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Novel thiosemicarbazide derivatives with 4-nitrophenyl group as multi-target drugs: α-glucosidase inhibitors with antibacterial and antiproliferative activity

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ABSTRACT

A series of thiosemicarbazides with 4-nitrophenyl group was obtained in the reaction of carboxylic acid hydrazides with isothiocyanates. All compounds were checked for their antibacterial and antiproliferative activity. Our results have shown that derivatives **6-8** possessed antibacterial activity against *S. aureus*, *S. epidermidis*, *S. mutans* and *S. sanguinis*, moderate cytotoxicity and good therapeutic safety *in vitro*. Additionally, compounds **1** and **4** significantly inhibited A549, HepG2 and MCF-7 cell division. Moreover, PASS software indicated that newly obtained compounds are potential α-glucosidase inhibitors. This was confirmed by *in vitro* studies. To investigate the mode of interaction with the molecular target compounds were docked to glucose binding site of the enzyme and exhibited a similar binding mode as glucose.

1. Introduction

In recent years the organo-nitrogen compounds are commonly designed and synthesized as biologically active compounds [1–3]. Small heterocyclic molecules, such as derivatives of pyrazole, pyridine, triazole, thiazole derivatives have interesting bioactivity profiles [4–6]. Moreover, linear derivatives of these compounds [7–9], including thiosemicarbazide derivatives can result in ground breaking discovery of new class of therapeutic agents [10]. Conjugated N-N-S tridentate ligand system of thiosemicarbazide (NH_2-C(S)-NH-NH_2) is very important for antibacterial, antitubercular, anticonvulsant, antifungal, antiproliferative and anticancer activity [11–15].

On the other hand, nitro group is a structural moiety that is often found in biologically active compounds. Nitroheterocyclic derivatives have a wide spectrum of activity including antibacterial, antifungal or anticancer properties [16,17]. A significant number of compounds have been found to be adequate in drug discovery process and pharmaceutical research exhibiting anti-inflammatory, anti-HIV, anti-tubercular, antiprotozoal, antibacterial, antifungal, antiproliferative and anticancer activity [18–24]. The nitro group affects biological properties of chemical compounds, usually by increasing their bactericidal ability and toxicity.

In the light of above, the aim of this study was to design, synthesize and study multi-target compounds containing thiosemicarbazide and nitro moieties with antibacterial and antiproliferative activity. Due to the limited amount of information on the activity of these compounds as a α-glucosidase inhibitors [25–30], we decided to test this mode of activity. The rationale of this study can be summarized as follows: (1) the possibility of

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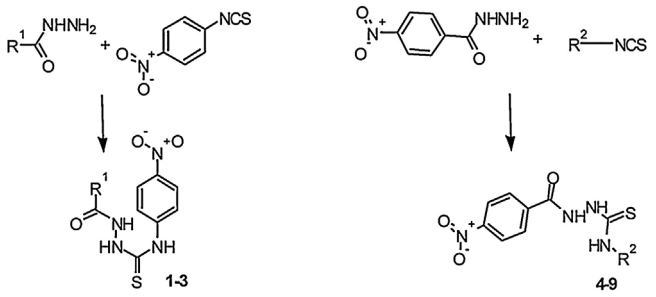

	R^1	R^2
1	2-pyridine	4-$NO_2C_6H_4$
2	3-pyridine	4-$NO_2C_6H_4$
3	4-pyridine	4-$NO_2C_6H_4$
4	4-$NO_2C_6H_4$	C_6H_{11}
5	4-$NO_2C_6H_4$	n-C_4H_9
6	4-$NO_2C_6H_4$	2-ClC_6H_4
7	4-$NO_2C_6H_4$	4-$NO_2C_6H_4$
8	4-$NO_2C_6H_4$	2,4-$Cl_2C_6H_3$
9	4-$NO_2C_6H_4$	$CH_2C_6H_5$

Scheme 1. Synthesis of the investigated compounds.

combination of two important bioactive groups, i.e. thiosemicarbazide and nitro moieties in one molecule; (2) the ability to design a group of multi-target compounds which is a hot topic in current medicinal chemistry and (3) the striking epidemiologic data about cancer, diabetes and drug-resistant bacterial infections.

2. Material end methods

2.1. Chemistry

The chemicals used for synthesis and analysis were purchased from Merck Co. or Sigma-Aldrich and used without further purification. Melting points were determined on a Fisher-Johns block and presented without any corrections. The NMR spectra were acquired using Bruker Avance 300 MHz spectrometer with TMS as an internal standard. The IR spectra were recorded on the Thermo Nicolet 6700 ATR device in the range of 500–3500 cm^{-1}. The mass spectra were obtained by Bruker microToF apparatus (CI-MS). The elementary analysis was performed with the application of Perkin-Elmer analyzer (940 Winter St., Waltham, MA, USA). The obtained results were within ±0.4% of the theoretical value. The purity of obtained compounds was checked by TLC on Merck Co. plates (aluminium oxide 60 F_{254} in a $CHCl_3/C_2H_5OH$ (10:1 or 10:2) solvent system with UV or in iodide visualization.

The carboxylic acid hydrazides were obtained by the general procedure described earlier [31]. Some of the tested compounds have been reported in literature [32–35].

2.2. General procedure for the preparation of 1,4-disubstituted thiosemicarbazide *1–9*

10 mmole of the 2–,3-,4-pyridine carboxylic acid hydrazide or 4-nitrophenyl carboxylic acid hydrazide, cyclohexyl, *n*-butyl, 4-nitrophenyl, 2,4-dichlorophenyl or benzyl isothiocyanate (10 mmole) and methanol (10 mL) were placed in a round bottom flask and heated at reflux for 30 min. After this time the precipitated compound was filtered, dried and crystallized from methanol-acetonitrile (1:1). (Supplementary material includes experimental data of all compounds).

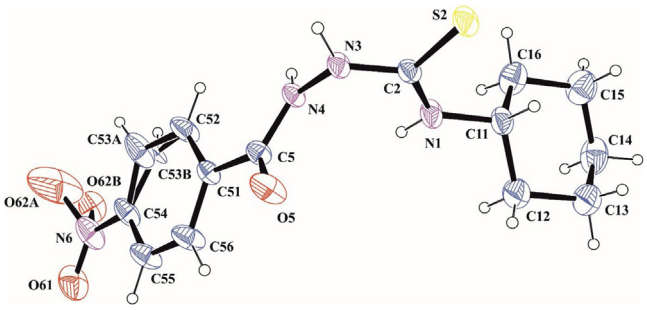

Fig. 1. The molecular structure of **4** compound, with atom labelling and displacement ellipsoids drawing at the 30% probability level.

2.3. X-ray analysis

X-ray data of compound **4** were collected on the Bruker SMART APEX II CCD diffractometer; crystal sizes 0.60 × 0.44 × 0.33 mm, CuKα (λ = 1.54178 Å) radiation, ω and φ scans, T = 293 K, absorption correction: multi-scan SADABS [36], T_{min}/T_{max} = 0.265/0.580. The structure was solved by direct methods using SHELXS97 [37]. All calculations were performed using WINGX version 1.64.05 package [38]. (Supplementary material includes experimental data of compound **4**).

2.4. Biological assays

2.4.1. Antibacterial assays

All synthesized compounds were screened for their *in vitro* antibacterial activity against aerobic Gram-positive *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228 and microaerobic Gram-positive *Streptococcus mutans* PCM 2502, *Streptococcus sanguinis* PCM 2335 bacterial strains. Pilot disc diffusion test and MIC, MBC assays were performed as described previously [39] and obtained data were compared to reference Cefepime (Maxipime).

2.4.2. Cytotoxicity assay and therapeutic index

The cytotoxicity assay and therapeutic index determination of synthesized compounds were performed using BJ cell line as described previously [40].

2.4.3. Cell proliferation assay and selectivity index

The cell proliferation assay was carried out using A549, HepG2, MCF-7 and BJ cell lines as described previously [36]. After 96-h

Table 1
Bacterial growth inhibition zones caused by new nitro derivatives **1**-**9**. *Compounds **2** and **5** showed no inhibition zone.

Comp.*	Bacterial growth inhibition zones [mm]			
	S. aureus	*S. epidermidis*	*S. mutans*	*S. sanguinis*
1	17	18	0	0
3	5	5	5	7
4	9	13	0	0
6	17	17	15	13
7	16	15	22	20
8	21	21	23	21
9	5	12	0	0
Cefepime	24	26	29	24

Table 2
Antibacterial and cytotoxic effects of new nitro derivatives: MIC, MBC/MIC ratio, CC_{50} and therapeutic index (TI). CC_{50}: the concentration that caused a 50% reduction of normal fibroblast viability; MIC: minimum inhibitory concentration; MBC: minimum bactericidal concentration; TI (therapeutic index): the ratio between CC_{50} and MIC values; NA– no activity in screening test; NT – not tested.

no	$CC_{50} \pm SD$ [μg/ml]	*S. aureus*			*S. epidermidis*			*S. sanguinis*			*S. mutans*		
		MIC [μg/ml]	TI	MBC/MIC	MIC [μg/ml]	TI	MBC/MIC	MIC [μg/ml]	TI	MBC/MIC	MIC [μg/ml]	TI	MBC/MIC
1	25.76 ± 1.15	62.5	0.41	16	31.25	0.82	8	NA	NA	NA	NA	NA	NA
4	24.21 ± 1.45	1000	0.02	NT	62.5	0.39	16	NA	NA	NA	NA	NA	NA
6	246.60 ± 1.03	125	1.97	2	62.5	3.95	8	31.25	7.86	4	15.62	15.79	2
7	95.49 ± 1.34	500	0.19	2	250	0.38	4	31.25	3.05	4	31.25	3.06	2
8	68.23 ± 1.03	31.25	2.18	2	31.25	2.18	8	15.62	4.36	2	7.81	8.74	2
Cefepime	>1000	0.488	nc	1	0.488	nc	2	0.122	nc	2	0.122	nc	2

nc – non cytotoxic in tested concentrations.

incubation, the inhibitory effect of tested compounds and Cisplatin was evaluated using SRB assay according to manufacturer protocol (Sigma Aldrich). Due to the fact that compounds which possess anticancer activity are often very toxic towards normal cells, the selectivity index (SI) was determined. It was defined as the ratio of IC_{50} of compounds in normal cells versus cancer cells [41–43]. Cisplatin was used as a reference drug [44].

2.4.4. α-glucosidase inhibition

The α-glucosidase inhibitory activity was examined according to Elya et al. [45] with some modifications, by measuring the release of *p*-nitrophenol from substrate *p*-nitrophenyl α-D-glucopyranoside (*p*-NPGP) (Sigma Aldrich). Acarbose (Sigma Aldrich) was used as a positive drug control. IC_{50} values were calculated by the graphic method

2.5. Statistical analysis

All *in vitro* experiments were performed in three independent measurements. The results were presented as mean values ± standard deviation (SD) or as mean values ± standard error of the mean (S.E.M). The data were analyzed using unpaired Students *t*-test. Differences were considered as significant with $P < 0.05$ (GraphPad Prism 5, Version 5.04 Software). The values of CC_{50} and IC_{50} were calculated via 4-parameter nonlinear regression analyses using GraphPad Prism 5, version 5.04.

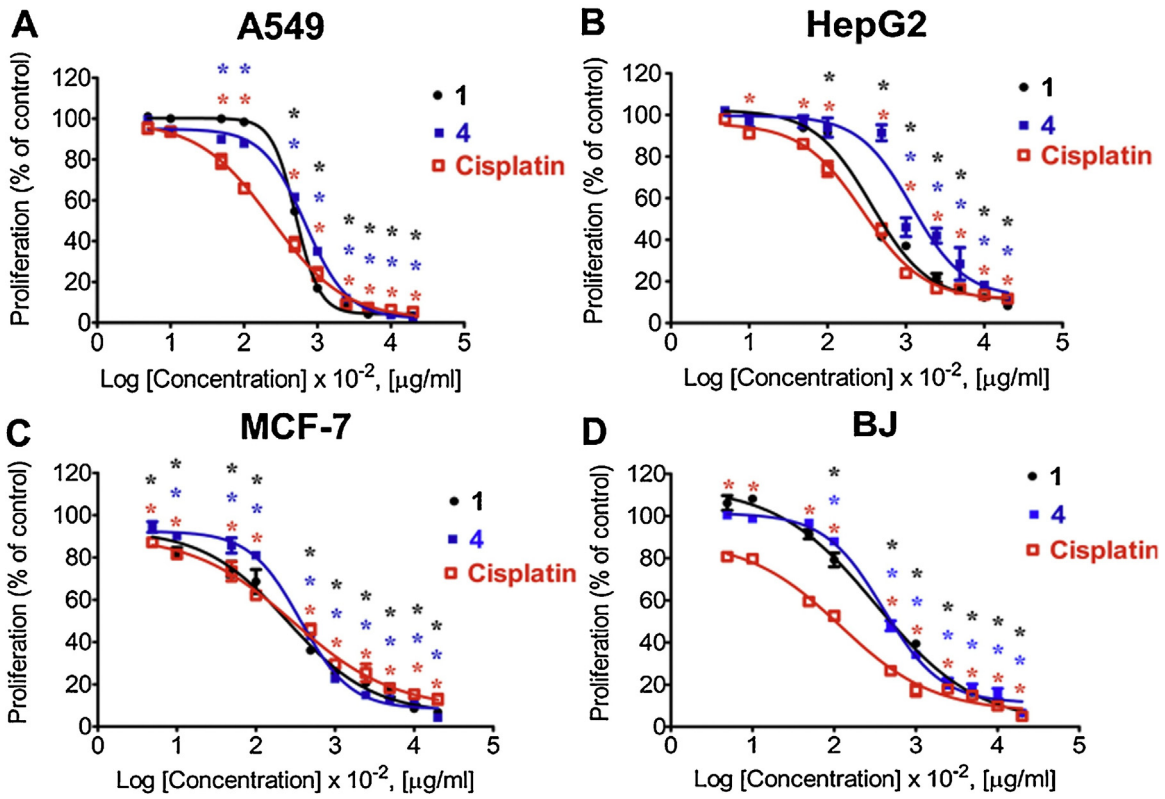

Fig. 2. Antiproliferative activity of derivatives **1**, **4** and Cisplatin on cancer cells: human lung adenocarcinoma (A), human hepatocellular carcinoma (B), human breast adenocarcinoma (C) and normal cells: normal human skin fibroblasts (D) after 96-h incubation. The results were presented as mean values ± S.E.M. from three separate experiments. *Statistically significance obtained at $P < 0.05$ compared to the control (unpaired Students t-Test).

Table 3
The IC_{50} and SI of **1, 4** derivatives and Cisplatin on cancer cell lines (A549, HepG2, MCF-7) and normal cell line (BJ) after 96-h incubation. The IC_{50} values were expressed as mean values ± SD from three separate experiments. IC_{50} – inhibitory concentration that caused 50% inhibition of cell proliferation; SI – the ratio between IC_{50} on normal cell line and IC_{50} on cancer cell line.

Comp.	$IC_{50} \pm SD$ [μg/mL]				SI		
	BJ	A549	HepG2	MCF-7	BJ/ A549	BJ/ HepG2	BJ/ MCF-7
1	2.87 ± 1.10	4.96 ± 1.96	3.64 ± 1.18	2.53 ± 1.21	0.58	0.79	**1.13**
4	3.09 ± 1.19	6.80 ± 1.25	11.88± 3.91	3.62 ± 1.07	0.45	0.26	0.85
Cisplatin	1.19 ± 0.56	2.38 ± 1.16	2.82 ± 1.79	2.97 ± 1.33	0.5	0.42	0.40

2.6. Molecular modeling

2.6.1. Homology modeling

Homology modeling was applied to construct molecular model of α-glucosidase from Saccharomyces cerevisiae (UniProt entry P53341) in complex with glucose. Sequence alignment was performed using Muscle software [46]. Homology modeling was performed using Modeller v. 9.17 [47]. X-ray structure of α-glucosidase from Geobacillus sp. HTA-462 (PDB ID: 2ZE0 [48]) was used as a template (sequence identity 40%). The glucose moiety was transferred to the enzyme catalytic site from the sucrose mutase-sucrose complex (PDB ID: 2PWE [49]). A population of 100 homology models was generated and subsequently assessed by the Modeller objective function and Discrete Optimized Protein Energy profiles [50]. The best model was subjected to quality assessments using the Schrödinger software module for Ramachandran plots.

2.6.2. Compound preparation

The investigated compounds were modeled using the LigPrep protocol from the Schrödinger Suite [51]. In order to sample different protonation states of ligands in physiological pH, Epik module was used with default settings [52].

2.6.3. Molecular docking

Molecular docking was performed using Glide from the Schrödinger suite of software [53]. The grid file was generated indicating glucose as a reference ligand. Molecular docking was performed using the SP (standard precision) protocol of Glide. 100 poses were generated for each ligand. PyMol v. 0.99 [54] was used for visualization of results.

3. Results and discussion

3.1. Chemistry

The starting reagents for the synthesis of thiosemicarbazides **1–9** were 2-, 3-, 4-pyridyl carboxylic acid hydrazide or 4-nitrophenyl carboxylic acid hydrazide. These compounds were obtained by the reaction of appropriate ester with hydrazine hydrate in anhydrous ethanol by the procedure described earlier [31]. The title compounds (**1–9**) were prepared in reaction of carboxylic acid hydrazide with isothiocyanate using methanol as a solvent (Scheme 1). All the synthesized compounds were characterized by spectroscopic methods. ^{1}H NMR spectra of thiosemicarbazide derivatives exhibited proton signals typical of the NH group in the range of 7.98–11.93 ppm. Also, in the IR spectra the signals from the group C=O and C=S are observed in the range 1600–1800 cm^{-1}. The above observations suggest that the compounds exist mainly in the thione form. This is consistent with the literature data [9,11].

For derivative **4** as a model compound X-ray structure determination was carried out It was performed in order to confirm the synthesis pathway, assumed molecular structure of **4** and identification of its tautomeric form in the crystalline phase. This compound crystallizes with the methanol solvent molecule lying on special position with twofold rotation symmetry. The structure and conformation of the molecule **4** in the crystal is shown in Fig. 1. In the crystalline state, the molecule exists in N1-amino/N3-amino/N4-amino/S2-thione/O5-keto tautomeric form, as evidenced by the C2–S2 and C5–O5 bond length of 1.213(3) and 1.684(2) Å, respectively, typical for the carbonyl and thione group [55]. Moreover, the positions of the amino-H atom are localized at the difference electron-density map in the vicinity of N1, N3 and N4 atoms. The carbonylthiosemicarbazide part of the molecule adopt *trans-cis-gauche-trans* conformation with the C11–N1–C2–N3, N1–C2–N3–N4, C2–N3–N4–C5 and N3–N4–C5–C51 of 174.2(2), −8.6(3), 103.9(2) and 171.73(18)°, respectively. The torsion angle O5–C5–C51–C56 of −20.6(4)° shows that the carbonyl group is slightly distorted from coplanarity with the benzene ring, similarly as disordered nitro group with the torsion angles C55–C54–N6–O61 of 6.0(5)°, C55–C54–N6–O62A of −152.8(4)° and C55–C54–N6–O62 B of 152.9(7)°. The thione C2=S2 group has *trans* and *cis* conformation with respect to N3–N4 and N1–C11 bonds, respectively; the respective torsion angles are 172.38(16) and

Table 4
Probability to inhibit (P_a) and not to inhibit (P_i) α-glucosidase calculated using software PASS.

Compound	P_a	P_i
1	0.919	0.009
2	0.901	0.012
3	0.915	0.010
4	0.886	0.014
5	0.777	0.034
6	0.927	0.009
7	0.926	0.009
8	0.915	0.010
9	0.900	0.012

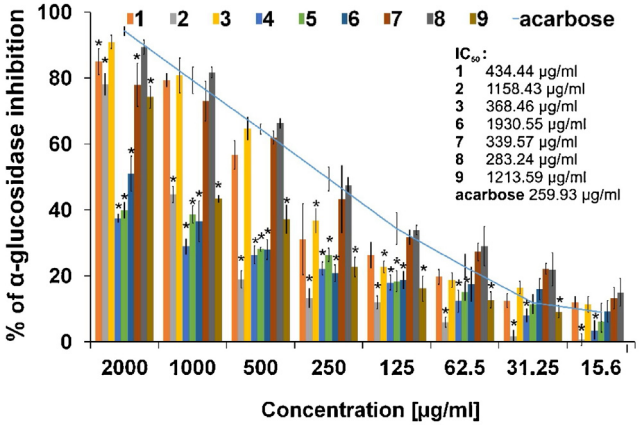

Fig. 3. The α-glucosidase inhibitory activity and estimation of IC_{50} of nitro derivatives and reference compound acarbose. The results were presented as mean values ± SD from three separate experiments. *Statistically significance obtained at $P < 0.05$ compared to the control (unpaired Students t-Test).

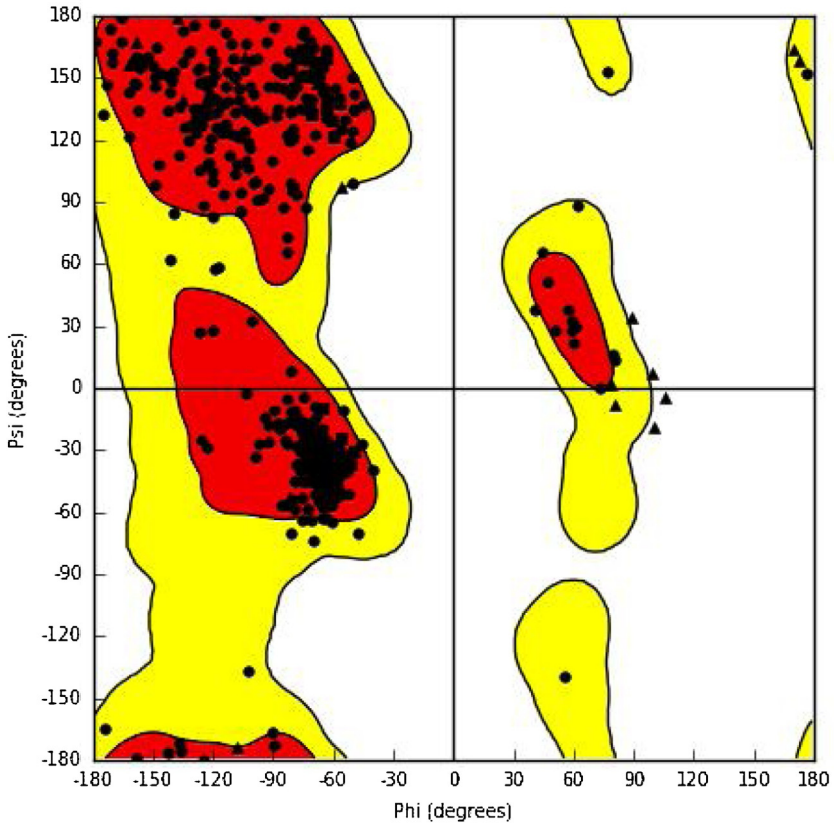

Fig. 4. Ramachandran plot for homology model of yeast α-glucosidase. Triangles denote glycine residues, squares – proline residues and circles – all other residues.

−6.9(4)°. The cyclohexyl ring adopts a chair conformation with puckering parameters of Q = 0.554(4) Å and θ = 178.3(4)° [56]. Other details can be found in Supplementary material.

3.2. Antibacterial activity

All newly synthesized compounds were tested for their antibacterial activity by a modified disc diffusion method. The main inhibition zones of both aerobic and microaerobic Gram-positive bacteria caused by thiosemicarbazides were found in a range of 13 mm to 23 mm (Table 1). Among tested substances, derivatives **6–8** had good activity against aerobic and microaerobic Gram-positive bacteria. Thus, the most active compounds were the thiosemicarbazides with

4-nitrophenyl group substituted in the position 1 and possessing in position 4 the following substituents: 2-chlorophenyl (**6**), 4-nitrophenyl (**7**) or 2,4-dichlorophenyl (**8**). This activity increases in a series 2-ClC_6H_4 < 4-$NO_2C_6H_5$ < 2,4-$Cl_2C_6H_3$ for *S. mutans* and *S. sanguinis*. For *S. aureus* and *S. epidermidis* the increase in activity is as follows: 4-$NO_2C_6H_5$ < 2-ClC_6H_4 < 2,4-$Cl_2C_6H_3$.

The minimum inhibitory concentration (MIC) was determined for each derivatives which exhibited the bacterial growth inhibition zones. Table 2 presents MIC values of the most potent molecules with good antibacterial activity. The MIC assay clearly confirmed results obtained during modified disc diffusion test and showed that compounds **6-8** (R^1 = 4-$NO_2C_6H_5$ and R^2 = 2-ClC_6H_4, 4-$NO_2C_6H_5$ or 2,4-$Cl_2C_6H_3$) had moderate to good activity against most of the tested bacteria. Compounds **6-8** display good inhibitory effect against tested microaerobic Gram-positive strains with MICs of 7.81–31.25 μg/mL. In turn, aerobic Gram-positive pathogens were well inhibited by **1** 4-(4-nitrophenyl)-1-(pyridine-2-yl)carbonylthiosemicarbazide (**1**), 4-(2-chlorophenyl)-1-(4-nitrophenyl)carbonylthiosemicarbazide (**6**) and 4-(2,4-dichlorophenyl)-1-(4-nitrophenyl)carbonylthiosemicarbazide (**8**) with MIC values ranging from 15.79 μg/mL to 125 μg/mL. It is known that an antibacterial agent is considered bacteriostatic when the MBC/MIC ratio is ≤ 4 while being bactericidal when the MBC/MIC ratio is > 4 [57]. According to the data summarized in Table 2 we found that thiosemicarbazides **6-8** showed bactericidal activity against *S. aureus*, *S. mutans* and *S. sanguinis*. The MBC values against *S. epidermidis* for compounds **6-8** and also **1** (R^1 = 2-pyridine and R^2 = 4-$NO_2C_6H_5$) were determined to be at least 4-fold higher than the MIC values, what confirms that compounds were bacteriostatic. However, cefepime remains the most active antimicrobial drug (MIC 0.122 μg/mL – 0.976 μg/mL) with high IB (1.08 to 34.6)

3.3. Cytotoxic activity

The compounds which exhibited antibacterial activity (**1, 4, 6, 7, 8**) were assessed for their cytotoxic activity against normal human skin fibroblast (BJ cell line) using MTT assay. The 1,4-disubstituted thiosemicarbazide derivatives showed different cytotoxic activities with CC_{50} values ranging from 24.21 to 246.60 μg/mL (Table 2). Among all tested compounds, the 4-cyclohexyl-1-(4-nitrophenyl) carbonylthiosemicarbazide (**4**) exhibited the highest cytotoxic activity with a CC_{50} value of 24.21 μg/mL. On the other hand, the 4-(2-chlorophenyl)-1-(4-nitrophenyl)-carbonylthiosemicarbazide (**6**) showed the lowest cytotoxic activity in comparison with the tested compounds. It is worth noting that compounds **4** and **6** possessed 4-$NO_2C_6H_4$ group at position 1 and C_6H_{11} (**4**) group and 2-ClC_6H_4 (**6**) group at position 4, respectively. The results suggest that the presence of 2-ClC_6H_4 substituted in the 4 position of

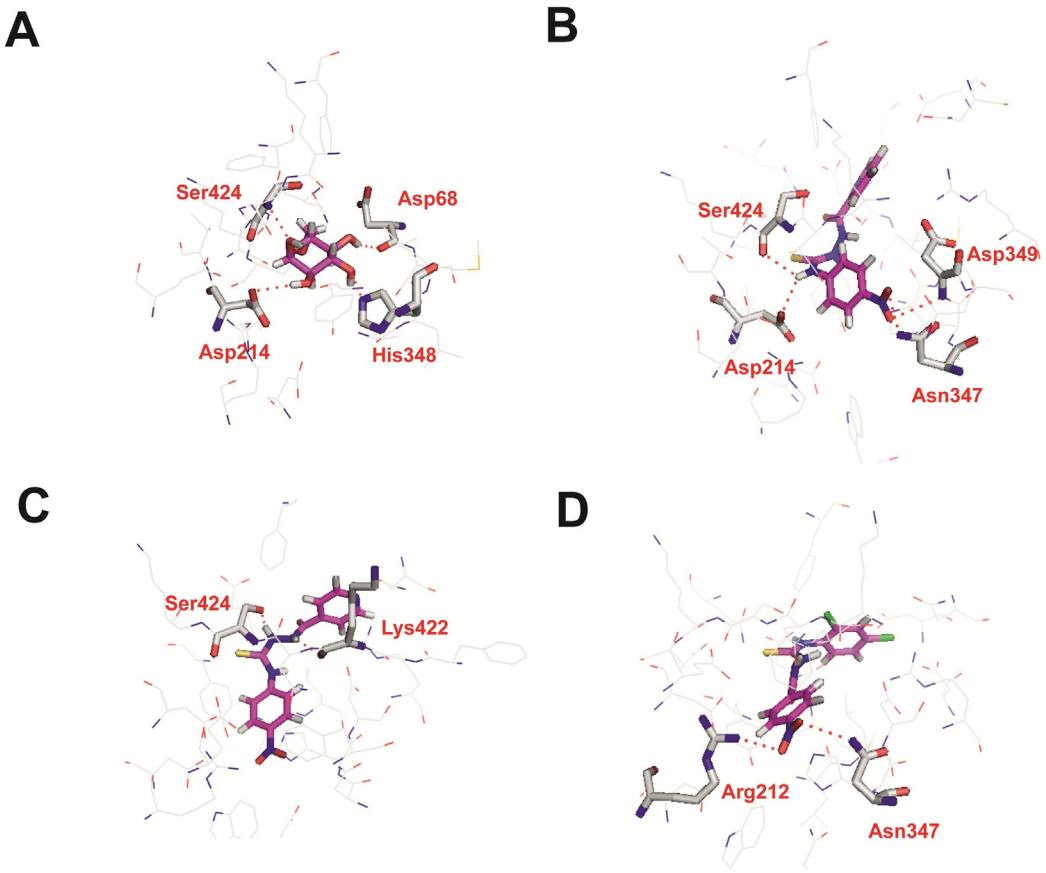

Fig. 5. Interactions of glucose and compounds **1**, **3** and **8** with yeast α-glucosidase. Protein shown with grey carbon atoms in wire representation while the residues forming hydrogen bonds (denoted as red dashed lines) are shown as sticks. Ligands shown as sticks with magenta carbon atoms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

thiosemikarbazide greatly increases cytotoxic activity of this derivatives. In order to estimate the in vitro therapeutic safety and efficacy of synthesized compounds, the therapeutic index (TI) was determined and summarized in Table 2. The high value of therapeutic index (TI) indicates that the tested compound is effective against bacterial strain and safe for eukaryotic cells Thus, the TI value below 1 determines a lack of therapeutic safety whereas TI value higher than 10 allows to perform in vivo evaluation [40,58,59]. Among the tested compounds, three of them (**6**, **7**, **8**) showed the TI values higher than 1. Nevertheless, compound **6** had the highest TI values against *S. epidermidis* (3.95), *S. sanguinis* (7.86) and *S. mutans* (15.79). Thus, derivative **6** is a promising candidate for treatment of *S. mutans* infections and due to excellent TI value can be administered to perform in vivo evaluation. Also in the case of cytotoxicity tests, the results obtained for cefepime were most favorable among all tested substances. Cefepime was non cytotoxic in tested concentration range (1000 μg/mL).

3.4. Antiproliferative activity

Among the synthesized compounds, 4-(4-nitrophenyl)-1-(pyridine-2-yl)carbonylthiosemi carbazide (**1**) and 4-cyclohexyl-1-(4-nitrophenyl)carbonylthiosemicarbazide (**4**) exhibited antiproliferative potency against cancer cells in a dose-dependent manner (Fig. 2A–C). Since the normal skin fibroblasts are widely used as a standard model of normal cells [60–62] in our study the BJ cells were used as a control of normal cells (Fig. 2D). In order to evaluate the antiproliferative selectivity of synthesized compounds towards cancer cells versus normal cells, the IC_{50} and SI values were calculated and summarized in Table 3. The SI value above 1 indicates that compound exhibited greater inhibition of cancer cells compared to normal cells. It was observed only for MCF-7 cells after incubation with derivative **1** (R^1 = 2-pyridyl and R^2 = 4-$NO_2C_6H_5$). Moreover, it should be noted that derivative **1** possessed antiproliferative selectivity greater than Cisplatin against all tested anticancer cells. The results indicate that thiosemicarbazide chain substituted in position 1 by pyridin–2 yl group and in position 4 by 4-$NO_2C_6H_4$ group cause that compounds possess high antiproliferative activity, which is more selective to cancer cells than to normal cells.

3.5. Inhibition of α-glucosidase activity in vitro and in silico

In order to check if the studied compounds may exhibit α-glucosidase inhibitory activity, PASS software was used [63]. The obtained results are shown in Table 4. It can be seen that all compounds except for compounds **4** (R^1 = 4-$NO_2C_6H_5$ and R^2 = C_6H_{11}) and **5** (R^1 = 4-$NO_2C_6H_5$ and R^2 = C_4H_9) have the probability to inhibit α-glucosidase equal or above 0.9. Due to results obtained using PASS software, it was decided to check inhibition of α-glucosidase by the studied compounds in vitro. The α-glucosidase from *S. cerevisiae* was used to demonstrate the inhibitory activity of new nitro compounds as previously reported [17]. The inhibitory activity of the compounds against α-glucosidase was tested using *p*-NPGP as a substrate and this was compared with a reference drug – acarbose. To determine the α-glucosidase inhibition ability in vitro IC_{50} values were

calculated. As can be observed in Fig. 3, the *in vitro* assay of α-glucosidase confirmed the results predicted using PASS software. The IC_{50} values of tested derivatives ranged from 283.24 μg/mL to 1930.55 μg/mL. Compound **8** was the most active (IC_{50} of 283,24 μg/mL), followed by **7** (with IC_{50} of 339.57 μg/mL), **3** (with IC_{50} of 368,46 μg/mL) and **1** (with IC_{50} of 434.44 μg/mL). There were no compounds which had IC_{50} lower than acarbose, but compounds **8**, **1** and **3** possess similar inhibitory activity to a reference compound acarbose on α-glucosidase in *in vitro* test.

In order to study the molecular interactions of most active compounds **1**, **3** and **8** with yeast α-glucosidase, we performed molecular docking. First homology model of yeast α-glucosidase was constructed using X-ray structure of α-glucosidase form Geobacillus sp. HTA-462 (PDB ID: 2ZE0 [45]) as a template. The quality of the homology model was confirmed by means of Ramachandran plot presented in Fig. 4. The interactions of yeast α-glucosidase with glucose as well as the results of molecular docking of compounds **1**, **3** and **8** to yeast α-glucosidase are presented in Fig. 5. The mode of interaction of glucose with yeast α-glucosidase resembles interactions reported for the form Geobacillus sp. HTA-462 (PDB ID: 2ZE0 [45]) was used as a template. Glucose forms hydrogen bond with side chains of Asp214 and His348 and with the main chain of Asp68 and Ser424. The mode of interaction of compounds **1**, **3** and **8** is similar. Compound **1** (4-(4-nitrophenyl)-1-(pyridine-2-yl)carbonylthiosemicarbazide) hydrogen-bonds to side chains of Asp214 and Asn347 and to the main chain of Asp349 and Ser424. Compound **3** (4-(4-nitrophenyl)-1-(pyridin-4-yl)carbonylthiosemicarbazide) interacts via hydrogen bond with the main chain of Lys422 and Ser 424. Finally, compound **8** (4-(2,4-dichlorophenyl)-1-(4-nitrophenyl)carbonylthiosemicarbazide**)** forms hydrogen bonds with side chains of Arg212 and Asn347.

4. Conclusion

In conclusion we obtained a series of new thiosemicarbazide derivatives with 4-nitrophenyl group. Good antibacterial activity is displayed by comounds containing 4-nitrophenyl group in the position 1 and 2-chlorophenyl (**6**) or 4-nitrophenyl (**7**) or 2,4-dichlorophenyl (**8**) group in the thiosemicarbazide chain in position 4. The most active compound (**8**) exhibited inhibitory effect against *S. mutants* with MIC value 7.81 μg/mL. It was confirmed that all compounds were bacteriostatic. Two compounds (**1**, **4**) exibited antiproliferative potency. 4-(4-Nitrophenyl)-1-(pyridine-2-yl)carbonylthiosemicarbazide (**1**) possess antiproliferative potential bigger than Cisplatin against tested anticancer celles. Compounds **1**, **3** and **8** possess similar inhibitory activity to a reference compound acarbose on α-glucosidase in *in vitro* test. Molecular docking for most active compounds to yeast α-glucosidase was performed and molecular interactions were determined.

Declaration of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biopha.2017.07.049.

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