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Investigation of Antioxidant, Anticholinesterase Inhibitory, Tyrosinase Inhibitory and Urease Inhibitory Activities of Some Hydrazone Derivatives

Bazı Hidrazon Türevlerinin Antioksidan, Antikolinesteraz İnhibisyon, Tirozinaz İnhibisyon ve Üreaz İnnhibisyon Aktivitelerinin Araştırılması

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Abstract

In this study was aimed to investigate antioxidant, anticholinesterase inhibitory, tyrosinase inhibitory and urease inhibitory activities of the synthesized fifteen hydrazone compounds (**1-15**). According to the antioxidant activity assay results, compound **8** (IC_{50}= 22.40±0.87 µM) and **6** (IC_{50}= 29.48±0.71 µM) showed the best lipid peroxidation inhibitory activity. The compound **6** and **8** exhibited better activity IC_{50} value of 21.34±0.11 and 25.33±0.27 µM, respectively, than standard BHT (IC_{50}= 54.97±0.99 µM) in DPPH free scavenging activity. Among the tested compound, compound **6** (IC_{50}= 11.71±0.28 µM) and **8** (IC_{50}= 16.45±0.31 µM) showed the best cation radical scavenging activity. Compound **6**, **11**, **10**, **8**, **1**, **7**, and **14** indicated the best CUPRAC capacity activity with an $A_{0.5}$ value of 15.12±0.00, 17.91±0.01, 21.18±0.00, 23.25±0.03, 24.29±0.01, 26.83±0.01, and 32.35±0.02 µM, respectively, than the standard antioxidants, *α*-tocopherol ($A_{0.5}$=40.55±0.04 µM) and BHA ($A_{0.5}$=32.71±0.02 µM) using as standards. Compound **14** (IC_{50}=11.38±0.44 µM) and **6** (IC_{50}=18.77±0.61 µM) showed the highest acetylcholinesterase inhibitory activity. Among them, compound **2**, **14**, **6**, **3**, **13**, **8**, **10**, **11**, and **12** were determined to have butyrylcholinesterase inhibitory activity IC_{50} value of 24.12±0.65, 27.46±0.44, 29.33±0.21, 30.26±0.05, 35.60±0.53, 37.42±0.48, 39.44±0.74, 42.75±0.22, and 45.40±0.76 µM, respectively, than the standard galantamine (IC_{50}=44.03±0.14 µM). According to the tyrosinase assay results, compound **3** (IC_{50}=12.20±0.44 mM), **4** (IC_{50}=14.76±0.90 mM), **15** (IC_{50}=16.28±0.41 mM), **5** (IC_{50}=18.39±0.87 mM), and **2** (IC_{50}=20.12±0.33 mM) exhibited the best tyrosinase inhibitory activity. Compound **6** (IC_{50}=19.20±0.48 mM) and **14** (IC_{50}=22.62±0.38 µM) founded that the urease inhibitory activity are significant when compared with the thiourea (IC_{50}=23.08±0.19 µM).

Key Words: Hydrazone, antioxidant activity, anticholinesterase inhibitory activity, tyrosinase inhibitory activity, urease inhibitory activity.

Öz

Bu çalışmada, 15 hidrazon molekülünün (**1-15**) antioksidan, antikolinesteraz inhibisyon, tirozinaz inhibisyon ve üreaz inhibisyon aktivitelerinin araştırılması amaçlandı. Antioksidan aktivite test sonuçlarına göre, **8** (IC_{50}=22.40±0.87 µM) ve **6** (IC_{50}=29.48±0.71 µM) numaralı moleküller en iyi lipit peroksidaz inhibisyon aktivitesine sahip olduğunu gösterdi. **6** ve **8** numaralı moleküllerin DPPH serbest radikal giderim aktivitesi, standart BHT'ya (IC_{50}=54.97±0.99 µM) göre sırasıyla 21.34±0.11 ve 25.33±0.27 µM'lik IC_{50} değerleri ile test edilen moleküllere istinaden daha iyi aktivite sergilediği saptandı. Test edilen moleküller arasında **6** (IC_{50}=11.71±0.28 µM) ve **8** (IC_{50}=16.45±0.31 µM) numaralı moleküllerin en iyi katyon radikal giderim aktivitesine sahip olduğunu bulundu. **6**, **11**, **10**, **8**, **1**, **7** ve **14** numaralı moleküller *α*-tokoferol ($A_{0.5}$=40.55±0.04 µM) ve BHA ($A_{0.5}$=32.71±0.02 µM) antioksidan standartlarına göre 15.12±0.00, 17.91±0.01, 21.18±0.00, 23.25±0.03, 24.29±0.01, 26.83±0.01 ve 32.35±0.02 µM'lik $A_{0.5}$ değerleriyle en iyi CUPRAC kapasite aktivitesine sahip olduğu saptandı. **14** (IC_{50}=11.38±0.44 µM) ve **6** (IC_{50}=18.77±0.61 µM) numaralı moleküller en iyi asetilkolinesteraz inhibisyon aktivitesi sergilerken, **2**, **14**, **6**, **3**, **13**, **8**, **10**, **11** ve **12** numaralı moleküllerin ise BChE standardı olarak kullanılan galantamine (IC_{50}=44.03±0.14 µM) göre sırasıyla 24.12±0.65, 27.46±0.44, 29.33±0.21, 30.26±0.05, 35.60±0.53, 37.42±0.48, 39.44±0.74, 42.75±0.22, and 45.40±0.76 µM'lik IC_{50} değerleriyle en iyi butirilkolinesteraz inhibisyon aktivitesine sahip oldukları belirlendi. Tiroazinaz test sonuçlarına göre, **3** (IC_{50}=12.20±0.44 mM), **4** (IC_{50}=14.76±0.90 mM), **15**

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(IC_{50}=16.28±0.41 mM), **5** (IC_{50}=18.39±0.87 mM), ve **2** (IC_{50}=20.12±0.33 mM) numaralı moleküllerin en iyi tirozinaz inhibisyon aktivitesi sergilendiği bulundu. Test edilen hidrazon moleküllerinin üreaz inhibisyon aktivitesi standart olarak kullanılan tiyoüre (IC_{50}=23.08±0.19 µM) ile mukayese edilediğinde **6** (IC_{50}=19.20±0.48 mM) ve **14** (IC_{50}=22.62±0.38 µM) numaralı hidrazon moleküllerinin daha iyi olduğu tespit edildi.

Anahtar Kelimeler: Hidrazon, antioksidan aktivite, antikolinesteraz inhibisyon aktivite, tirozinaz inhibisyon aktivite, üreaz inhibisyon aktivite.

1. Introduction

Hydrazones are a special member of Schiff bases which have azomethine (-HC = N-) group. They are an important class of organic compounds with the general $R_2C=NNH_2$ form. The presence of two connected nitrogen atoms separate hydrazones from the member of Schiff bases such as imines, oximes etc. The pharmacological effects of hydrazone derivative compounds such as antimicrobial (Vicini et al. 2002), analgesic (Salgın-Gökşen et al. 2007), antihypertensive (Emilsson and Selander 1988), antiinflamatuar (Todeschini et al. 1998), anticonvulsant (Dimmock et al. 1999), antidepressant Ergenç and Günay 1998), anticancer (Gürsoy and Güzeldemirci-Ulusoy 2007), antimalarial (Melnyk et al. 2006) and antiviral (El-Sabbagh and Rady 2009) are at the forefront.
In recent years, researchers have been directed to develop new agents, due to the emerging resistance against antioxidant, anticholinesterase, anti-tyrosinase and anti-urease drugs as well as many existing drugs used in clinical practice. For this reason, in the present study 15 hydrazone molecules were synthesized by condensing the substituted aromatic aldehydes with hydrazine monohydrates in order to develop an agent with a less toxic and more efficient biological activity. Antioxidant, anticholinesterase, tyrosinase and urease inhibitory activities of the synthesized molecules were investigated.

2. Material and Methods

All chemicals used in synthesis and biological activity assays were purchased from E. Merck (Darmstadt, Germany), Sigma-Aldrich (St. Louis, MO, USA), FlukaChemie (FlukaChemie GmbH, Sternheim, Germany). The reactions and the purities of the compounds were monitored by thin layer chromatography (TLC) on silica gel 60 F254 aluminum sheets purchased from Merck (Darmstadt, Germany). The synthesized hydrazone compounds (**1-15**) were reported in previous studies (Stroh 1957, Shastin et al. 2001, Maaskant 1937, Bower and Doyle 1957, Meister 1908, Abou Off et al. 1973, Troger and Lange 1920, Tuktarov et al. 2012, Takase and Kai 1997, Niyaz et al. 2009, Reed et al. 1950, Yoneda et al. 1976, Kern et al. 1957). The biological activity tests were done by using Molecular Devices Spectra Max PC340 microplate reader (Sunnyvale, CA, USA).

2.1 Synthesis of Hydrazone Molecules

Substitute aromatic aldehyde (0.05 mol) in ethanol was added to the solution of of hydrazine monohydrate (0.1 mol, 98%). The mixture was refluxed on a water bath for 30 min. After cooling, the precipitate was collected, washed with distilled water, and recrystallized from ethanol (Rollas et al. 2002). The synthesis pathway of hydrazone molecules involved in this study is shown in Fig. 1.

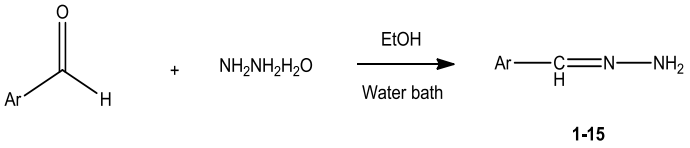


Figure 1. Synthesis pathway of hydrazone molecules.

2.2 Pharmacology

The antioxidant, anticholinesterase inhibitory, tyrosinase inhibitory and urease inhibitory activities of synthesized hydrazone compounds (**1-15**) were evaluated. Solutions of test compounds were prepared at concentrations of 50, 100, 200 and 400 µM in ethanol. The sample concentration, which provides 50% biological activity (IC_{50}), was calculated from the graph of % activity aganist sample concentration.

2.2.1 Antioxidant Activity Assays of Synthesized Hydrazone Molecules

In the total antioxidant activity assay, the synthesized hydrazone compounds (1-15) were evaluated using *β*-carotene-linoleic acid model assay system (Miller 1971). Additionally, the free radical scavenging activity and cation radical scavenging activity was determined by DPPH (1,1-diphenyl-2-picrylhydrazyl) (Blois 1958) and $ABTS^{+\cdot}$ (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (Re et al. 1989) assay, respectively. The cupric-reducing antioxidant capacity was determined according to the method of Apak et al. (2004). DMSO was used as negative control while *α*-tocopherol, butylated hydroxyl toluene (BHT) and butylated hydroxyl anisole (BHA) were used as positive controls.

2.2.2 Determination of Acetylcholinesterase- (AChE), Butyrylcholinesterase- (BChE), Tyrosinase and Urease Inhibitory Activity

AChE and BChE inhibitory activities of synthesized hydrazone compounds (**1-15**) were measured by the spectrophotometric method using Ellman et al. (1961) procedure. Galantamine was used as a positive control for both assays. The tyrosinase inhibitory activity assay was evaluated using as previously described methods (Hearing 1987 and Khatib et al. 2005). The urease inhibitory activity assay was determined according to the method of Weatherburn (1967).

2.3 Statistical Analysis

Results of the antioxidant activity (Table 1), anticholinesterase, tyrosinase, and urease inhibitory activities data (Table 2) were determined by the averages of triplicate analyses. Data were recorded as mean ± standard error of mean (SEM). Significant differences between means were determined by Student's *t*-test, while $p<0.05$ were regarded as significant.

Table 1. The code, name and Ar structure of hydrazone molecules.

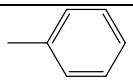
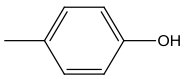
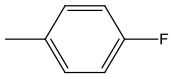
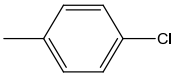
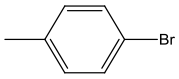
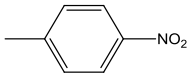
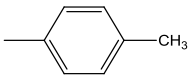
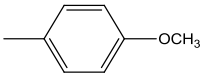
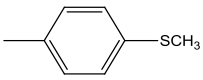
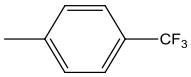
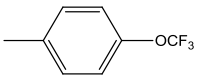
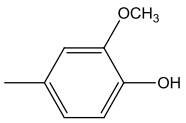
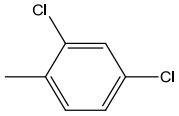
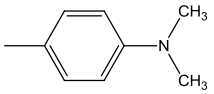
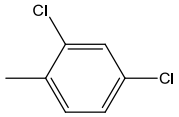
Compound Code	Compound Name	Ar
1	Phenylhydrazone (Stroh 1957)	
2	4-(hydroxyphenyl)hydrazone (Franzen and Eichler 1911)	
3	4-(fluorophenyl)hydrazone (Shastin et al. 2001)	
4	4-(chlorophenyl)hydrazone (Maaskant 1937)	
5	4-(bromophenyl)hydrazone (Bower and Doyle 1957)	
6	4-(nitrophenyl)hydrazone (Meister 1908)	
7	4-(methylphenyl)hydrazone (Abou Off et al. 1973)	
8	4-(methoxyphenyl)hydrazone (Troger and Lange 1902)	
9	4-(thiomethoxyphenyl)hydrazone (Tuktarov et al. 2012)	
10	4-(trifluoromethylphenyl)hydrazone (Takase and Kai 1997)	
11	4-(trifluorometoksiphenyl)hydrazone (Niyaz et al. 2009)	
12	3-methoxy-4-hydroxyphenylhydrazone (Reed et al.1950)	
13	3,4-(dichlorophenyl)hydrazone (Yoneda et al. 1976)	
14	4-(dimethylaminophenyl)hydrazone (Kern et al. 1957)	
15	2,4-(dichlorophenyl)hydrazone (Kern et al. 1957)	

Table 2. Antioxidant activity results of compounds (**1-15**)[a].

Compound	Antioxidant Activity Assay			
	β-carotene/linoleic acid assay (IC_{50} μM)	DPPH· assay (IC_{50} μM)	$ABTS^{+\cdot}$ assay (IC_{50} μM)	CUPRAC assay ($A_{0,5}$ μM)
1	42.10±0.29	35.91±0.14	37.04±0.93	24.29±0.01
2	63.34±0.12	61.23±0.05	45.82±0.27	54.36±0.01
3	56.20±0.47	53.60±0.18	44.30±0.18	52.02±0.02
4	60.72±0.75	58.69±0.66	46.99±0.70	57.40±0.00
5	77.38±0.46	75.92±0.74	51.05±0.64	61.63±0.02
6	29.48±0.71	21.34±0.11	11.71±0.28	15.12±0.00
7	39.82±0.14	38.06±0.46	33.72±0.61	26.83±0.01
8	22.40±0.87	25.33±0.27	16.45±0.31	23.25±0.03
9	47.64±0.32	47.45±0.19	41.07±0.16	47.75±0.00
10	35.35±0.20	28.77±0.85	20.04±0.66	21.18±0.00
11	33.63±0.81	32.09±0.40	23.21±0.40	17.91±0.01
12	50.46±0.55	44.83±0.32	39.48±0.25	41.58±0.00
13	73.77±0.26	71.28±0.37	53.44±0.21	64.77±0.01
14	45.73±0.68	43.09±0.09	29.50±0.45	32.35±0.02
15	69.22±0.13	64.74±0.44	48.64±0.33	59.99±0.01
α-Tocopherol[b]	4.50±0.09	12.26±0.07	4.87±0.45	40.55±0.04
BHT[b]	2.34±0.09	54.97±0.99	2.91±0.55	4.00±0.04
BHA[b]	1.34±0.04	19.40±0.47	4.10±0.06	35.71±0.02

[a]IC_{50} and $A_{0.5}$ values represent the means ± S.E.M. of three parallel measurements ($p<0.05$).

[b]Reference compound.

3. Results

3.1 Antioxidant Activity of Synthesized Compounds

The antioxidant activity results of hydrazine compounds (**1-15**) are given in Table 2. Compound **8** (IC_{50}= 22.40±0.87 μM) and **6** (IC_{50}= 29.48±0.71 μM) showed the highest lipid peroxidation inhibitory activity, followed by **11**, **10**, and **7** with IC_{50} value of 33.63±0.81, 35.35±0.20, and 39.82±0.14 μM, respectively. In DPPH assay results, compounds **6**, **8**, **10**, **11**, **1**, **7**, **14**, **12**, **9**, and **3** exhibited better activity with IC_{50} value of 21.34±0.11, 25.33±0.27, 28.77±0.85, 32.09±0.40, 35.91±0.14, 38.06±0.46, 43.09±0.09, 44.83±0.32, 47.45±0.19, and 53.60±0.18 μM, respectively, than the positive standard BHT (IC_{50}= 54.97±0.99 μM). In ABTS assay results, among the all tested compounds, the compounds **6** (IC_{50}= 11.71±0.28 μM), **8** (IC_{50}= 16.45±0.31 μM), **10** (IC_{50}= 20.04±0.66 μM), **11** (IC_{50}= 23.21±0.40 μM), and **14** (IC_{50}= 29.50±0.45 μM) showed the best cation radical scavenging activity. In the CUPRAC assay results, compounds **6**, **11**, **10**, **8**, **1**, **7**, and **14** indicated the best activity with $A_{0.5}$ value of 15.12±0.00, 17.91±0.01, 21.18±0.00, 23.25±0.03, 24.29±0.01, 26.83±0.01, and 32.35±0.02 μM, respectively, than the positive standards *α*-tocopherol ($A_{0.5}$=40.55±0.04) and BHA ($A_{0.5}$=32.71±0.02).

3.2 Acetyl- and Butyryl-cholinesterase Inhibitory Activity of Synthesized Compounds

The anticholinesterase inhibitory activity results of hydrazine compounds (**1-15**) are given in Table 3. Compounds **14** (IC_{50}= 11.38±0.44 μM) and **6** (IC_{50}= 18.77±0.61 μM) showed the highest acetylcholinesterase inhibitory activity, followed by **2**, **13**, and **3** with IC_{50} value of 22.93±0.17, 24.17±0.11, and 25.64±0.52 μM, respectively. In BChE assay, compounds **2**, **14**, **6**, **3**, **13**, **8**, **10**, **11**, and **12** exhibited better activity with IC_{50} value of 24.12±0.65, 27.46±0.44, 29.33±0.21, 30.26±0.05, 35.60±0.53, 37.42±0.48, 39.44±0.74, 42.75±0.22, and 45.40±0.76 μM, respectively, than the positive standard galantamine (IC_{50}= 44.03±0.14 μM).

3.3 Tyrosinase Inhibitory Activity of Synthesized Compounds

The tyrosinase inhibitory activity results of hydrazine compounds (**1-15**) are given in Table 3. As can be seen from the tyrosinase assay results among the all tested samples, the compounds **3** (IC_{50}=12.20±0.44 mM), **4** (IC_{50}=14.76±0.90 mM), **15** (IC_{50}=16.28±0.41 mM), **5** (IC_{50}=18.39±0.87 mM), and **2** (IC_{50}=20.12±0.33 mM) showed the best tyrosinase inhibitory activity.

Table 3. Anticholinesterase, tyrosinase and urease inhibitory activity results of compounds (**1-15**)[a].

Compound	Anticholineterase inhibitory activity		Tyrosinase inhibitory activity	Urease inhibitory activity
	AChE assay (IC_{50} µM)	BChE assay (IC_{50} µM)	Tyrosinase assay (IC_{50} mM)	Urease assay (IC_{50} µM)
1	51.36±1.04	53.71±0.26	38.43±0.60	69.33±0.69
2	22.93±0.17	24.12±0.65	20.12±0.33	72.26±0.57
3	25.64±0.52	30.26±0.05	12.20±0.44	59.73±0.62
4	57.41±0.29	55.68±0.88	14.76±0.90	55.60±0.39
5	72.83±0.19	60.34±0.21	18.39±0.87	45.10±0.64
6	18.77±0.61	29.33±0.49	28.43±0.59	19.20±0.48
7	49.23±0.38	47.36±1.26	35.72±0.82	63.49±0.68
8	34.47±0.72	37.42±0.48	22.55±0.16	66.08±0.14
9	55.04±0.68	51.09±0.61	40.60±0.75	65.75±0.09
10	44.19±0.26	39.44±0.74	25.30±0.66	24.67±0.94
11	39.80±0.35	42.75±0.22	33.10±0.41	39.75±0.52
12	53.85±1.11	45.40±0.76	42.98±0.52	37.30±0.43
13	24.17±0.11	35.60±0.53	48.86±0.35	43.83±0.66
14	11.38±0.44	27.46±0.44	45.02±1.08	22.62±0.38
15	64.26±0.88	62.80±0.95	16.28±0.41	49.87±0.21
Galantamine[b]	4.48±0.78	46.03±0.14	NT	NT
Kojic acid[b]	NT	NT	0.64±0.12	NT
L-mimosine[b]	NT	NT	0.67±0.06	NT
Thiourea[b]	NT	NT	NT	23.08±0.19

[a]IC_{50} values represent the means ± S.E.M. of three parallel measurements ($p<0.05$).

[b]Reference compound.

NT: Not tested

3.3. Urease Inhibitory Activity of Synthesized Compounds

The urease inhibitory activity results of hydrazine compounds (**1-15**) are also shown in Table 3. In the urease assay, compounds **6** and **14** exhibited the best activity with IC_{50} value of 19.20±0.48 and 22.62±0.38 µM, respectively, than the thiourea positive standard (IC_{50}=23.08±0.19).

4. Discussion

The *in vitro* antioxidant activity, anticholinesterase, tyrosinase and urease inhibitory activities of synthesized hydrazone compounds (**1-15**) have been reported in this study for the first time. The hydrazone compounds (**1-15**) indicated good activity against antioxidant assays. In general, compound **6** and **8** exhibited better activity in all antioxidant assays. Compound **14** and **6** have founded more anticholinesterase agents by indicating inhibitory activity against acetylcholinesterase and butyrylcholinesterase enzymes. Therefore, compound **14** and **6** may be used as anticholinesterase pharmaceutical agents in AChE and BChE, respectively. It can be concluded that the particularly compound **3** can be the potential candidate for the treatment of melanin biosynthesis related skin diseases, likely hyper-pigmentation of human as well as animals. Compound **6** (IC_{50}=19.20±0.48 µM) and **14** (IC_{50}=22.62±0.38 µM) are important to note that the urease inhibitory activities of are significant when compared with the thiourea (IC_{50}=23.08±0.19 µM). Nowadays clinically, compound **6** and **8** can be used as a naturally occurring product to prevent or treat the symptoms caused by urease.

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