Clinical and Experimental Health Sciences

Synthesis, Characterization and Biological Evaluation of Novel Thiourea Derivatives

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Received: 25.02.2022 **Accepted:** 09.03.2022

ABSTRACT

Objective: A new series of 4-[3-(substitutedphenyl)thioureido]-*N*-(6-chloropyrazin-2-yl)benzenesulfonamide were synthesized from sulfaclozine.

Methods: All compounds were characterized by IR, ^{1}H-NMR spectroscopic methods and elemental analysis. In addition to the antioxidant activity of the synthesis series, enzyme inhibition activities such as anticholinesterase, tyrosinase, α-amylase and α-glycosidase were determined for the first time in this study.

Results: According to these biological activity test results, compound **2a** in the DPPH, **2c** in the $ABTS^{\cdot+}$ assay exhibited more antioxidant activity than reference standard. All thiourea derivatives demonstrated good BChE inhibitory activity than galantamine. Among the compounds, **2e** and **2f** showed the best tyrosinase enzyme inhibition activity, while **2g** had the best α-amylase and α-glucosidase enzyme inhibition activity. In addition, we evaluated the druglikeness properties of compounds and their oral bioavailability were also found to be high.

Conclusion: Thiourea derivatives exhibited remarkable antioxidant activity and enzyme inhibition activity against tyrosinase, cholinesterase, α-amylase and α-glucosidase.

Keywords: Thiourea, antioxidant, anticholinesterase, tyrosinase, α-amylase.

1. INTRODUCTION

Thiourea functional groups have numerous pharmacological activities such as antidiabetic, antibacterial, antifungal, antiviral, anticancer, anticonvulsant, antidiabetic (1-3). Thioureas play an important role in the regulation of various pharmacological activities by increasing potency and selectivity or by modulating of physicochemical properties. The pharmacological activity of thioureas results from specific interactions between proteins, enzymes, receptor targets and drugs. For example, the protons on the two nitrogens act as hydrogen bond donors, while the C=S fragment of thiourea acts as a hydrogen bond acceptor (4). Therefore, thiourea compounds are widely used in the search for new drug candidates (5). An another biologically active group is also sulfonamides (6). There are many active pharmaceutics used in different activities due to sulfonamide structure (7). For example, sulfadizine (antibacterial), darunavir (antiviral), celecoxib (anti-inflammatory), clopamide (diuretic), zonisamide (anticonvulsant) (8).

Kollu et al. demonstrated high antioxidant activities of thioureas after DPPH and FRAP experiments (9). In another study, Yiğit et al. indicated that compounds with thiourea structure show better activity than the reference drug tacrine against enzymes such as achethylcholinesterase (AChE) and butyrylcholinesterase (BChE) (10). Not only but also antidiabetic activity of sulfonamides and thioureas were reported in the literature (11,12). In our previous study, we also demonstrated the high anticancer activities of compounds with both thiourea and sulfonamide structures (13).

Keeping in view of significant biological activities of thiourea derivatives, we aimed to synthesize and show different biological activities of some novel thiourea derivatives. For this purpose, all synthesized compounds investigated for their

DOI: 10.33808/clinexphealthsci.1062872

antioxidant activity by DPPH, ABTS, β-carotene-linoleic acid and CUPRAC method, antidiabetic activity with α-amylase and α-glucosidase inhibitory assays, anticholinesterase activity against AChE and BChE, tyrosinase inhibitory activity.

2. METHODS

Sulfaclozine sodium monohydrate was granted by Medicavet A.Ş. All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA). To monitor the reaction progress and determine the purities of the synthesized compounds were used thin-layer chromatography (TLC) (Merck, Darmstadt, Germany). Melting points were determined by SMP II melting point apparatus (Cole-Parmer Ltd. Staffordshire, UK). IR spectra were recorded on a Schimadzu FTIR-8400S spectrophotometer (Shimadzu Corp., Kyoto, Japan). ^{1}H-NMR spectra were recorded on a Bruker Avance DPX-400 spectrometer (Billerica, MA, USA). Tetramethylsilane as the internal standard and DMSO-d_6 as the solvent were used for ^{1}H-NMR spectrum. Elemental analyses were performed with GmbH varioMICRO CHNS (Langenselbold, Germany).

2.1. Chemistry

General procedure for the preparations of thioureas (**2a**-**2h**)

Firstly, sulfaclozine sodium monohydrate (1 mmol) was dissolved in 3 mL of water. When HCl (5%) was added dropwise, sulfaclozine began to precipitate and the precipitate was washed with plenty of water.

Then, sulfaclozine (1 mmol) was dissolved in anhydrous acetone (10 mL). 1 mmol of substituted phenylisothiocyanate was added on it. The mixture was refluxed for 10 hours. Excess solvent was evaporated under vacuum. The precipitated product was crystallized from methanol (14).

4-amino-*N*-(6-chloropyrazin-2-yl)benzenesulfonamide (**1**)

Yield: 85%; m.p. 235.2-235.3 °C. IR (n, cm^{-1}): 3348, 3234 (N-H), 3028 (aromatic =C-H), 1633 (C=N), 1325, 1139 (SO_2), 1089 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 6.17 (s, 2H, – NH_2), 6.60 (d, *J*=8.8 Hz, 2H, Ar-H), 7.65 (dd, *J*=8.6 Hz, 2H, Ar-H), 8.28 (d, *J*=16.4 Hz, 2H, pyrazine protons), 11.52 (s, 1H, – SO_2NH). Anal. Calcd for $C_{10}H_9ClN_4O_2S$: C 42.18, H 3.19, N 19.68. Found: C 42.23, H 3.19, N 19.67 %.

N-(6-Chloropyrazin-2-yl)-4-(3-phenylthioureido) benzenesulfonamide (**2a**)

Yield: 75%; m.p. 202.3-202.5 °C. IR (n, cm^{-1}): 3255 (N-H), 3043 (aromatic =C-H), 1585 (C=N), 1338, 1157 (SO_2), 1240 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.15 (s, 1H, Ar-H), 7.42 (m, 4H, Ar-H), 7.84 (m, 4H, Ar-H), 8.34 (d, *J*=19.5, 2H, pyrazine protons), 10.17 (d, *J*=19.2 Hz, 2H, NH), 11.90 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{14}ClN_5O_2S_2$: C 48.63, H 3.36, N 16.68. Found: C 48.43, H 3.35, N 16.74 %.

N-(6-Chloropyrazin-2-yl)-4-(3-[4-fluorophenyl]thioureido) benzenesulfonamide (**2b**)

Yield: 78%; m.p. 249.5-249.9 °C. IR (n, cm^{-1}): 3205 (N-H), 3028 (aromatic =C-H), 1599 (C=N), 1325, 1149 (SO_2), 1226 (C=S), 1091 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.20 (d, *J*=8.4 Hz, 2H, Ar-H), 7.46 (s, 2H, Ar-H), 7.84 (m, 4H, Ar-H), 8.34 (d, *J*=19.8 Hz, 2H, pyrazine protons), 10.15 (d, *J*=36.6 Hz, 2H, NH), 11.92 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{13}ClFN_5O_2S_2$: C 46.63, H 2.99, N 15.99. Found: C 46.81, H 3.01, N 15.92 %.

4-[3-(4-Chlorophenyl)thioureido]-*N*-(6-chloropyrazin-2-yl) benzenesulfonamide (**2c**)

Yield: 70%; m.p. 173.0-173.4 °C. IR (n, cm^{-1}): 3205 (N-H), 3020 (aromatic =C-H), 1599 (C=N), 1323, 1151 (SO_2), 1230 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.46 (m, 4H, Ar-H), 7.80 (m, 4H, Ar-H), 8.35 (d, *J*=19.3 Hz, 2H, pyrazine protons), 10.24 (d, *J*=23.6 Hz, 2H, NH), 11.93 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{13}Cl_2N_5O_2S_2$: C 44.94, H 2.88, N 15.41. Found: C 45.19, H 2.92, N 15.33 %.

4-[3-(4-Bromophenyl)thioureido]-*N*-(6-chloropyrazin-2-yl) benzenesulfonamide (**2d**)

Yield: 80%; m.p. 189.5-189.9 °C. IR (n, cm^{-1}): 3205 (N-H), 3030 (aromatic =C-H), 1599 (C=N), 1325, 1141 (SO_2), 1230 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.54 (m, 4H, Ar-H), 7.69 (m, 2H, Ar-H), 7.89 (t, 2H, Ar-H), 8.34 (d, *J*=19.1, 2H, pyrazine protons), 10.23 (d, *J*=19.2 Hz, 2H, NH), 11.93 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{13}BrClN_5O_2S_2$: C 40.93, H 2.63, N 14.04. Found: C 40.81, H 2.62, N 14.10 %.

N-(6-Chloropyrazin-2-yl)-4-(3-*p*-tolylthioureido) benzenesulfonamide (**2e**)

Yield: 83%; m.p. 186.3-186.7 °C. IR (n, cm^{-1}): 3194 (N-H), 3012 (aromatic =C-H), 1593 (C=N), 1336, 1149 (SO_2), 1228 (C=S), 1089 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 2.30 (s, 3H, CH_3), 7.25 (m, 4H, Ar-H), 7.84 (m, 4H, Ar-H), 8.34 (d, *J*=19.1 Hz, 2H, pyrazine protons), 10.08 (d, *J*=16.0 Hz, 2H, NH), 11.91 (s, 1H, – SO_2NH). Anal. Calcd for $C_{18}H_{16}ClN_5O_2S_2$: C 49.82, H 3.72, N 16.14. Found: C 49.99, H 3.71, N 16.19 %.

N-(6-Chloropyrazin-2-yl)-4-[3-(4-methoxyphenyl)thioureido] benzenesulfonamide (**2f**)

Yield: 82%; m.p. 166.3-166.7 °C. IR (n, cm^{-1}): 3211 (N-H), 3007 (aromatic =C-H), 1583 (C=N), 1336, 1151 (SO_2), 1236 (C=S), 1089 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 3.77 (s, 3H, OCH_3), 6.96 (d, *J*=8.8 Hz, 2H, Ar-H), 7.37 (d, *J*=8.8 Hz, 2H, Ar-H), 7.83 (m, 4H, Ar-H), 8.30 (d, *J*=19.1 Hz, 2H, pyrazine protons), 10.00 (d, *J*=25.8 Hz, 2H, NH), 11.91 (s, 1H, – SO_2NH). Anal. Calcd for $C_{18}H_{16}ClN_5O_3S_2$: C 48.05, H 3.58, N 15.57. Found: C 48.19, H 3.60, N 15.49 %.

N-(6-Chloropyrazin-2-yl)-4-(3-(4-ethylphenyl)thioureido) benzenesulfonamide (**2g**)

Yield: 75%; m.p. 183.9-184.4 °C. IR (n, cm^{-1}): 3205 (N-H), 3022 (aromatic =C-H), 2962 (C-H), 1583 (C=N), 1338, 1138 (SO_2), 1230 (C=S), 1087 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 1.13 (t, 3H, CH_2CH_3), 2.43 (q, 2H, CH_2CH_3), 7.26 (m, 4H, Ar-H), 7.77 (m, 2H, Ar-H), 7.88 (m, 2H, Ar-H), 8.31 (d, *J*=19.5, 2H, pyrazine protons), 10.09 (d,

J=18.6 Hz, 2H, NH), 11.90 (s, 1H, – SO_2NH). Anal. Calcd for $C_{19}H_{18}ClN_5O_2S_2$: C 50.94, H 4.05, N 15.63. Found: C 51.08, H 4.02, N 15.66 %.

N-(6-Chloropyrazin-2-yl)-4-[3-(2,6-dichlorophenyl) thioureido]benzenesulfonamide (**2h**)

Yield: 73%; m.p. 148.3-148.6 °C. IR (n, cm^{-1}): 3211 (N-H), 3010 (aromatic =C-H), 1585 (C=N), 1336, 1149 (SO_2), 1232 (C=S), 1084 (aromatic C-Cl). ^{1}H-NMR (400 MHz, DMSO-d_6, ppm): *d* 7.37 (m, 1H, Ar-H), 7.60 (t, 2H, Ar-H), 7.91 (m, 4H, Ar-H), 8.31 (d, *J*=20.2 Hz, 2H, pyrazine protons), 10.44 (d, *J*=25.8 Hz, 2H, NH), 11.94 (s, 1H, – SO_2NH). Anal. Calcd for $C_{17}H_{12}Cl_3N_5O_2S_2$: C 41.77, H 2.47, N 14.33. Found: C 41.88, H 2.48, N 14.70 %.

2.2.Biological Activity

The all experiments were carried out in triplicate. The DMSO was used as a negative control to follow the reaction. The bleaching rate was calculated from the absorbance's differences versus time. The sample concentration providing 50% inhibition activity (IC_{50}) for other all assays while 0.5 absorbance ($A_{0.5}$) for CUPRAC assay were calculated from the graph of bleaching rate (%) against sample concentrations. All biological activity measurements were using a 96-well microplate reader (SpectraMax 340PC^{384}, Molecular Devices, USA).

In vitro Antioxidant Activity

The antioxidant activity of thiourea derivatives **2a-2h** was determined using four complimentary assays, namely, β-carotene bleaching method, DPPH free radical scavenging activity, ABTS cation radical scavenging activity, and cupric reducing antioxidant capacity (CUPRAC). The αlpha-tocopherol (α-TOC) and butylatedhydroxytoluene (BHT) were used as standard to compare the activity.

Determination of the lipid peroxidation inhibitory activity of the thiourea derivatives

The lipid peroxidation inhibitory activity was evaluated using the β-carotene-linoleic acid model assay system (15) with slight changes. The method is based on the occurrence of lipid peroxidation from linoleic acid in singlet oxygen saturated water. Then the radical degrades the color of b-carotene followed using 470 nm wavelength. The more color exhibits more powerful lipid peroxidation inhibitor capacity. Briefly, the reactive was prepared by mixing β-carotene (0.5 mg) in 1 mL of chloroform was added to 25 µL of linoleic acid, and 200 mg of Tween 40 emulsifier in a bottle. After chloroform evaporated, the mixture was dissolved using 100 mL pure water saturated with singlet oxygen. Briefly, 160 mL of prepared reactive was mixed with 40 mL of thiourea derivatives **2a-2h** dissolved in DMSO at different concentrations. The zero-time absorbance was measured at 470 nm, and the measurement was done in every 30 minutes up to the absorbance of control reduces under 0.1 absorbance in 96 well plate cell length. The measurement took almost 2 hours.

Determination of DPPH free radical scavenging activity of the thiourea derivatives

The DPPH free radical scavenging activity was performed according to Blois (16), with slight modifications. Briefly, 160 mL of 0.004% of DPPH in ethanol was mixed with 40 mL of thiourea derivatives **2a-2h** dissolved in ethanol at different concentrations. The absorbance was measured at 517 nm after 30 min incubation in dark.

Determination of ABTS cation radical scavenging activity of the thiourea derivatives

The ABTS cation radical scavenging activity was performed according to Re et al. (17), with slight modifications (18). The ABTS cation radical was prepared using the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, and then stored in the dark at room temperature for 12 h. Before usage, the occured ABTS cation radical solution was diluted 1:88 ration with ethanol to get 0.700 ± 0.025 absorbance at 734 nm. Then briefly, 160 mL of prepared reactive was mixed with 40 mL of thiourea derivatives **2a-2h** dissolved in DMSO at different concentrations. The absorbance was measured at 734 nm after 10 min incubation.

Determination of Cupric reducing antioxidant capacity of the thiourea derivatives

Cupric reducing antioxidant capacity (CUPRAC) assay was performed according to Apak et al. (19). Briefly, 50 µL 10 mM Cu (II), 50 µL 7.5 mM neocuproine, and 60 µL NH_4Ac buffer (1 M, pH 7.0) were added to each well in a 96 well plate. To the mixture, 40 µL of thiourea derivatives **2a-2h** at various concentrations were added. After 1 h, incubation absorbance was recorded at 450 nm.

In vitro Enzyme Inhibitory Activities

Galantamine for anticholinesterase, kojic acid and L-mimosine for tyrosinase, acarbose for α-amylase and α-glucosidase were used as positive standard to compare the inhibitory activity.

Determination of anticholinesterase activity of the thiourea derivatives

The acetylcholinesterase, (AChE, Type-VI-S, EC 3.1.1.7, 425.84 U/mg), enzyme obtained from electric eel, was bought ready-made from Sigma aldrich by the suppliers in Turkey. Horse reddish butyrylcholinesterase (BChE, EC 3.1.1.8, 11.4 U/mg) were used to determine the anticholinesterase activity of DMSO thiourea derivatives **2a-2h** where acetylthiocholine iodide and butyryl-thiocholine chloride were employed as substrates using spectroscopic method (20). Briefly, 130 mL sodium phosphate buffer (100 mM, pH 8.0), 10 mL thiourea

derivatives **2a-2h** at different concentrations, and 20 mL AChE or BChE enzymes in buffer were mixed. After incubation for 15 min at 25 °C, 20 mL 0.5 mM DTNB (5,50-dithiobis (2-nitrobenzoic acid) and 20 mL acetylthiocholine iodide (0.71 mM) or butyryl-thiocholine chloride (0.2 mM) were added. Then the absorbance was measured at 412 nm.

Determination of tyrosinase inhibitory activity of the thiourea derivatives

The mushroom tyrosinase (EC 232-653-4, 250 KU) was used to determine the inhibitory activity of the thiourea derivatives **2a-2h** where L-DOPA was substrate according to DOPAchrome method (21) with slight modifications. Briefly, 150 µL of 50 mM sodium phosphate buffer (pH 6.8), 10 µL of thiourea derivatives **2a-2h** in DMSO, 20 µL of tyrosinase enzyme solution (13.3 U/well) were added in a 96-well plate. After 10 min incubation at 37 °C, L-DOPA (0.5 mM) was added to start the enzymatic reaction. The enzymatic reaction formation of DOPA chrome was monitored using 475 nm wavelength at 37 °C for 10 min.

Determination of a-amylase inhibitory activity of the thiourea derivatives

a-Amylase inhibitory activity of the thiourea derivatives **2a-2h** was tested by using the spectroscopic method with slight changes Quan et al. (22). Briefly, 25 µL sample solution in different concentrations and 50 µL a-amylase solution (0.1 U/mL) in phosphate buffer (20 mM pH=6.9 phosphate buffer prepared with 6 mM NaCl) were mixed in a 96-well microplate. The mixture was pre-incubated for 10 min at 37 °C. After pre-incubation, 50 µL starch solution (0.05 %) was added and incubated for more 10 min at 37 °C. The reaction was stopped by addition of 25 µL HCl (0.1 M) and then 100 µL Lugol solutions were added for monitoring. 96-well microplate reader was used to measure absorbance at 565 nm.

Determination of a-glucosidase inhibitory activity of the thiourea derivatives

a-Glucosidase inhibitory activity of the thiourea derivatives **2a-2h** was determined using the spectroscopic method with slight modifications (23). Briefly, 50 µL phosphate buffer (10 mM pH=6.9), 25 µL PNPG (*p*-nitrophenyl-*a*-*D*-glucopyranoside) in phosphate buffer (10 mM pH=6.9), 10 µL sample solution and 25 µL a-glucosidase (0.1 U/mL) in phosphate buffer (10 mM pH=6.0) were mixed in a 96-well microplate. After 20 minutes incubation at 37 °C, 90 µL sodium carbonate (100 mM) was added into the each well to stop the enzymatic reaction. Absorbance of the 96-well microplate reader was recorded at 400 nm.

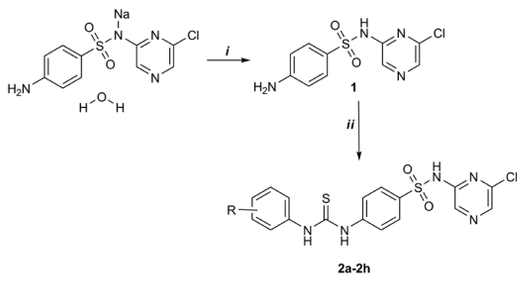
***In Silico* Prediction of Druglikeness**

The druglikeness properties such as Lipinski and Veber rules were calculated by using Swissadme online server (http://www.swiss adme.ch/).

3. RESULTS

3.1. Chemistry

In this study, novel thiourea derivatives were synthesized as indicated in Figure 1. Firstly, sulfaclozine was prepared by washing sulfaclozine sodium monohydrate with diluted hydrochloride acid (5%). Then, new thiourea derivatives were obtained by heating sulfaclozine with the same molar ratio of substituted isothiocyanate in the presence of anhydrous acetone. The confirmation of all thioureas was carried out by IR, ^{1}H-NMR spectroscopic methods and elemental analysis.



Comp.	R
2a	4-H
2b	4-F
2c	4-Cl
2d	4-Br
2e	4-CH_3
2f	4-OCH_3
2g	4-C_2H_5
2h	2,6-diCl_2

Figure 1. *The synthetic route of thiourea derivatives. Reagents: (i) 5% HCl, water; (ii) substituted phenylisothiocyanate, acetone.*

In the Infrared spectrum, the C=S stretching bands belonging to the thiourea group were detected in the range of 1226-1240 cm^{-1}. The thiourea N-H, aromatic =C-H and pyrazine C=N streching bands were observed in the region of 3194-3255 cm^{-1}, 3007-3043 cm^{-1} and 1583-1599 cm^{-1}, respectively. The asymmetric and symmetric SO_2 streching bands of the sulfonamide structure were assigned in the range of 1323-1338 cm^{-1} and 1138-1157 cm^{-1}, respectively.

In the ^{1}H-NMR spectrum, the observation of NH peaks belonging to the thiourea group with the 2H integral values in the range of 10.00-10.44 ppm supported the synthesis of thiourea groups. In addition, the disappearance of the amine peaks seen at 6.17 ppm was an another important indicator for thiourea synthesis. The aromatic protons in the pyrazine ring were detected in the range of 8.30-8.35 ppm, the other aromatic protons resonated in the range of 6.96-7.91 ppm. The sulfonamide NH peaks appeared at 11.90-11.94 ppm. Elemental analyses (C, H, N) were in accordance within ±0.4% of theoretical values.

3.2. Biological Activity

The antioxidant activity assays results of the synthesized thiourea derivatives were given in Table 1. The activity of the synthesis products was found to exhibit much better activity than the compound used for the synthesis of thiourea derivatives. According to the β-carotene-linoleic acid assay result, it was determined that compounds **2b** and **2e** exhibited best lipid peroxidation inhibition in the tested series. In the DPPH$^{\cdot}$ assay, all the thiourea derivatives exhibited better activity than BHT used as the positive standard of the assay,

while compounds **2a**, **2b**, **2c**, **2e** and **2f** were also found to exhibit tremendous activity than the α-TOC, which is other positive standard of the assay. In the $ABTS^{\cdot+}$ assay, among all the thiourea derivatives, compound **2c** showed greater activity than both standards with an IC_{50} value of 1.08±0.44 µM. In the CUPRAC assay, it was determined that all synthesized thiourea derivatives exhibited more effective activity than a-TOC. The most active compound in the series was found to be **2c** (IC_{50}=7.46±0.02 µM) in the tested activity.

The enzyme inhibition activity assays results of the synthesized thiourea derivatives were given in Table 2. It was determined that the anticholinesterase inhibitory activity of the synthesized thiourea derivatives showed better activity than AChE when the AChE and BChE assay results were compared with the galantamine, which is the standard of the assay, in general. In the AChE assay, compound **2c** showed the best activity within the thiourea series. In the BChE assay, on the other hand, all synthesized compounds were determined to be more active than galantamine. Also, in this assay, the thiourea derivative showed a tremendous activity of **2h** and was found to be approximately 3.5 times more active than galantamine. In the tyrosinase enzyme inhibition activity of the synthesized thiourea derivatives, compounds **2e** and **2f** came to the fore as the derivatives exhibiting the best activity.

In α-amylase and α-glucosidase enzyme inhibition activity, known as antidiabetic assay, compound **2g** is the most active within thiourea derivatives in both assays and competes with the acarbose being standard of the assays.

Table 1. *Antioxidant activity results of compound* **1** *and* **2a-2h**[a]

Compound	β-carotene-linoleic acid assay IC_{50} (µM)	$DPPH^{\cdot}$ assay IC_{50} (µM)	$ABTS^{\cdot+}$ assay IC_{50} (µM)	CUPRAC assay $A_{0.50}$ (µM)
1	>250	>250	>250	130.60±0.03
2a	55.62±0.97	2.25±0.01	26.52±1.84	8.88±0.01
2b	27.03±0.79	9.90±0.39	6.54±0.71	11.29±0.02
2c	34.10±0.95	11.21±1.07	1.08±0.44	7.46±0.02
2d	33.51±0.56	22.94±1.35	9.02±0.58	12.66±0.00
2e	27.84±0.14	10.29±0.69	56.71±0.95	7.73±0.01
2f	56.98±0.09	12.47±0.39	43.49±0.63	11.39±0.00
2g	37.64±0.39	19.54±1.03	12.58±0.24	7.60±0.00
2h	41.44±1.60	46.28±0.69	47.34±1.51	10.73±0.02
α-TOC[b]	4.79±0.14	12.90±0.57	5.14±0.29	40.45±0.01
BHT[b]	2.45±0.29	54.82±0.76	2.80±0.47	3.88±0.03

[a]*Values expressed are the mean ± SEM of three parallel measurements (p<0.05).*
[b]*Reference compounds.*

Table 2. *Enzyme inhibition activities of compounds* **1** *and* **2a-2h**[a]

Compound	Anticholinesterase Activity		Tyrosinase Activity IC_{50} (mM)	α-Amylase Activity IC_{50} (µM)	α-Glucosidase Activity IC_{50} (µM)
	AChE IC_{50} (µM)	BChE IC_{50} (µM)			
1	31.12±1.56	24.57±1.22	NA	>250	132.23±1.73
2a	10.58±0.11	20.03±0.57	14.56±1.72	115.35±0.05	110.66±1.90
2b	11.38±0.89	28.28±1.38	16.03±1.42	90.21±1.51	131.53±1.40
2c	8.58±0.48	21.89±0.27	11.86±0.70	100.41±1.19	119.49±0.73
2d	43.33±0.64	30.65±1.07	19.96±1.46	125.42±0.02	135.59±1.26
2e	13.38±0.50	17.47±1.34	6.71±0.89	110.66±0.79	132.13±1.44
2f	32.37±0.47	33.92±1.00	5.08±0.58	113.94±0.96	124.80±0.59
2g	29.25±0.46	15.37±0.77	15.64±0.78	65.41±0.34	106.06±0.31
2h	25.52±0.78	14.57±1.18	13.79±0.32	130.12±0.37	163.46±0.74
Galantamine[b]	5.07±0.62	48.02±0.21	NT	NT	NT
Kojic acid[b]	NT	NT	0.71±0.54	NT	NT
L-mimosine[b]	NT	NT	0.79±0.09	NT	NT
Acarbose[b]	NT	NT	NT	25.14±0.60	63.95±1.29

[a]*Values expressed are the mean ± SEM of three parallel measurements (p<0.05).*
[b]*Reference compounds. NT: Not tested. NA: Not active.*

Table 3. *Druglikeness properties of the synthesized compounds.*

Code	Lipinski rule of five				Veber rule	
	MW (g/mol)	cLog P	*n*-ON	n-OHNH	*n*-ROTB	TPSA
2a	419.91	0.94	4	3	7	136.48
2b	437.90	1.73	5	3	7	136.48
2c	454.35	1.85	4	3	7	136.48
2d	498.80	1.56	4	3	7	136.48
2e	433.93	1.58	4	3	7	136.48
2f	449.93	1.06	5	3	8	145.71
2g	447.96	1.81	4	3	8	136.48
2h	488.80	1.94	4	3	7	136.48

MW: molecular weight, cLog P: calculated partition coefficient, n-ON: number of hydrogen bond acceptors, n-OHNH: number of hydrogen bond donors, n-ROTB: number of rotatable bonds. TPSA: Topological polar surface area, Calculations were performed using SwissAdme online server (http://www.swissadme.ch).

In Silico Prediction of Druglikeness

To evaluate the druglikeness of a drug is used the Lipinski and Veber rules. A good strategy for predicting oral bioavailability if the compound meets Lipinski and Veber rules. Compounds that do not meet these rules may cause physicochemical and pharmacokinetic problems (24). According to Lipinski, compounds must meet at least three of following rules: (i) molecular weight (MW) ≤ 500 Da, (ii) Partition coefficient values (log P) ≤ 5, (iii) number of hydrogen bond donors (n-OHNH) ≤ 5, number of hydrogen bond acceptors (*n*-ON) ≤ 10 (25). On the other hand, the criteria of Veber rules are rotatable bonds (*n*-ROTB) ≤ 10 and polar surface area (TPSA) ≤ 140 Å^2 (26-28). These results were given in Table 3. The thiourea derivatives comply the Lipinski and Veber rules except compound **2f** (only one violation; TPSA>140).

The pink area in bioavailability radar indicates the optimum range for six physicochemical properties such as polarity, saturation, size, flexibility, lipophilicity and solubility. All physicochemical properties except for saturation of the compounds are in the pink area. The bioavailability radar of compounds **2a**-**2h** was given in Figure 2.

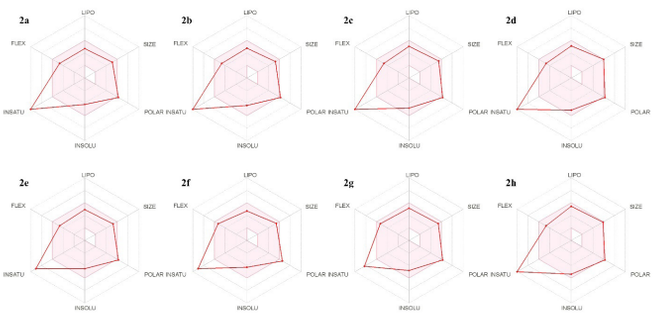


Figure 2. *The bioavailability radar of all compounds (The pink area represents the optimal range for each property. LIPO: Lipophilicity, SIZE: Molecular weight, POLAR: Total Polar Surface Area, INSOLU: Insolubility in water by log S scale, INSATU: Insaturation by carbon fraction in sp3 hybridization, FLEX: Flexibility as per rotatable bonds).*

4. DISCUSSION

In this study, the characteristic C=S bands in the IR spectrums demonstrated the synthesis of thiourea structures successfully. In addition, the disappearance of the free amine peaks and the observation of thiourea peaks with 2H integral value in the ^{1}H-NMR spectrum were other factors that proved the synthesis of thiourea structures.

According to antioxidant activity results, especially in DPPH and APTS$^+$ assays, more effective compounds were found than the reference standard. The evaluation of the anticholinesterase enzyme activity, all compounds showed higher activity than the standard against BChE. Compound **2c** was the most effective derivatives against the AChE enzyme, with an IC_{50} value of 8.58 µM (IC_{50} value for galantamine, 5.07 µM). Against the tyrosinase enzyme, compounds **2e** and **2f** were the most active compounds in this series. Against the enzymes α-amylase and α-glucosidase, which are related to antidiabetic activity, the compound **2g** showed the most promising activity. Therefore, thiourea derivatives exhibited remarkable biological activities in this study.

According to *in silico* prediction, the majority of all compounds did not violate druglikeness properties such as Lipinski and Veber rule. The bioavailability radar, defines a bioavailable drug candidate, showed that compounds are slightly outside the pink area on one side only, due to the inconformity of saturation. On the other hand, all thiourea derivatives have bioavailability score of 0.55, which means good pharmacokinetic properties.

5. CONCLUSION

As a result of the development of resistance to many drugs used in the clinic after a long period or the limited use of drugs due to various side effects, the need for the synthesis of more effective and selective drugs is increasing day by day. Therefore we synthesized some novel thiourea derivatives based on sulfaclozine and screened their antioxidant activity and enzyme inhibitory activity against cholinesterase (AChE and BChE), tyrosinase, α-amylase and α-glucosidase. The antioxidant activity results showed that compounds **2b** and **2c** may be candidate compounds for the elimination of free radicals in the body due to various reasons. The anticholinesterase activity results indicated that all compounds exhibited higher BChE inihibitory activity than galantamine. Compounds **2e** and **2f**, which exhibited the best activity in tyrosinase inhibition activity, remarked among the synthesis derivatives as potential compounds in the treatment of skin diseases related to melanin biosynthesis. The results of α-amylase and α-glycosidase enzyme inhibition activities also showed that thiourea derivatives could be a lead compound for antidiabetic activity.

Acknowledgement

The starting compound, sulfaclozine sodium monohydrate, was obtained from Medicavet A.Ş.

Funding

The authors declare that they have no competing financial interest

Conflicts of interest

The authors declare that they have no conflict of interest.

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How to cite this article: Tok F, Cakir C, Cam D, Kirpat MM, Sicak Y. Synthesis, Characterization and Biological Evaluation of Novel Thiourea Derivatives. Clin Exp Health Sci 2022; 12: 533-540. DOI: 10.33808/clinexphealthsci.1062872