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Araştırma / Original Paper

Some Urea and Thiourea Derivatives Bearing 1,2,4-Triazole Ring and Their Anti-Acetylcholinesterase Activities

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ÖZET

1,2,4-Triazol halkası taşıyan bazı üre ve tiyoüre türevleri ve anti-asetilkolinesteraz aktiviteleri

Amaç: Yirmi adet farklı tiyoüre ve üre türevleri sentezlenmiş ve asetilkolinesteraz enzimini (AChE) inhibe etme yetenekleri Ellman'ın modifiye spektrofotometrik yöntemi ile değerlendirilmiştir.
Yöntem: Anti-asetilkolinesteraz aktivite tayini Ellman'ın modifiye edilmiş spektrofotometrik yöntemi kullanılarak yapılmıştır. Bu spektrofotometrik yöntem bir kromojenik reaktif olan 5,5- dithio-bis-(2-nitrobenzoik asit) ile salınan tiyokolinin renkli bir ürün vermesi esasına dayanır.
Bulgular: Sentezlenen bileşiklerin (**1a-e, 2a-e, 3a-e ve 4a-4e**) anti-asetilkolinesteraz aktivite tayini Ellman'ın modifiye edilmiş spektrofotometrik yöntemi kullanılarak yapılmıştır. Test edilen bileşikler arasında, (4-{[(4-triflorometilfenil)karbamoil]amino} fenil)asetik asit (**1d**), en yüksek aktivite gösteren bileşik olmuştur. Bileşik **1d**'nin 0.1mM konsantrasyonda inhibisyon oranı %48.55 olarak hesaplanmıştır.
Sonuç: Anti-asetilkolinesteraz aktivite tarama sonuçları incelediğinde, fenil halkasının 4. konumunda triflorometil grubu taşıyan bileşik **1d**'nin kaydadeğer anti-asetilkolinesteraz aktivite gösterdiği tespit edilmiştir. Aktivite sonuçları incelendiğinde, fenil halkası üzerinde halojen taşıyan yapıların anti-asetilkolinesteraz aktiviteyi arttırıcı yönde katkı sağladığı gözlenmektedir.
Anahtar sözcükler: Üre, tiyoüre, 1,2,4-triazol, anti-asetilkolinesteraz aktivite

ABSTRACT

Some urea and thiourea derivatives bearing 1,2,4-triazole ring and their anti-acetylcholinesterase activities

Objective: Twenty different urea and thiourea derivatives were synthesized and evaluated for their ability to inhibit acetylcholinesterase (AChE) using a modification of Ellman's spectrophotometric method.
Methods: Anti-acetylcholinesterase activity was evaluated by using a modification of Ellman's spectrophotometric method. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent 5,5-dithio-bis-(2-nitrobenzoic acid).
Results: The anti-acetylcholinesterase effects of the compounds (**1a-e, 2a-e, 3a-e and 4a-4e**) were determined by modified Ellman's spectrophotometric method. Among these compounds, (4-{[(4-trifluoromethylphenyl)carbamoyl]amino}phenyl)acetic acid (**1d**), was found as the most active compound. The inhibition percentages were calculated 48.55% at 0.1 mM concentrations for compound **1d**.
Conclusion: The anti-acetylcholinesterase activity screening indicated that among the tested compounds, **1d** bearing 4-trifluoromethyl group on the phenyl ring, showed noteworthy anti-acetylcholinesterase activity. Based on the activity results, it appears that halogen atoms on the phenyl ring have made good contribution to the anti-acetylcholinesterase activity.
Keywords: Urea, thiourea, 1,2,4-triazole, anti-acetylcholinesterase activity

INTRODUCTION

Acetylcholinesterase (AChE) has proven to be the most viable therapeutic target for symptomatic improvement in Alzheimer's disease (AD) because cholinergic deficit is a consistent and early finding in AD. Inhibition of AChE was considered to be achievable as a therapeutic target because of proven efficacy of inhibition of peripheral AChE as a

treatment for myasthenia gravis (MG) proving that the approach was feasible. However, selective inhibition of the central nervous system (CNS) AChE has initially been proved to be daunting. Before tacrine, physostigmine, the classic AChE inhibitor was investigated as a treatment for AD. Physostigmine was subsequently abandoned because of poor tolerability. Four drugs are currently available for AD treatment: galantamine, rivastigmine, donepezil, and memantine. The first three are AChE inhibitors and memantine is not (1).

For a quarter of a century, the pathogenesis of AD has been linked to a deficiency in the brain neurotransmitter acetylcholine (ACh). This was based on observations that correlated cholinergic system abnormalities with intellectual impairment (2). Subsequently, the 'cholinergic hypothesis' of AD gained considerable acceptance. It stated that a serious loss of cholinergic function in the central nervous system contributed to cognitive symptoms (3). Over the years, both evidence for and challenges to the relationship between acetylcholine dysfunction and AD have been put forward (4).

A progressive reduction in cholinergic neurons in some areas of the brain such as cortex and hippocampus is related to the deficits in memory and cognitive function in AD. This observation led to the development of therapeutic agents that function as AChE inhibitors in central nervous system. In fact, these agents prolong the duration of action of acetycholine and render symptomatic relief in this disorder (5-9). In addition urea and thiourea play important role for anti-AChE activity (10-11) on the another hand 1,2,4 triazoles have anti-AChE activity (12-14). In the present study, we evaluated various urea and tiourea derivatives for their anti-AChE activity.

MATERIALS AND METHODS

Synthesis of Test Compounds

A series of new thiourea and urea derivatives bearing 1,2,4-triazole ring were prepared according to Figure 1. The

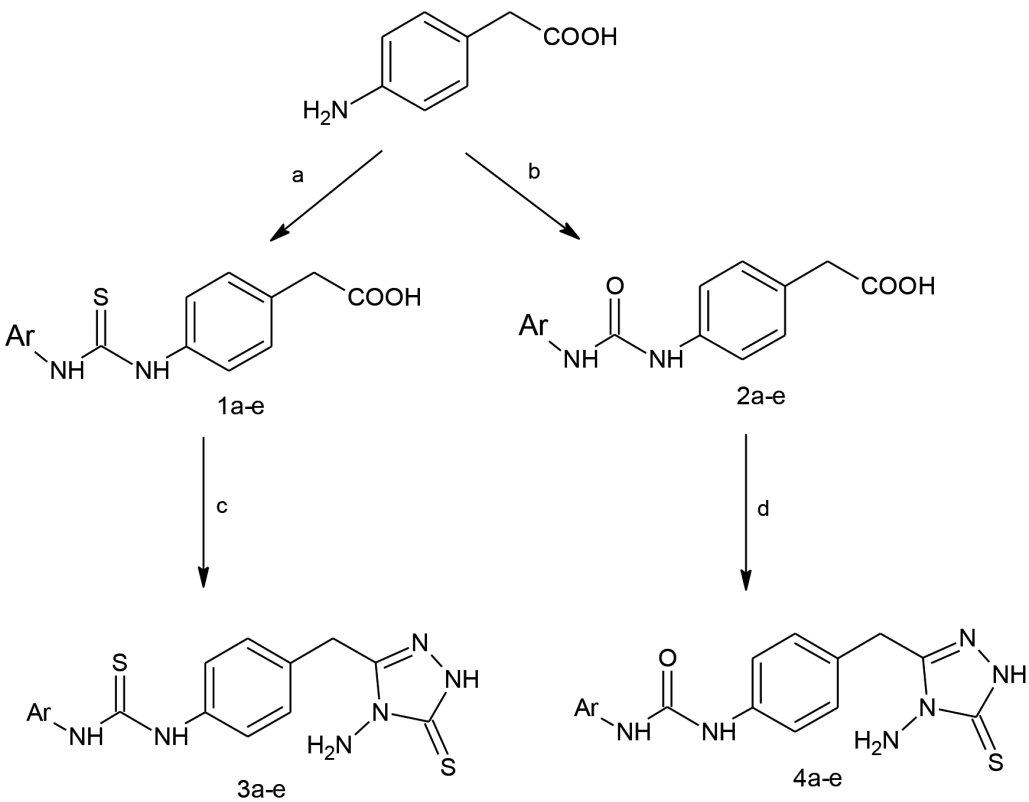

Figure 1: General Synthetic Route for Title Compounds **1a-e, 2a-e, 3a-e** and **4a-e**.
Reagents and conditions: (a) Ar-NCS, 100°C; (b) Ar-NCO, 100°C; (c), (d) NH2NHCSNHNH2, 130-140°C

Table 1: Structure and Physical Data of Title Compounds **1a-e, 2a-e, 3a-e** and **4a-e**.

Comp.	Ar	M.P. (°C)	M.F.	M.W.
1a	2,4,6-Cl-C_6H_2	214-215	$C_{15}H_{11}Cl_3N_2O_2S$	389.68
1b	2,6-Cl_2-C_6H_4	206-207	$C_{15}H_{13}ClN_2O_2S$	320.79
1c	4-CH_3S-C_6H_4	193-194	$C_{16}H_{16}N_2O_3S$	316.37
1d	4-CF_3-C_6H_5	240-241	$C_{15}H_{13}N_3O_4S$	331.34
1e	4-NO_2-C_6H_5	200-201	$C_{15}H_{13}FN_2O_2S$	304.34
2a	2,4,6-Cl-C_6H_2	276-277	$C_{15}H_{11}Cl_3N_2O_3$	373.62
2b	2,6-Cl_2-C_6H_4	258-259	$C_{15}H_{13}ClN_2O_3$	304.73
2c	4-CH_3S-C_6H_4	234-235	$C_{16}H_{16}N_2O_4$	300.31
2d	4-CF_3-C_6H_5	250-251	$C_{15}H_{13}N_3O_5$	315.28
2e	4-NO_2-C_6H_5	245-246	$C_{15}H_{13}FN_2O_3$	288.27
3a	2,4,6-Cl-C_6H_2	227-228	$C_{16}H_{13}Cl_3N_6S_2$	459.80
3b	2,6-Cl_2-C_6H_4	206-207	$C_{16}H_{15}ClN_6S_2$	390.85
3c	4-CH_3S-C_6H_4	228-230	$C_{17}H_{18}N_6OS_2$	386.56
3d	4-CF_3-C_6H_5	240-241	$C_{15}H_{17}N_7O_2S_2$	401.47
3e	4-NO_2-C_6H_5	234-236	$C_{16}H_{15}FN_6S_2$	374.46
4a	2,4,6-Cl-C_6H_2	237-238	$C_{16}H_{13}Cl_3N_6OS$	443.74
4b	2,6-Cl_2-C_6H_4	258-259	$C_{16}H_{15}ClN_6OS$	374.85
4c	4-CH_3S-C_6H_4	180-182	$C_{17}H_{19}N_6O_2S$	371.43
4d	4-CF_3-C_6H_5	214-216	$C_{16}H_{15}N_7O_3S$	385.40
4e	4-NO_2-C_6H_5	240-241	$C_{16}H_{15}FN_6OS$	358.39

Comp: Compound, Ar: Substituted phenyl, M.P.: Melting Point, M.W.: Molecular Weight

thiourea derivatives (**1a-e**) were prepared by reacting 4-(aminophenyl) acetic acid with corresponding isothiocyanate. Compounds **2a-e** were prepared by refluxing equimolar 4-(aminophenyl) acetic acid and various isocyanates in acetone. The reaction of the compounds **1a-e** and **2a-e** with thiocarbohydrazide in oil bath afforded the corresponding 1,2,4-triazoles (**3a-e** and **4a-e**). Physicochemical and spectroscopic characterization of all compounds have been previously described (15,16). The purities of the synthesized compounds were checked by reversed phase HPLC (Chromasil C_{18} 3.6x150 mm column using acetonitrile and water (70:30 v/v) as the eluent). All compounds showed a single and sharp peak with a retention time of 4.120-6.269 min. Physical and chemical properties of all compounds are presented in Table 1.

Pharmacology

AChE Inhibition

All compounds were subjected to a slightly modified method of Ellman's test (17) in order to evaluate their potency to inhibit the AChE. The spectrophotometric method is based on the reaction of released thiocholine to give a coloured product with a chromogenic reagent 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB). AChE, (E.C.3.1.1.7 from Electric Eel, 500 units), and Donepezil hydrochloride were purchased from Sigma–Aldrich (Steinheim, Germany). Potassium dihydrogen phosphate, DTNB, potassium hydroxide, sodium hydrogen carbonate, gelatine, acetylthiocholine iodide (ATC) were obtained from Fluka (Buchs, Switzerland). Spectrophotometric measurements were performed on a 1700 Shimadzu UV-1700 UV–Vis spectrophotometer. Cholinesterase activity of the compounds (**1a-e, 2a-a, 3a-e** and **4a-e**) was measured in 100 mM phosphate buffer (pH 8.0) at 25°C, using ATC as substrate, respectively. DTNB (10 mM) was used in order to observe absorbance changes at 412 nm. Donepezil hydrochloride was used as a positive control (18).

Enzymatic assay

Enzyme solutions were prepared in gelatin solution (1%), at a concentration of 2.5 units/mL. AChE and compound solution (50 µL) which is prepared in 2 % DMSO at a concentration range of 10^{-1}-10^{-6} mM were added to 3.0 mL phosphate buffer (pH 8±0.1) and incubated at 25 °C for 5 min. The reaction was started by adding DTNB (50 µL) and ATC (10 µL) to the enzyme-inhibitor mixture. The production

Table 2: AChE inhibition (%) of the tested compounds and their IC_{50} values

Comp.	AChE Inhibition (%)		
	1 mM	0.1 mM	IC_{50} (mM)
1a	ND	20.21±2.12	> 1
1b	24.77±2.23	ND	> 1
1c	ND	ND	ND
1d	ND	48.55±3.52	> 1
1e	17.83±1.20	ND	ND
2a	ND	ND	ND
2b	ND	ND	ND
2c	15.46±1.56	12.59±3.28	> 1
2d	ND	ND	ND
2e	ND	ND	ND
3a	ND	ND	ND
3b	10.25±1.40	ND	> 1
3c	ND	ND	ND
3d	ND	11.84±3.45	> 1
3e	3.29±1.05	ND	> 1
4a	ND	ND	ND
4b	ND	ND	ND
4c	ND	ND	ND
4d	ND	5.64±1.37	> 1
4e	ND	2.74±1.45	> 1
Donepezil	99.01±4.89	95.52±5.01	0.054±0.002μM

ND: not determined, IC_{50}: The half maximal inhibitory concentration

of the yellow anion was recorded for 10 min at 412 nm. As a control, an identical solution of the enzyme without the inhibitor is processed following the same protocol. The blank reading contained 3.0 mL buffer, 50 μL 2% DMSO, 50 μL DTNB and 10 μL substrate. All processes were assayed in triplicate. The inhibition rate (%) was calculated by the following equation:

Inhibition % = $(A_C - A_I) / A_C \times 100$

Where AI is the absorbance in the presence of the inhibitor, AC is the absorbance of the control and AB is the absorbance of blank reading. Both of the values are corrected with blank-reading value. SPSS for Windows 15.0 was used for statistical analysis. Data were expressed as Mean ± SD.

RESULTS

The anti-AChE effects of the compounds (**1a-e, 2a-e, 3a-e** and **4a-4e**) were determined by modified Ellman's spectrophotometric method (Table 2). Among these compounds, (4-{[(4-trifluoromethylphenyl)carbamoyl] amino}phenyl)acetic acid (**1d**), was found as the most active compound. The inhibition percentages were calculated 48.55% at 0.1 mM concentrations for compound **1d**. IC_{50} values could not be defined for none of the compounds. The inhibition percentages were not determined for compounds **1c, 2a, 2b, 2d, 2e, 3a, 3c, 4a, 4b, 4c,** and these compounds were evaluated as inactive at two tested concentrations. Compound **1a** bearing 2, 4, 6 trichloro phenyl moiety and compound **1b** bearing 2,6-dichloro phenyl moiety exhibited anti-acetylcholinesterase activity with nearly 20% inhibition value. Compound **2c** showed moderate activity with the inhibition percentages 15.46 and 12.59 at 1M and 0.1 mM concentrations. The other compounds **3b, 3d, 3e, 4d** and **4e** showed relatively weak activity and the inhibiton values were found less than 11.90%. Standard drug Donepezil was studied at lower concentrations for the purpose of finding IC_{50} value and it was determined as 0.054 μM. None of the compounds showed comparable activity with donepezil and significant anti-AChE activity contrary to expectations.

CONCLUSION

In conclusion, a series of thiourea and urea derivatives have been synthesized and screened for their anti-AChE activity. The anti-AChE activity screening indicated that among the tested compounds, **1d** bearing 4-trifluoromethyl

group on the phenyl ring, showed noteworthy anti-AChE activity. Based on the activity results, it appears that halogen atoms on the phenyl ring have made good contribution to the anti-AChE activity.

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