–d** derivatives may occur in thione or thiol form. However, in our case the signal of C=S group in compounds **2a–d** proved that they were obtained in thione form. The thione-thiol tautomerism was not observed. It is consistent with the studies recently conducted in our department which were focused on the cyclization mechanism of thiosemicarbazide derivatives[33,34]. In the Mannich base derivatives **3a–e**, **4a–e**, **5a–e**, **6a–e** the CH_2 group was observed at about 50–69 ppm. All other aliphatic and aromatic signals were observed at expected regions.

The synthetic pathway leading to the new Mannich base derivatives **3a–e**, **4a–e**, **5a–e**, **6a–e** was carried out according to the steps shown in Scheme 1. The substituents of all obtained compounds **1–6** are presented in Table 1.

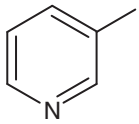
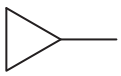
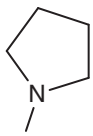
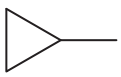
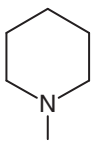
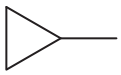
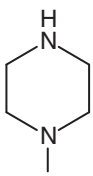
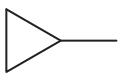
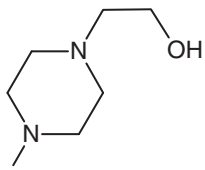
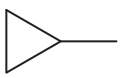
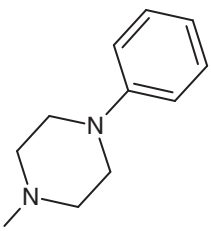
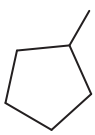
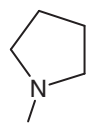
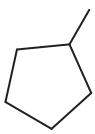
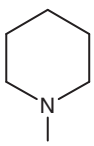
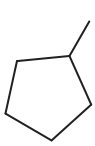
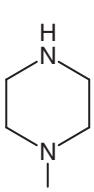
Table 1. Substituents of compounds **1–6**.

Compound	R_1	R_2	R_3
1a		–	–
1b		–	–
1c		–	–
1d		–	–
2a		4-Cl	–
2b		2,4-diCl	–
2c		4-Cl	–

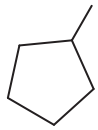
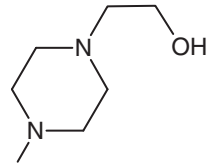
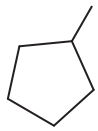
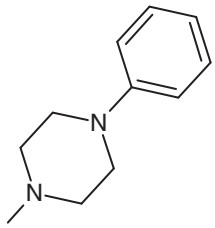
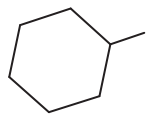
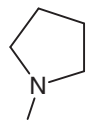
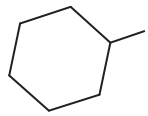
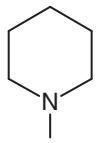
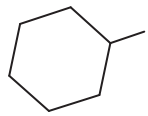
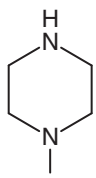
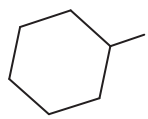
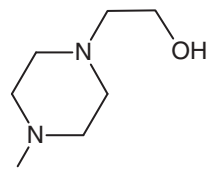
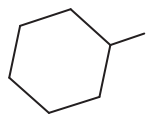
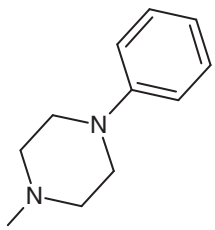
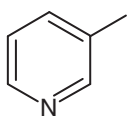
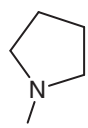
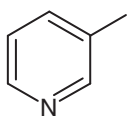
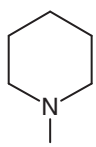
(*continued*)



Scheme 1. Synthetic route for new Mannich base derivatives **3a–3e**, **4a–4e**, **5a–5e**, **6a–6e**.

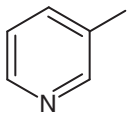
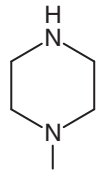
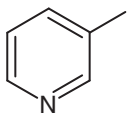
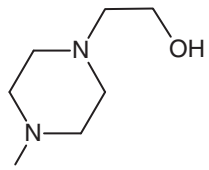
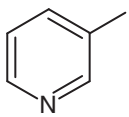
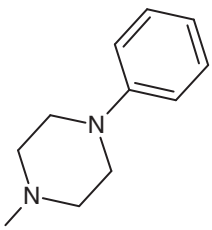
Compound	R_1	R_2	R_3
2d		H	–
3a		4-Cl	
3b		4-Cl	
3c		4-Cl	
3d		4-Cl	
3e		4-Cl	
4a		2,4-diCl	
4b		2,4-diCl	
4c		2,4-diCl	

(*continued*)

Compound	R_1	R_2	R_3
4d		2,4-diCl	
4e		2,4-diCl	
5a		4-Cl	
5b		4-Cl	
5c		4-Cl	
5d		4-Cl	
5e		4-Cl	
6a		H	
6b		H	

(*continued*)

Table 1. Continued

Compound	R_1	R_2	R_3
6c		H	
6d		H	
6e		H	

Antiproliferative activity

The purpose of this study was to evaluate *in vitro* antiproliferative activities against chosen cell lines of the 12 compound based on of Mannich bases containing 1,2,4-triazole system. Evaluation of cell cycle progression is essential for investigations in many scientific fields. Measurement of [3H] thymidine incorporation as cells enter S phase has been a traditional method for detection of cell proliferation. Precentage of cells viability, before the foundation of the culture, was about 87–96%. The amount of BrdU was determined according to OD. The higher the OD, the higher the BrdU concentration in the sample. Results were presented as the percentage of cell viability in comparison to control. The influence of various concentrations of new synthesized compounds on the proliferative activity of chosen cell cultures is summarized in Table 2.

The investigated compounds proved to be low toxic for checked cell lines, since applied in concentrations of 50, 100 and 150 μM caused only slight viability decreases in examined cell cultures. Only 1 of the 12 investigated compounds was found to be little biologically active *in vitro*. We have noticed, that compound **6b** evoked slight dose-dependent viability decreases in T47D, HeLa and A549 cell lines (15–20% growth inhibition was observed comparison to control). In case of HeLa cell line, except compound **6b**, it was noticed that compound **6a**, in the

Table 2. Effects of tested compounds (**3a**, **3b**, **3c**, **3e**, **4a**, **4b**, **4d**, **5c**, **5e**, **6a**, **6b**, **6e**) on the viability of chosen cell lines (%).

Number of derivative and dose	L929			GMK			A549			HeLa			TOV-112D			T47D		
	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h
Control	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3a–50 μM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5
3a–100 μM	0	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	5	5
3a–150 μM	0	0	0	0	0	0	5	5	0	0	0	0	0	0	10	0	5	5
3b–50 μM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0
3b–100 μM	0	0	0	0	0	0	0	5	5	0	0	0	0	5	10	5	5	5
3b–150 μM	0	0	0	0	0	0	0	5	10	5	5	5	5	10	10	5	10	10
3c–50 μM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3c–100 μM	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	5	5
3c–150 μM	0	0	0	0	0	0	0	0	0	5	5	5	0	0	0	10	5	5
3e–50 μM	0	0	0	0	0	0	0	5	5	0	0	0	0	0	5	5	5	5
3e–100 μM	0	0	0	0	0	0	5	5	5	5	5	0	0	5	10	10	10	10
3e–150 μM	0	0	0	0	0	0	10	10	10	5	5	5	5	5	10	15	15	15
4a–50 μM	0	0	0	0	0	0	0	5	10	0	0	0	0	5	5	5	5	5
4a–100 μM	0	0	0	0	0	0	5	10	15	2	5	5	5	10	10	10	10	10
4a–150 μM	0	0	0	0	0	0C	5	10	15	5	10	10	5	10	10	15	15	15
4b–50 μM	0	0	0	0	0	0	5	0	2	0	0	0	0	0	5	5	5	5
4b–100 μM	0	0	0	0	0	0	5	10	10	0	0	0	5	10	10	15	15	15
4b–150 μM	0	0	0	0	0C	0C	10	15	20	10	10	15	10	10	5	20	20	20
4d–50 μM	0	0	0	0	0	0	0	5	5	0	0	0	0	0	5	10	10	5
4d–100 μM	0	0	0	0	0	0	0	10	10	5	5	5	0	5	10	15	15	10
4d–150 μM	0	0	0	0	0	0	10	15	15	10	10	10	0	10	15	15	15	15
5c–50 μM	0	0	0	0	0	0	0	5	5	0	0	0	0	0	5	5	10	10
5c–100 μM	0	0	0	0	0	0	5	10	15	10	10	10	0	5	10	10	10	10
5c–150 μM	0	0	0	0	0	0	5	10	15	15	15	15	5	15	15	20	20	20
5e–50 μM	0	0	0	0	0	0	2	5	10	0	0	0	0	5	5	15	15	15
5e–100 μM	0	0	0	0	0	0	2	10	15	5	5	5	5	10	15	20	20	20
5e–150 μM	0	0	0	0	0	0C	5	15	20	10	10	10	5	15	20	20	20	20
6a–50 μM	0	0	0	0	0	0	0	2	5	5	5	5	5	5	5	5	5	5
6a–100 μM	0	0	0	0	0	0	5	10	10	10	10	10	10	10	10	10	10	10
6a–150 μM	0	0	0	0	0	0	5	10	10	20	20	20	15	20	20	25	25	20
6b–50 μM	0	0	0	0	0	0	0	5	5	5	5	5	0	0	5	5	5	5
6b–100 μM	0	0	0	0	0	0	5	10	10	15	15	15	5	5	5	15	10	10
6b–150 μM	0	0	0	0	0	0	20	25	25	20	20	20	5	0	5	20	20	20
6e–50 μM	0	0	0	0	0	0	0	5	5	0	0	0	5	5	5	10	10	10
6e–100 μM	0	0	0	0	0	0	5	5	10	0	5	5	5	5	5	15	15	15
6e–150 μM	0	0	0	0	0	0	5	10	15	0	5	5	10	10	10	20	20	20

L929, Murine aneuploid fibrosarcoma cell line; GMK, Green monkey kidney cell line; A549, Adenocarcinomic human alveolar basal epithelial cells; HeLa, Human cervical cancer cells; TOV-112D, Human ovarian cancer cell line; T47D, Human ductal breast epithelial tumor cell line.

Table 3. The influence of synthesized compounds **1–6** on the growth of Gram-positive and Gram-negative bacteria of on the basis of MIC (μg/ml).

Compound	Sa6538	Sa25923 (MSSA)	Sa43300	Se12228	Bs6633	Bc10876	Ml10240	Ec25922	Kp13883	Pm12453	Pa27853
1a	62.5	62.5	62.5	250	125	62.5	31.25	nd	nd	nd	nd
1b	62.5	62.5	62.5	250	125	62,5	31.25	nd	nd	nd	nd
1c	31.25	31.25	31.25	31.25	250	62.5	31.25	nd	nd	nd	nd
2a	125	125	125	62.5	125	125	31.25	nd	nd	nd	nd
2c	62.5	125	62.5	125	125	125	62.5	nd	nd	nd	nd
3a	500	500	250	250	250	250	125	1000	500	1000	1000
3b	500	500	250	250	500	500	250	1000	500	1000	1000
3e	500	500	500	500	500	500	500	nd	500	nd	nd
4a	500	500	125	500	500	125	62.5	1000	500	1000	1000
4b	500	500	250	500	1000	125	62.5	>1000	1000	>1000	>1000
4d	1000	125	500	1000	1000	125	125	nd	500	nd	nd
4e	>1000	500	500	500	250	500	250	nd	500	nd	nd
5c	125	125	125	125	125	62.5	125	nd	nd	nd	nd
5e	nd	nd	nd	nd	nd	250	62.5	nd	nd	nd	nd
6a	500	500	500	500	500	500	500	1000	500	500	1000
6b	500	500	500	500	500	500	500	1000	250	500	1000
6e	500	1000	500	500	500	500	500	1000	500	500	1000

nd, not determined; Sa6538, *Staphylococcus aureus* ATCC 6538; Sa25923, *Staphylococcus aureus* ATCC 25923; Sa43300, *Staphylococcus aureus* ATCC 43300; Se12228, *Staphylococcus epidermidis* ATCC 12228; Bs6633, *Bacillus subtilis* ATCC 6633; Bc10876, *Bacillus cereus* ATCC 10876; Ml10240, *Micrococcus luteus* ATCC 10240; Ec25922, *Escherichia coli* ATCC 25922; Pm12453, *Proteus mirabilis* ATCC 12453; Pa27853, *Pseudomonas aeruginosa* ATCC 27853.

concentration of 100 and 150 μM also caused small decreases (10 and 20%, respectively) in cell viability versus control.

We have noted, that T47D cell line was the most susceptible cancer cell line, among the others, on compounds' influencing. The slight cytotoxic activity against T47D cell line in the set of checked compounds (except compound **6b**) were found also for compounds number **5e** and **6a**. In the concentration of 50, 100 and 150 μM, these compounds showed 15–25% growth inhibition against breast tumor cell line after 24, 48 and 72 h of incubation. The other compounds that affected on this cell line, but to a much lesser extent, were compound number **4b**, **4d**, **5c**, **6a** and **6e**.

No visual changes in cell viability against TOV-112D cell line, L929 and GMK cell line during tested compound treatment, were not observed. Our study revealed that some of our synthesized compounds possess minor antiproliferative activity.

Microbiology

On the basis of the preliminary results obtained by agar plate method, it was shown that some of the newly synthesized compounds listed in Table 3 have shown a potential activity against reference strains mainly of Gram-positive bacteria. On the basis of MIC estimation some compounds was found to possess good, moderate or mild bioactivity against Gram-positive bacterial species with MIC ranging from 31.25 to 500 μg/ml (Table 3). The highest activity possessed compounds **1a**, **1b**, **1c**, **2a**, **2c** and **5c** (MIC = 31.25–250 μg/ml). *M. luteus* ATCC 102740 was the most sensitive to the selected derivatives (MIC = 31.25–250 μg/ml). In our experiments, MIC of cefuroxime was 0.24–1.95 μg/ml for *Staphylococcus* species and 0.49–31.25 μg/ml for the other Gram-positive bacteria.

Among the tested compounds, only seven (**3a**, **3b**, **4a**, **4b**, **6a**, **6b** and **6e**) had mild or moderate inhibitory effect on the Gram-negative bacteria growth with MIC values ranging from 250 to ≥1000 μg/ml.

In general, our obtained thiosemicarbazide derivatives showed better activity as compared to cyclic compounds, what is consistent with the literature findings[35]. According to the literature, the Mannich base derivatives antimicrobial activity is strongly dependent on the kind of amine substituent in N2 position[36], what can also be observed in our case study. Our results have shown that the tested derivatives exhibited good or moderate activity against both pathogenic (e.g. *S. aureus*) and opportunistic (e.g. *S. epidermidis*, *M. luteus*, *B. subtilis* or *B. cereus*) Gram-positive bacteria and may be of value for searching new derivatives showing better antimicrobial activity.

Conclusions

In the current study, we synthesized and characterized by ^{1}H NMR, ^{13}C NMR and elemental analyses a new series of Mannich base derivatives containing 1,2,4-triazole moiety.

Twelve of obtained compounds were analyzed for their *in vitro* antiproliferative acitivity against six cancer cell lines. Our study revealed that synthesized compounds **5e**, **6a**, **6e** possess minor antiproliferative activity and some further studies in this direction need to be done.

All the newly prepared compounds were also screened for their *in vitro* antimicrobial activity by agar dilution technique against 11 reference strains of Gram-positive and Gram-negative bacteria. Our antimicrobial study revealed that compounds **1a**, **1b**, **1c**, **2a**, **2c** and **5c** had good or moderate activity against the reference Gram-positive bacteria and the compounds **3a**, **3b**, **4a**, **4b**, **6a**, **6b** and **6e** had mild or moderate inhibitory effect on the Gram-negative bacteria growth. Obtained compounds may be regarded as precursor compounds for searching new derivatives showing better antimicrobial activity against pathogenic or opportunistic bacteria. Nowadays due to several serious problems such as growing drug resistance of bacteria or undesirable side effects of drugs it is very important to find new substances with antimicrobial activity.

Declaration of interest

The project was partially supported by the Research Grant for Young Scientists (MNmb25). The authors declare no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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