Journal of Research in Pharmacy

Research Article

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Synthesis, characterization and biological evaluation of some novel sulfonylurea derivatives

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Received: 8 August 2023 / Revised: 31 August 2023 / Accepted: 1 September 2023

ABSTRACT: In this study, some new sulfonylurea derivatives based on the sulfanilamide compound were synthesized. IR, ^{1}H-NMR, ^{13}C-NMR spectroscopic methods and elemental analysis data were used to confirm the structures of the synthesized compounds. ABTS, DPPH, Cuprac and β-Carotene/linoleic acid assays were performed to evaluate the antioxidant activities of sulphonylurea derivatives. The activities of these compounds against some enzymes (acetylcholinesterase, butrylcholinesterase, tyrosinase, α-amylase and α-glucosidase) were also investigated. Compounds **S7**, **S8**, **S13** and **S14** showed inhibitory activity against α-amylase enzyme with IC_{50} values of 227.84±1.48-298.27±8.61 µM. In addition, the drug-likeness properties and solubility of the compounds were determined by computational programs.

KEYWORDS: Sulfonylurea; antioxidant; cholinesterase; tyrosinase; α-amylase; α-glucosidase.

1. INTRODUCTION

Sulfonylurea consists of a sulfonyl group (-S(=O)$_2$) with a sulfur atom attached to the nitrogen atom of a urea group [1]. Sulfonylurea group shows various pharmacological activities such as antibacterial, antimalarial, anticancer, antidiabetic and antiherbicide depending on the substituents it carries on sulfur and nitrogen atoms [2-5]. In fact, there is a class of antidiabetic drugs that carry the sulfonylurea group [6]. Sulfonylurea group antidiabetic agents such as tolbutamide, chlorpropamide, tolazamide, acetohexamide, glybenclamide and glipizide stimulate the beta cells of the pancreas to release insulin and thus lower blood sugar [7] (Figure 1). Idrees et al. synthesized sulfonylurea structures that can strongly inhibit carbonic anhydrase enzyme but do not show toxic effects on normal human cells [8]. In another study, Ceras et al. reported that the sulfonylurea compound bearing the naphthalene ring they synthesized was a potent histamine receptor inhibitor [9]. Nan et al. reported that 4-phenoxycinoline structures bearing sulfonylurea structure showed strong tyrosine kinase inhibitory activity and antitumor activity on some cancer cells [10]. Studies on new sulfonylurea derivatives with both antidiabetic activity and molecules with different biological activity, such as Sulofenur (anticancer) and Chlorsulfuron (herbicide), are continuing [11].

The presence of donor and acceptor moieties in sulfonylurea groups facilitates their participation in various non-covalent interactions, in particular hydrogen bonding. Therefore, the synthesis and structural properties of sulfonylureas have attracted attention in recent years [12]. When comparing the solubility of urea compounds with that of sulfonylurea compounds, low solubility usually results in low systemic exposure and poor *in vivo* activity. Low solubility is a significant problem, and can be addressed at different stages of the drug discovery process [13]. Early-stage intervention is molecular design, later-stage formulation modification may be an option, but none of these have any guarantee of success [14,15]. Therefore, the structures of the molecules were designed based on the knowledge of the high solubility of sulfonylurea compounds compared to urea compounds.

In the light of the above information; starting from 4-aminobenzenesulfonamide, amide structure in the first step and sulfonylurea structures in the second step were synthesized. The structures of the synthesized

How to cite this article: Tok F, Cihangir H, Şan K, Çakır C, Tuna K, Sıcak Y, Öztürk M, Koçyiğit-Kaymakçıoğlu B. Synthesis, characterization and biological evaluation of some novel sulfonylurea derivatives. J Res Pharm. 2023; 27(6): 2416-2424.

ISSN: 2630-6344

http://dx.doi.org/10.29228/jrp.529

compounds were elucidated by spectroscopic methods and their antioxidant, anticholinesterase, antityrosinase and antidiabetic activities were investigated.

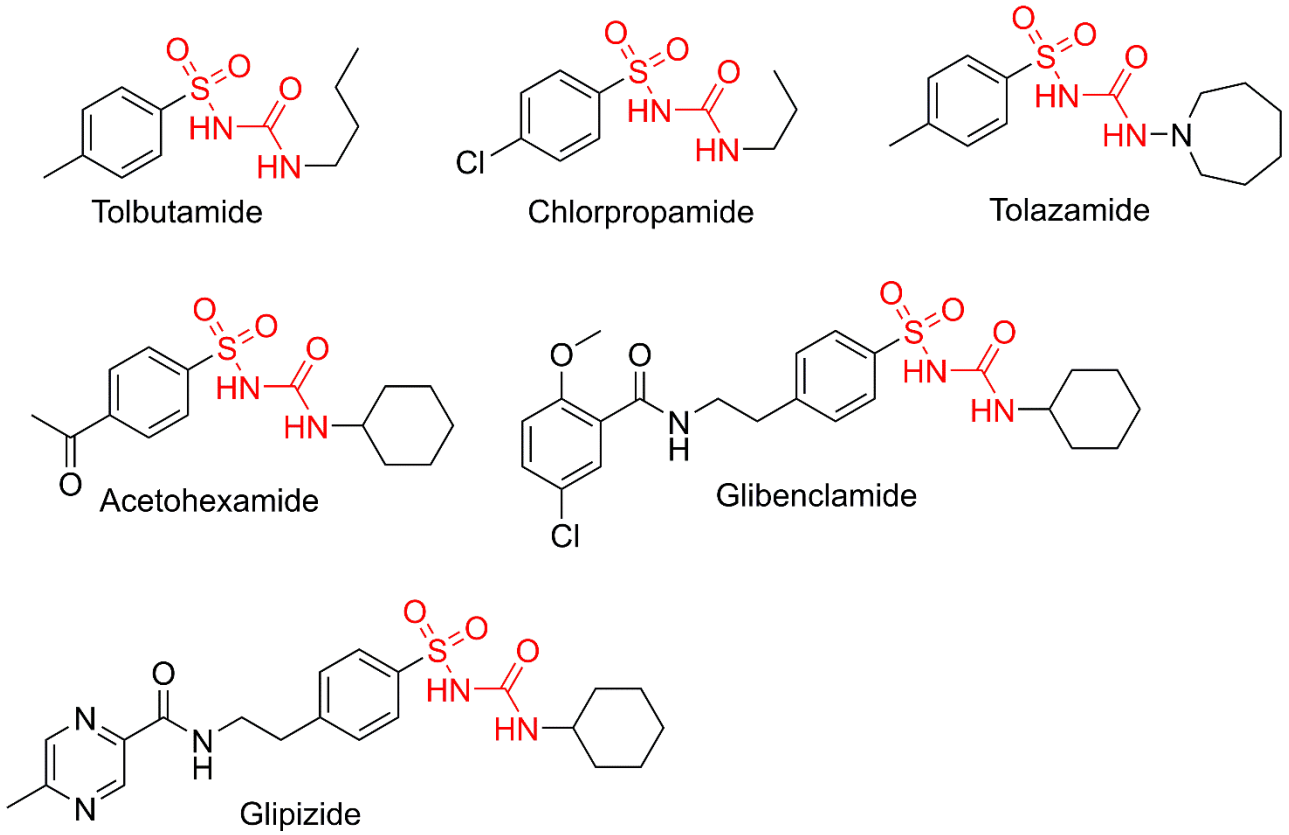
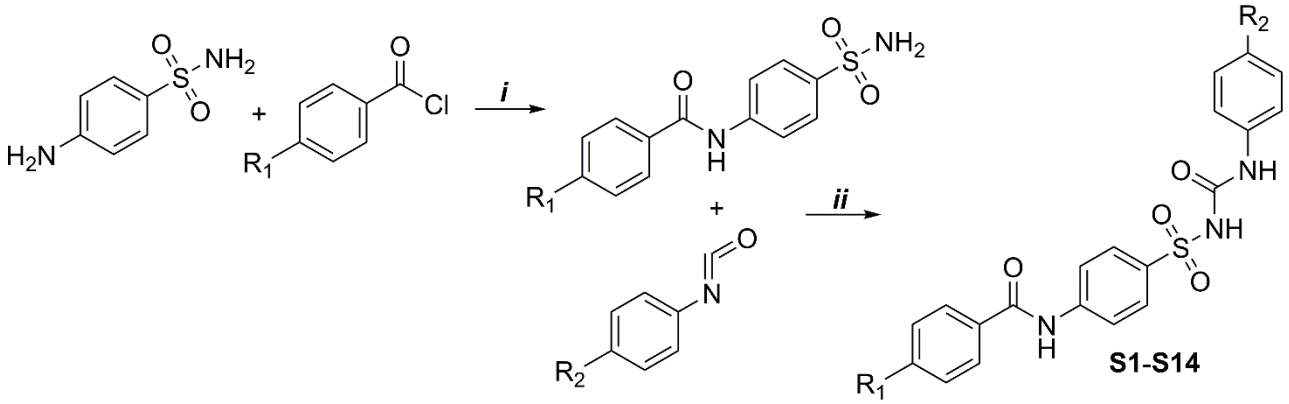


Figure 1. Some antidiabetics having sulfonylurea groups.

2. RESULTS

2.1. Chemistry

The amide structure was obtained by oxidation of 4-aminobenzamide molecule with various benzoyl chlorides in a basic medium. Subsequently, molecules with sulfonylurea structure (**S1-S14**) were synthesized by nucleophilic addition reaction of sulfonamide group with 4-chlorophenylisocyanate or 4-methylphenylisocyanate in a basic medium (Scheme 1). The purity of all compounds was confirmed by thin layer chromatography and elemental analysis. The structures of the synthesized compounds were proved by IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic methods.



Scheme 1. The synthetic pathway of target molecules. (i) pyridine, acetone (ii) potassium carbonate, DMF.

In the IR spectrum of the compounds with sulfonylurea structure, two sharp C=O bands in the range of 1649-1680 cm^{-1} and 1629-1639 cm^{-1} were found to be important for the proof of the structure. Apart from this, the characteristic NH stretching bands of the sulfonylurea structure were detected in the range of 3165-3377 cm^{-1}.

When the ^{1}H-NMR spectrum of the sulfonylurea compounds was analyzed, the amide proton was detected in the lowest energy range between 10.41-10.89 ppm. Among the NH protons forming the sulfonylurea group, the one connected to the sulfone group resonated in the aromatic region and the one connected to the aromatic structure resonated at 8.49-8.88 ppm, respectively.

In the ^{13}C-NMR spectrum of sulfonylurea compounds, amide and sulfonylurea carbonyls were observed in the range of 163.91-166.44 ppm and 150.08-153.11 ppm, respectively.

2.2. Biological activity

ABTS, DPPH, CUPRAC and β-carotene/linoleic acid assays were carried out to screen the antioxidant activity of the synthesized sulfonylurea derivatives. The antioxidant activity of the compounds could not be determined when compared with the standard drug BHA. Only compounds **S7** and **S13** showed IC_{50} values of 387.14 µM and 335.10 µM in the CUPRAC assay, respectively (Table 1).

Table 1. Antioxidant activities of synthesized **S1-S14**[a]

Compounds	R_1	R_2	Antioxidant activity			
			ABTS^{+} Assay (IC_{50} µM)	DPPH Assay (IC_{50} µM)	CUPRAC ($A_{0.5}$ µM)	β-Carotene/linoleic acid assay (IC_{50} µM)
S1	H	Cl	NA	NA	>400	NA
S2	H	CH_3	NA	>400	>400	NA
S3	F	Cl	NA	NA	>400	NA
S4	F	CH_3	NA	>400	>400	NA
S5	Cl	Cl	NA	NA	>400	NA
S6	Cl	CH_3	NA	NA	NA	NA
S7	CH_3	Cl	NA	NA	387.14±0.19	NA
S8	CH_3	CH_3	NA	NA	>400	NA
S9	NO_2	Cl	NA	>400	>400	NA
S10	NO_2	CH_3	NA	NA	>400	NA
S11	OCH_3	Cl	NA	>400	>400	NA
S12	OCH_3	CH_3	NA	>400	NA	NA
S13	Br	Cl	NA	>400	335.10±0.84	NA
S14	Br	CH_3	NA	NA	>400	NA
BHA[b]			2.49±0.05	5.93±0.20	5.74±0.41	0.66±0.02

Abbreviation: BHA, 2-tert-Butyl-4-hydroxyanisole.
[a] Values expressed herein are mean ± SEM of three parallel measurements. $p<0.05$.
NT: not tested. NA: not active. [b]Reference compounds.

The activities of the synthesized compounds against acetylcholinesterase (AChE), butrylcholinesterase (BChE), tyrosinase, α-amylase and α-glucosidase enzymes were also investigated. Although compounds **S7**, **S8**, **S13** and **S14** showed a little inhibitory activity against the α-amylase enzyme, none of the remaining compounds showed significant inhibitor activity (Table 2).

Table 2. Enzyme inhibition activities of synthesized **S1-S14**[a]

Compounds	Anticholinesterase activity		Tyrosinase Activity IC_{50} (mM)	Antidiabetic inhibitory activities	
	AChE (IC_{50} µM)	BChE (IC_{50} µM)		α-Amylase Inhibitory Activity (IC_{50} µM)	α-Glucosidase Inhibitory Activity (IC_{50} µM)
S1	NA	NA	NA	>400	NA
S2	NA	NA	NA	>400	NA
S3	NA	NA	NA	>400	NA
S4	NA	NA	NA	>400	NA
S5	NA	NA	NA	>400	NA
S6	NA	NA	NA	>400	NA
S7	NA	NA	NA	280.16±2.80	NA
S8	NA	NA	NA	298.27±8.61	NA

S9	NA	NA	NA	>400	NA
S10	NA	NA	NA	>400	NA
S11	NA	NA	NA	>400	NA
S12	NA	NA	NA	>400	NA
S13	NA	NA	NA	227.84±1.48	NA
S14	NA	NA	NA	267.67±2.76	NA
Galantamin[b]	1.82±0.30	4.62±0.12	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	62.92 ± 1.84	201.07±0.55

[a] Values expressed herein are mean ± SEM of three parallel measurements. $p<0.05$.
NT: not tested. NA: not active. [b]Reference compounds.

2.3. *In silico* studies

One of the prerequisites for synthesized compounds to be drug candidate compounds is drug-likeness properties [16]. Lipinski and Veber's rules, which are a result of the physicochemical properties of compounds, are one of the most important drug-like properties [17]. All of the compounds synthesized in this study are in full compliance with the Lipinski rule. Only **S9** and **S10**, which carry a nitro group on the aromatic ring, deviate from the Veber rule, but the other compounds are in full compliance with the Veber rule (Table 3).

Solubility is extremely important for oral absorption of the drug. Compounds with poor solubility cause poor oral absorption and poor oral bioavailability [15]. The solubility of the synthesized compounds can be estimated by theoretically calculated log S values. Only **S13** has poor solubility while all other compounds have moderate solubility.

Table 3. Drug-likeness properties of synthesized **S1-S14**[a]

Compounds	Lipinski filter				Veber filter		Solubility class
	MW	clogP	num. H-bond acceptors	num. H-bond donors	*n*-ROTB	TPSA	Log S
S1	429.88	2.90	4	3	8	112.75	-5.24
S2	409.46	2.90	4	3	8	112.75	-4.80
S3	447.87	3.54	5	3	8	112.75	-5.40
S4	427.45	3.27	5	3	8	112.75	-4.96
S5	464.32	3.38	4	3	8	112.75	-5.83
S6	443.90	3.38	4	3	8	112.75	-5.39
S7	443.90	3.38	4	3	8	112.75	-5.39
S8	423.48	3.12	4	3	8	112.75	-5.10
S9	472.88	2.22	6	3	8	146.89	-5.72
S10	452.46	2.22	6	3	8	146.89	-5.28
S11	459.90	2.86	5	3	9	121.98	-5.31
S12	439.48	2.60	5	3	9	121.98	-4.87
S13	508.77	3.49	4	3	8	112.75	-6.15
S14	488.35	3.49	4	3	8	112.75	-5.71

MW: Molecular weight, clogP: partition coefficient, num. H-bond acceptors: number of hydrogen bond acceptors, num. H-bond donors: number of hydrogen bond donors, *n*-ROTB: number of rotatable bonds, TPSA: total polar surface area. MW≤500, clogP≤4.15, num. H-bond acceptors≤10 and num. H-bond donors≤5 for Lipinski filter. *n*-ROTB<10 and TPSA<140 for Veber filter. log S scale: Insoluble<-10<poorly<-6<moderately<-4<soluble<-2<very<0<highly.

3. CONCLUSION

Sulfonylurea derivatives are an important functional group considered in drug discovery processes. For this reason, some new compounds based on 4-aminobenzenesulfonamide compound have been synthesized and their structures have been elucidated. Antioxidant activity studies such as ABTS, DPPH, Cuprac and β-Carotene/linoleic acid assays were carried out to screen the biological activity of the synthesized compounds but no significant activity was observed. In addition, the activities of the synthesized compounds against enzymes responsible for different biological effects such as cholinesterase (AChE and BChE), tyrosinase, α-amylase and α-glucosidase were screened. Among these enzymes, only compounds **7** and **8** carrying methyl and compounds **13** and **14** carrying bromine substituents on the aromatic ring showed low activity against α-

amylase. All of the compounds were found to comply with Lipinski's rule, except **S9** and **S10** which were found to comply with Veber's rule. The solubility of the compounds, which is an important parameter for oral bioavailability, was found to be moderate except for compound **S13**.

4. MATERIALS AND METHODS

4.1. Chemistry

All chemicals used in this study were purchased from Sigma-Aldrich. Melting points were determined by Schmelzpunktbestimmer SMP II. IR spectra were recorded at FTIR-8400S Shimadzu. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a Bruker Avance III HD 600 MHz instrument at 600 MHz for ^{1}H-NMR and 151 MHz for ^{13}C-NMR (decoupled) in DMSO-d_6 with TMS as an internal standard for protons. The purity of the compounds was controlled by thin layer chromatography (TLC) and elemental analysis. Elemental analyses were obtained using Leco CHNS-932.

*4.1.1. General synthesis procedure of target compounds (**S1**-**S14**)*

1 mmol sulfanilamide and 2 mmol pyridine were dissolved in 15 ml acetone with stirring. The temperature of the mixture was lowered below 0 ˚C and substituted benzoyl chloride (1 mmol) was added dropwise to the solution. The mixture was stirred in a magnetic stirrer at room temperature for 5 hours. The excess solvent was evaporated under a vacuum. The resulting product was purified by column chromatography [18].

5.8 mmol sulfonamide derivative and 1.2 mmol potassium carbonate in 20 ml DMF stirred at 100 ˚C for 1 hour. Then 5.8 mmol 4-chlorophenylisocyanate or 4-methylphenylisocyanate was added to the mixture and stirred at 100 ˚C for 10 hours. After checking by thin layer chromatography, the mixture was poured on crushed ice and neutralized with conc.HCl. The precipitate obtained was dried and crystallized from ethanol [19].

N-(4-(*N*-((4-Chlorophenyl)carbamoyl)sulfamoyl)phenyl)benzamide (**S1**)

White solid, yield: 77%, m.p. = 280 °C. IR (vmax, cm^{-1}): 3362, 3290, 3255, 1651, 1631, 1589, 1558, 1516, 1296, 1155, 829. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.58 (s, 1H, amide NH), 8.86 (s, 1H, sulfonylurea NH), 7.98 (t, *J* = 7.2 Hz, 4H), 7.84 – 7.80 (m, 2H), 7.65 – 7.60 (m, 1H), 7.56 (t, *J* = 7.5 Hz, 2H), 7.51 – 7.46 (m, 2H), 7.36 – 7.29 (m, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.44 (amide CO), 152.82 (sulfonylurea CO), 142.61, 139.20, 139.00, 134.95, 132.39, 129.10, 128.94, 128.25, 127.00, 126.00, 120.33, 120.32. Anal. calcd. for $C_{20}H_{16}ClN_3O_4S$: C 55.88, H 3.75, N 9.78, S 7.46. Found: C 58.54, H 4.52, N 10.57, S 7.36 %.

N-(4-(*N*-(*p*-Tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S2**)

White solid, yield: 80%, m.p. = 290 °C. IR (vmax, cm^{-1}): 3360, 3290, 3255, 3032, 2916, 2856, 1651, 1639, 1591, 1564, 1514, 1296, 1155, 829. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.57 (s, 1H, amide NH), 8.50 (s, 1H, sulfonylurea NH), 8.00 – 7.94 (m, 4H), 7.81 (dd, *J* = 8.8, 1.9 Hz, 2H), 7.62 (td, *J* = 7.4, 1.8 Hz, 1H), 7.59 – 7.53 (m, 2H), 7.35 – 7.28 (m, 3H), 7.07 (d, *J* = 8.0 Hz, 2H), 2.24 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 166.44 (amide CO), 153.10 (sulfonylurea CO), 142.61, 139.20, 137.67, 134.96, 132.39, 130.99, 129.62, 128.95, 128.26, 126.99, 120.31, 118.70, 20.80. Anal. calcd. for $C_{21}H_{19}N_3O_4S$: C 61.60, H 4.68, N 10.26, S 7.83. Found: C 58.35, H 4.50, N 10.47, S 7.23 %.

N-(4-(*N*-((4-Chlorophenyl)carbamoyl)sulfamoyl)phenyl)-4-fluorobenzamide (**S3**)

White solid, yield: 75%, m.p. = 262 °C. IR (vmax, cm^{-1}): 3321, 3277, 3225, 3105, 1676, 1631, 1589, 1537, 1504, 1298, 1155, 845. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.59 (s, 1H, amide NH), 8.86 (s, 1H, sulfonylurea NH), 8.07 (dd, *J* = 8.6, 5.5 Hz, 2H), 7.96 (d, *J* = 8.5 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.49 (d, *J* = 8.6 Hz, 2H), 7.40 (t, *J* = 8.6 Hz, 2H), 7.33 (d, *J* = 8.5 Hz, 2H), 7.31 (s, 1H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.56, 165.31, 163.91 (amide CO), 152.82 (sulfonylurea CO), 150.06, 142.51, 139.27, 138.99, 136.61, 131.39, 131.37, 131.09, 131.02, 129.10, 127.01, 126.00, 124.38, 120.36, 120.33, 115.98, 115.84. Anal. calcd. for $C_{20}H_{15}ClFN_3O_4S$: C 53.64, H 3.38, N 9.38, S 7.16. Found: C 55.74, H 3.93, N 10.06, S 7.81 %.

4-Fluoro-*N*-(4-(*N*-(*p*-tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S4**)

White solid, yield: 81%, m.p. = 257 °C. IR (vmax, cm-1): 3317, 3223, 3097, 2912, 1680, 1639, 1591, 1554, 1506, 1334, 1157, 845. 1H NMR (600 MHz, DMSO-d_6) δ 10.58 (s, 1H, amide NH), 8.50 (s, 1H, sulfonylurea NH), 8.09 – 8.04 (m, 2H), 7.97 – 7.92 (m, 2H), 7.84 – 7.79 (m, 2H), 7.40 (td, *J* = 8.7, 1.7 Hz, 3H), 7.35 – 7.28 (m, 2H), 7.07 (d, *J* = 8.0 Hz, 2H), 2.24 (s, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 165.56, 165.31, 163.91 (amide CO), 153.10 (sulfonylurea CO), 150.07, 142.50, 139.27, 137.66, 131.39, 131.37, 131.09, 131.03, 131.00, 129.62, 127.00, 124.39, 120.36, 118.71, 115.99, 115.85, 20.79. Anal. calcd. for $C_{21}H_{18}FN_3O_4S$: C 59.01, H 4.24, N 9.83, S 7.50. Found: C 61.71, H 4.68, N 10.34, S 7.28 %.

4-Chloro-*N*-(4-(*N*-((4-chlorophenyl)carbamoyl)sulfamoyl)phenyl)benzamide (**S5**)

White solid, yield: 70%, m.p. = 267 °C. IR (vmax, cm-1): 3377, 3298, 3165, 3095, 1664, 1631, 1589, 1527, 1315, 1155, 831.1H NMR (600 MHz, DMSO-d_6) δ 10.64 (s, 1H, amide NH), 8.86 (s, 1H, sulfonylurea NH), 8.02 (d, *J* = 8.1 Hz, 2H), 7.96 (d, *J* = 8.4 Hz, 2H), 7.83 (d, *J* = 8.4 Hz, 2H), 7.64 (d, *J* = 8.1 Hz, 2H), 7.49 (d, *J* = 8.4 Hz, 2H), 7.36 – 7.30 (m, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 165.32 (amide CO), 152.82 (sulfonylurea CO), 150.06, 142.41, 139.37, 139.00, 137.26, 136.60, 133.63, 130.23, 129.09, 129.02, 127.02, 126.01, 124.37, 120.41, 120.33. Anal. calcd. for $C_{20}H_{15}Cl_2N_3O_4S$: C 51.74, H 3.26, N 9.05, S 6.90. Found: C 54.29, H 3.83, N 9.57, S 7.35 %.

4-Chloro-*N*-(4-(*N*-(*p*-tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S6**)

White solid, yield: 85%, m.p. = 282 °C. IR (vmax, cm-1): 3377, 3304, 3171, 3076, 2914, 1664, 1639, 1591, 1527, 1315, 1155, 831. 1H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H, amide NH), 8.10 – 7.91 (m, 6H), 7.91 – 7.74 (m, 4H), 7.74 – 7.58 (m, 4H), 2.35 (s, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 165.30 (amide CO), 150.08 (sulfonylurea CO), 142.41, 139.38, 137.25, 133.64, 130.24, 129.61, 129.03, 127.02, 124.38, 120.39, 118.69, 20.80. Anal. calcd. for $C_{21}H_{19}N_3O_4S$: C 61.60, H 4.68, N 10.26. Found: C 60.10, H 3.90, N 16.25 %. Anal. calcd. for $C_{21}H_{18}ClN_3O_4S$: C 56.82, H 4.09, N 9.47, S 7.22. Found: C 50.76, H 3.85, N 9.13, S 9.49 %.

N-(4-(*N*-((4-Chlorophenyl)carbamoyl)sulfamoyl)phenyl)-4-methylbenzamide (**S7**)

White solid, yield: 88%, m.p. = 275 °C. IR (vmax, cm-1): 3358, 3306, 3255, 3032, 1649, 1633, 1591, 1519, 1296, 1157, 825. 1H NMR (400 MHz, DMSO-d_6) δ 10.48 (s, 1H, amide NH), 8.87 (s, 1H, sulfonylurea NH), 8.03 – 7.72 (m, 6H), 7.50 – 7.26 (m, 7H), 2.40 (s, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 166.21 (amide CO), 152.82 (sulfonylurea CO), 142.70, 142.50, 139.08, 139.02, 132.05, 129.46, 129.10, 128.30, 126.97, 125.98, 120.32, 120.27, 21.51. Anal. calcd. for $C_{21}H_{18}ClN_3O_4S$: C 56.82, H 4.09, N 9.47, S 7.22. Found: C 59.22, H 4.63, N 9.96, S 6.30 %.

4-Methyl-*N*-(4-(*N*-(p-tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S8**)

White solid, yield: 70%, m.p. = 295 °C. IR (vmax, cm-1): 3358, 3306, 3255, 3032, 1649, 1633, 1591, 1516, 1294, 1157, 827. 1H NMR (600 MHz, DMSO-d_6) δ 10.48 (s, 1H, amide NH), 8.50 (s, 1H, sulfonylurea NH), 7.97 (d, *J* = 8.5 Hz, 2H), 7.90 (d, *J* = 7.8 Hz, 2H), 7.81 (d, *J* = 8.4 Hz, 2H), 7.38 – 7.28 (m, 5H), 7.07 (d, *J* = 8.0 Hz, 2H), 2.39 (s, 2H), 2.24 (s, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 166.22 (amide CO), 153.11 (sulfonylurea CO), 142.71, 142.51, 139.08, 137.67, 132.05, 131.00, 129.62, 129.46, 128.30, 126.97, 120.28, 118.71, 21.50, 20.79. Anal. calcd. for $C_{22}H_{21}N_3O_4S$: C 62.40, H 5.00, N 9.92, S 7.57. Found: C 66.77, H 5.87, N 10.58, S 6.05 %.

N-(4-(*N*-((4-Chlorophenyl)carbamoyl)sulfamoyl)phenyl)-4-nitrobenzamide (**S9**)

White solid, yield: 77%, m.p. = 256 °C. IR (vmax, cm-1): 3356, 3292, 3254, 3078, 1662, 1629, 1597, 1531, 1325, 1153, 827. 1H NMR (400 MHz, DMSO-d_6) δ 10.89 (s, 1H, amide NH), 8.86 (s, 1H, sulfonylurea NH), 8.44 – 8.37 (m, 2H), 8.21 (dd, *J* = 9.0, 2.3 Hz, 2H), 7.97 (dd, *J* = 8.9, 2.4 Hz, 2H), 7.84 (dd, *J* = 8.8, 2.4 Hz, 2H), 7.52 – 7.45 (m, 2H), 7.40 – 7.30 (m, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 164.80 (amide CO), 152.82 (sulfonylurea CO), 149.81, 142.11, 140.59, 139.73, 139.02, 129.84, 129.10, 127.08, 125.97, 124.08, 120.54, 120.31. Anal. calcd. for $C_{20}H_{15}ClN_4O_6S$: C 50.59, H 3.18, N 11.80, S 6.75. Found: C 52.48, H 3.41, N 12.26, S 6.57 %.

4-Nitro-*N*-(4-(*N*-(*p*-tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S10**)

White solid, yield: 72%, m.p. = 248 °C. IR (vmax, cm-1): 3342, 3304, 3254, 3050, 2918, 1662, 1639, 1595, 1564, 1327, 1155, 827. 1H NMR (600 MHz, DMSO-d_6) δ 10.88 (s, 1H, amide NH), 8.49 (s, 2H, sulfonylurea NH), 8.42 – 8.37 (m, 1H), 8.26 – 8.19 (m, 2H), 7.99 – 7.94 (m, 2H), 7.90 – 7.82 (m, 2H), 7.35 – 7.30 (m, 3H), 7.12 – 7.05 (m, 2H), 2.24 (s, 3H). 13C NMR (151 MHz, DMSO-d_6) δ 164.82 (amide CO), 153.10 (sulfonylurea CO), 149.81, 142.10, 140.59,

139.73, 137.66, 131.00, 129.83, 129.62, 127.08, 124.08, 120.55, 118.70, 67.49, 20.79. Anal. calcd. for $C_{21}H_{18}N_4O_6S$: C 55.50, H 3.99, N 12.33, S 7.05. Found: C 61.59, H 5.08, N 12.67, S 5.16 %.

N-(4-(*N*-((4-Chlorophenyl)carbamoyl)sulfamoyl)phenyl)-4-methoxybenzamide (**S11**)

White solid, yield: 70%, m.p. = 266 °C. IR (vmax, cm^{-1}): 3346, 3277, 3201, 3080, 2954, 1660, 1633, 1587, 1543, 1315, 1157, 825. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.41 (s, 1H, amide NH), 8.88 (s, 1H, sulfonylurea NH), 7.97 (dd, *J* = 15.5, 8.7 Hz, 4H), 7.80 (d, *J* = 8.7 Hz, 2H), 7.48 (d, *J* = 8.8 Hz, 1H), 7.34 (d, *J* = 8.6 Hz, 4H), 7.09 (d, *J* = 8.7 Hz, 2H), 3.86 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.73 (amide CO), 162.65, 152.82 (sulfonylurea CO), 142.84, 139.02, 138.93, 130.27, 129.10, 126.96, 126.92, 125.98, 120.32, 120.21, 114.17, 55.95. Anal. calcd. for $C_{21}H_{18}ClN_3O_5S$: C 54.84, H 3.95, N 9.14, S 6.97. Found: C 56.38, H 4.49, N 9.49, S 7.82 %.

4-Methoxy-*N*-(4-(*N*-(*p*-tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S12**)

White solid, yield: 70%, m.p. = 288 °C. IR (vmax, cm^{-1}): 3346, 3277, 3203, 3066, 2956, 1660, 1633, 1587, 1566, 1315, 1157, 825. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.41 (s, 1H, amide NH), 8.50 (s, 1H, sulfonylurea NH), 7.98 (ddd, *J* = 19.8, 8.9, 2.0 Hz, 4H), 7.83 – 7.78 (m, 2H), 7.35 – 7.30 (m, 2H), 7.29 (s, 1H), 7.08 (t, *J* = 7.6 Hz, 4H), 3.85 (s, 3H), 2.24 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.75 (amide CO), 162.66, 153.11 (sulfonylurea CO), 142.83, 138.93, 137.67, 131.00, 130.27, 129.62, 127.13, 126.96, 126.92, 120.22, 118.71, 114.17, 55.94, 20.79. Anal. calcd. for $C_{22}H_{21}N_3O_5S$: C 60.13, H 4.82, N 9.56, S 7.29. Found: C 62.11, H 5.06, N 10.09, S 6.53 %.

4-Bromo-*N*-(4-(*N*-((4-chlorophenyl)carbamoyl)sulfamoyl)phenyl)benzamide (**S13**)

White solid, yield: 80%, m.p. = 268 °C. IR (vmax, cm^{-1}): 3292, 3230, 3097, 2954, 1660, 1629, 1587, 1556, 1315, 1157, 825. ^{1}H NMR (600 MHz, DMSO-d_6) δ 10.64 (s, 1H, amide NH), 8.86 (s, 2H, sulfonylurea NH), 7.95 (dd, *J* = 11.2, 8.3 Hz, 2H), 7.83 (d, *J* = 8.5 Hz, 2H), 7.78 (d, *J* = 7.9 Hz, 2H), 7.52 – 7.47 (m, 2H), 7.33 (dd, *J* = 10.5, 3.7 Hz, 4H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.45 (amide CO), 152.82 (sulfonylurea CO), 142.40, 139.38, 139.00, 138.97, 134.00, 131.96, 130.39, 129.09, 127.02, 126.21, 126.00, 120.41, 120.33. Anal. calcd. for $C_{20}H_{15}BrClN_3O_4S$: C 47.42, H 2.97, N 8.26, S 6.30. Found: C 51.25, H 3.46, N 9.15, S 4.33 %.

4-Bromo-*N*-(4-(*N*-(*p*-tolylcarbamoyl)sulfamoyl)phenyl)benzamide (**S14**)

White solid, yield: 85%, m.p. = 253 °C. IR (vmax, cm^{-1}): 3302, 3050, 2914, 1650, 1637, 1593, 1564, 1307, 1157, 813. ^{1}H NMR (400 MHz, DMSO-d_6) δ 10.63 (s, 1H, amide NH), 8.50 (s, 1H, sulfonylurea NH), 7.94 (ddt, *J* = 6.5, 4.3, 2.1 Hz, 3H), 7.80 (ddd, *J* = 10.8, 8.8, 2.1 Hz, 2H), 7.32 (dd, *J* = 9.1, 2.7 Hz, 4H), 7.16 – 7.00 (m, 4H), 2.24 (s, 3H). ^{13}C NMR (151 MHz, DMSO-d_6) δ 165.43 (amide CO), 153.09 (sulfonylurea CO), 142.39, 139.38, 137.69, 134.00, 131.97, 130.96, 130.40, 129.62, 127.02, 126.21, 120.39, 118.69, 20.80. Anal. calcd. for $C_{21}H_{18}BrN_3O_4S$: C 51.65, H 3.72, N 8.60, S 6.56. Found: C 52.77, H 4.92, N 9.59, S 4.84 %.

4.2. Biological activity

4.2.1. In vitro antioxidant activities

The antioxidant activities of **S1**-**S14** derivatives were determined using four complimentary assays, namely, ABTS cation radical scavenging activity, DPPH free radical scavenging activity, cupric reducing antioxidant capacity (CUPRAC) and β-carotene bleaching method. The antioxidant activity methods were applied as reported in our previous research [20,21]. The 2-tert-butyl-4-hydroxyanisole (BHA) was used as the standard to compare the activity.

4.2.2. In vitro enzyme inhibitory activities

The AChE and BChE inhibitory activities of all obtained derivatives (**S1–S14**) were assessed using the modified Ellman method, as described in the our previous studies [22,23]. The tyrosinase enzyme inhibition procedure was applied using the modified Hearing method, as reported in our previous research papers [24,25]. The α-amylase and α-glucosidase inhibitory activities of synthesized compounds were also evaluated using the spectroscopic method with slight changes by Quan et al. and Kim et al. [26,27]. Galantamine for

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

4.3. *In silico* studies

SwissAdme online server was used to evaluate the drug-like properties of the synthesized compounds (http://www.swissadme.ch/index.php, access date: 29.07.2023). The theoretical values of the synthesized compounds such as molecular weight, partition coefficient, hydrogen donor and acceptor numbers, number of rotatable bonds and total polar surface area were calculated by the program.

Acknowledgements: This work was partly supported by a TUBITAK 2209-A Research Projects Program. (No: 1919B012219955).

Author contributions: Concept – F.T., B.K.K.; Design – F.T., Y.S., M.Ö., B.K.K.; Supervision – M.Ö., B.K.K.; Resources – F.T., Y.S., M.Ö., B.K.K.; Materials – F.T., Y.S., M.Ö., B.K.K.; Data Collection and/or Processing – F.T., H.C., K.Ş., C.Ç., K.T., Y.S., M.Ö., B.K.K.; Analysis and/or Interpretation – F.T., H.C., K.Ş., C.Ç., K.T., Y.S., M.Ö., B.K.K.; Literature Search – F.T., H.C., K.Ş., C.Ç., K.T., Y.S., M.Ö., B.K.K.; Writing – F.T., Y.S., M.Ö., B.K.K.; Critical Reviews – F.T., Y.S., M.Ö., B.K.K.

Conflict of interest statement: The authors declared no conflict of interest.

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