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ORIGINAL ARTICLE / ÖZGÜN MAKALE

SYNTHESIS, CHARACTERIZATION AND CHOLINESTERASE INHIBITORY EFFECTS OF IBUPROFEN-BASED SPIROTHIAZOLIDINONES

İBUPROFEN TÜREVİ SPİROTHİAZOLİDİNONLARIN SENTEZİ, KARAKTERİZASYONU VE KOLİNESTERAZ İNHİBİTÖR ETKİLERİ

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ABSTRACT

Objective: *A novel class of spirothiazolidinone derivatives were designed, synthesized, and assessed as possible acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitors for Alzheimer's disease therapy.*
Material and Method: *In this study, novel spirocyclic compounds were synthesized by one-pot reaction. IR, ^{1}H NMR, ^{13}C NMR, LC-MS and elemental analysis techniques have all been used to clarify the chemical structures of the compounds. AChE and BuChE inhibitory effects of compounds (4a-e and, 5a-e) were assayed according to Ellman's method using galantamine as reference.*
Result and Discussion: *According to the inhibition data, compound 4e demonstrated the most promising activity against AChE, with an IC_{50} value of 39.53±1.94 µM, while compound 4b exhibited the most potential against BuChE, with an IC_{50} value of 95.97±1.79 µM.*
Keywords: *Acetylcholinesterase, butyrylcholinesterase, ibuprofen, spirothiazolidinone, synthesis*

ÖZ

Amaç: *Alzheimer hastalığına karşı potansiyel asetilkolinesteraz (AChE) ve butirilkolinesteraz (BuChE) inhibitörleri olarak bir seri yeni spirotiyazolidinon türevi tasarlanmış, sentezlenmiş ve inhibisyon etkileri değerlendirilmiştir.*
Gereç ve Yöntem: *Bu çalışmadan kazanılan yeni spiro bileşikler tek kap yöntemi ile sentez edilmiştir. Bileşiklerin kimyasal yapılarını aydınlatmak için IR, ^{1}H NMR, ^{13}C NMR, LC-MS ve elementel analiz yöntemleri kullanılmıştır. Bileşiklerin (4a-e ve 5a-e), AChE ve BuChE enzimleri üzerindeki inhibisyon etkileri galantamin referans olarak kullanılarak Ellman yöntemine göre analiz edilmiştir.*
Sonuç ve Tartışma: *İnhibisyon sonuçlarına göre, en umut verici bileşik AChE'ye karşı IC_{50} değeri 39.53±1.94 µM olan 4e ve BuChE'ye karşı IC_{50} değeri 95.97±1.79 µM olan 4b bileşikleri olarak kaydedilmiştir.*
Anahtar Kelimeler: *Asetilkolinesteraz, butirilkolinesteraz, ibuprofen, sentez, spirotiyazolidinon*

INTRODUCTION

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A type of dementia known as Alzheimer's disease gradually impairs all cognitive functions, particularly memory. It is identified microscopically by aberrant protein accumulation in the brain [1]. Cholinergic neurotransmission is crucial for regulating cognitive functions, particularly in learning and memory [2]. Cholinesterases (ChE), namely acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) are involved in cholinergic transmission. AChE and BuChE inhibitors indirectly enhance cholinergic transmission by inhibiting the enzyme that hydrolyzes acetylcholine (ACh). Tacrine, the first-generation AChE inhibitor, was withdrawn from use due to its hepatotoxic effects. Donepezil, galantamine and rivastigmine (as shown in Figure 1), which were developed later, and currently approved by the FDA for Alzheimer's disease, are frequently used in the treatment [3].

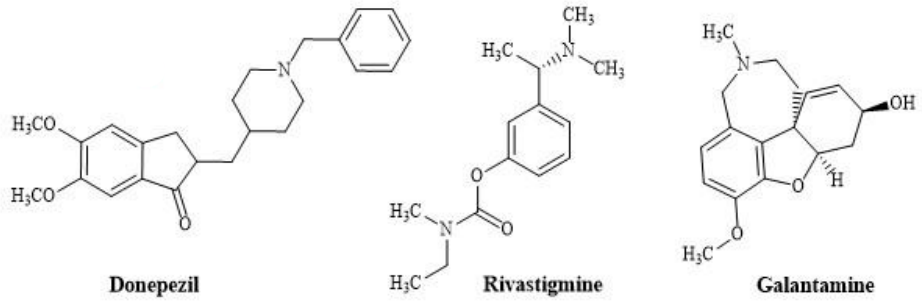


Figure 1. The structures of ChE inhibitors used for the treatment of AD

The chiral 2-arylpropionic acid (2-APA) derivative, ibuprofen, also known as (±)-(R,S)-α-methyl-4-(2-methylpropyl)benzeneacetic acid, is a well-known and often used nonsteroidal anti-inflammatory drug (NSAID) (as shown in Figure 2) [4]. The first discovery of the anti-inflammatory effects of ibuprofen was made in 1961 by Dr. Stewart Adams and the drug was introduced on the market in 1969 in the UK for the treatment of rheumatic arthritis [5].

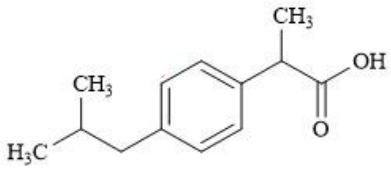


Figure 2. The structure of ibuprofen

Long-term usage of NSAIDs reportedly lowers the risk of Alzheimer's disease [6]. Its effects may be through decreasing the concentrations of amyloid beta peptide (Aβ) (1-42), which is the amyloidogenic form, and it may be associated with behavioral changes observed in AD. *In vitro* studies showed that mouse treated with ibuprofen had a 90% reduction in Aβ plaque and ibuprofen treatment in mouse (regardless of cyclooxygenase-2 inhibition) reduced oxidative stress and plaque load without improving cognitive function by preventing inhibition of the NADPH oxidase 2 complex [7].

4-Thiazolidinones are active compounds that play a crucial role in pharmacological effects. There are numerous studies demonstrating that compounds containing the 4-thiazolidinone ring have antituberculosis [8], anticancer [9,10], anti-inflammatory [11], antidiabetic [12], hypnotic [13], and antiviral activities [14]. Additionally, there are studies on the AChE and BuChE inhibition effects of these compounds are available [15,16].

As previously noted, there is existing literature suggesting that NSAIDs like ibuprofen may help reduce the risk of Alzheimer's disease [6]. The spirothiazolidinone core also has significant potential in

the development of biologically important compounds [17,18]. Encouraged by the above data, it was anticipated that a combination of ibuprofen and spirothiazolidinone structures in one molecule could be interesting to the development of new agents that inhibit cholinesterase activity. In this study, novel 2-[4-(2-methylpropyl)phenyl]propanehydrazide derivatives incorporating a spirothiazolidinone ring were designed, synthesized and assessed for their AChE and BuChE inhibitor effects *in vitro*.

MATERIAL AND METHOD

Chemistry

Chemicals and Instruments

All of the chemicals used were purchased from Aldrich without subsequent purification. The Shimadzu IR Affinity-1 FTIR spectrometer was used to record infrared (IR) spectra. ^{1}H and ^{13}C NMR (APT) spectra were acquired in $CDCl_3$ using a "Bruker 500 MHz" instrument. Chemical shifts are reported as δ (ppm), while coupling constants (*J)* are provided in hertz (Hz). TMS used as the internal standard. Agilent 1260 Infinity II LC/MS mass spectrometer was used to record the mass spectra with electrospray ionization. The Buchi B-540 melting point equipment was used to determine melting points. Elemental analyzer (Thermo Finnigan Flash EA 1112) was used for microanalyses. (sp: spirodecan)

Methyl 2-[4-(2-methylpropyl)phenyl]propanoate (2)

A solution containing sulfuric acid (98%) (0.1 mol) and racemic acid (0.01 mol) in 50 ml of methanol was refluxed over night. The mixture was neutralized with sodium bicarbonate solution (0.01 mol), then extracted with chloroform, and finally dried over anhydrous sodium sulfate. A yellowish oil was obtained by evaporating the solvents in vacuum (CAS number: 61566-34-5) [19].

2-[4-(2-Methylpropyl)phenyl]propanehydrazide (3)

A mixture of 2 (0.1 mol), $H_2NNH_2.H_2O$ (98%, 0.2 mol), and ethanol (50 ml) was heated under reflux for 8 hours. The resulting solid was filtered and utilized without additional purification (CAS number: 127222-69-9) [20].

General procedure for the synthesis of 2-[4-(2-methylpropyl)phenyl]-*N*-[2,8-(non)substituted-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl]propanamides (4, 5)

Using a Dean–Stark water separator, a mixture containing 3 (0.0025 mol), a suitable cyclohexanone (0.0025 mol), and either mercaptoacetic acid or 2-mercaptopropionic acid (0.01 mol) in dry toluene was refluxed for 6–8 hours. In vacuo, extra toluene was evaporated. The solid separated was collected, washed with sodium bicarbonate solution solution and water. After drying, the solid crystallized again from ethanol.

2-[4-(2-Methylpropyl)phenyl]-*N*-(3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (4a)

White crystals. mp: 80-84 °C. yield 80%. IR(KBr): ν_{max} 3417 (NH), 1716 (CO), 1676 (NHCO). ^{1}H-NMR ($CDCl_3$/500 MHz): 7.29 (3H, d, *J*= 7.8 Hz, phenyl $C_{2,6\text{-}H}$ and NH), 7.16 (2H, d, *J* = 8.2 Hz, phenyl $C_{3,5\text{-}H}$), 3.72 (1H, q, *J* = 7.1 Hz, COCH), 3.54, 3.48 (2H, 2d, *J*= 15.9 Hz, $COCH_2$), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.89-1.67; 1.60-1.43 (14H, m, sp-H, CH and CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3). ^{13}C-NMR ($CDCl_3$/125 MHz): 174.1, 168.8, 141.1, 137.7, 129.7, 127.4, 73.1, 45.0, 44.9, 37.3, 37.2, 30.1, 28.5, 24.4, 23.1, 23.0, 22.3, 18.2. LC/MS (m/z): $C_{21}H_{30}N_2O_2S$ requires 374.54 found $[M + H]^+$: 375.2. Anal. calcd. for $C_{21}H_{30}N_2O_2S$ (374.54) C= 67.34, H= 8.07, N= 7.48. Found C= 67.02, H= 8.03, N= 7.46.

2-[4-(2-Methylpropyl)phenyl]-*N*-(8-methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (4b)

White powder. mp: 88-90 °C. yield 76%. IR(KBr): ν_{max} 3415 (NH), 1726 (CO), 1678 (NHCO). ^{1}H-NMR (500 MHz): 7.30 (1H, s, NH), 7.28 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-}H}$), 7.15 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-}H}$), 3.72 (1H, q, *J* = 7.1 Hz, COCH), 3.55, 3.48 (2H, 2d, *J*= 15.9 Hz, $COCH_2$), 2.48 (2H, d,

J= 7.2 Hz, CH_2), 1.88-1.72; 1.64-1.55; 1.27-1.09 (13H, m, sp-H, CH and CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.88 (3H, d, *J*= 7.0 Hz, sp-CH_3). ^{13}C-NMR(125 MHz): 174.1, 168.9, 141.1, 137.7, 129.7, 127.4, 72.9, 44.9, 44.9, 37.0, 36.9, 31.5, 31.4, 31.1, 30.1, 28.5, 22.3, 22.2, 21.7, 18.1. LC/MS (m/z): $C_{22}H_{32}N_2O_2S$ requires 388.56 found $[M + H]^+$: 389.2. Anal. calcd. for $C_{22}H_{32}N_2O_2S$ (388.57) C= 68.00, H= 8.30, N= 7.21. Found C= 68.09, H= 8.25, N= 7.22.

2-[4-(2-Methylpropyl)phenyl]-*N*-(8-ethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (4c)

White powder. mp: 98-101 °C. yield 81%. IR(KBr): ν_{max} 3410 (NH), 1720 (CO), 1676 (NHCO). ^{1}H-NMR (500 MHz): 7.29 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.27 (1H, s, NH), 7.16 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 3.72 (1H, q, *J* = 7.1 Hz, COCH), 3.55, 3.48 (2H, 2d, *J*= 15.9 Hz, $COCH_2$), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.89-1.69; 1.60-1.53; 1.23-1.10 (15H, m, sp-H, CH, CH_2 and CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.88 (3H, t, *J*= 7.0 Hz, CH_2CH_3). ^{13}C-NMR(125 MHz): 174.0, 168.8, 141.1, 137.7, 129.7, 127.4, 73.2, 45.0, 44.9, 37.7, 37.0, 36.9, 30.1, 29.2, 28.5, 22.3, 18.1, 11.4. LC/MS (m/z): $C_{23}H_{34}N_2O_2S$ requires 402.59 found $[M + H]^+$: 403.1. Anal. calcd. for $C_{23}H_{34}N_2O_2S$ (402.59) C= 68.62, H= 8.51, N= 6.96. Found C= 68.98, H= 8.32, N= 6.90.

2-[4-(2-Methylpropyl)phenyl]-*N*-(8-propyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (4d)

White crystals. mp: 89-94 °C. yield 85%. IR(KBr): ν_{max} 3433 (NH), 1705 (CO), 1678 (NHCO). ^{1}H-NMR (500 MHz): 7.28 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.26 (1H, s, NH), 7.15 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 3.71 (1H, q, *J* = 7.1 Hz, COCH), 3.54, 3.48 (2H, 2d, *J*= 15.9 Hz, $COCH_2$), 2.47 (2H, d, *J*= 7.2 Hz, CH_2), 1.88-1.67; 1.60-1.55; 1.30-1.24; 1.19-1.10 (17H, m, sp-H, CH, CH_2 and CH_3), 0.92 (6H, d, *J*= 7.0 Hz, CH_3), 0.87 (3H, t, *J*= 7.0 Hz, CH_2CH_3). ^{13}C-NMR (125 MHz): 174.0, 168.8, 141.1, 137.7, 129.7, 127.4, 73.2, 45.0, 44.9, 38.6, 37.0, 36.9, 35.7, 30.5, 30.1, 29.7, 29.5, 29.4, 28.5, 26.7, 22.3, 20.7, 20.0, 18.2, 14.2. LC/MS (m/z): $C_{24}H_{36}N_2O_2S$ requires 416.61 found $[M + H]^+$: 417.2. Anal. calcd. for $C_{24}H_{36}N_2O_2S$ (416.62) C= 69.19, H= 8.71, N= 6.72. Found C= 69.38, H= 8.50, N= 6.70.

2-[4-(2-Methylpropyl)phenyl]-*N*-(8-*tert*-butyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (4e)

White powder. mp: 178-179 °C. yield 75%. IR(KBr): ν_{max} 3261 (NH), 1716 (CO), 1685 (NHCO). ^{1}H-NMR (500 MHz): 7.29 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.23 (1H, s, NH), 7.16 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 3.71 (1H, q, *J* = 7.1 Hz, COCH), 3.54, 3.48 (2H, 2d, *J*= 15.9 Hz, $COCH_2$), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.90-1.71; 1.59-1.54; 1.27-1.24 (13H, m, sp-H, CH and CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.84 (9H, s, *tert*-butyl CH_3). ^{13}C-NMR (125 MHz): 174.1, 168.8, 141.1, 137.7, 129.7, 127.4, 73.1, 46.5, 45.0, 44.9, 37.4, 37.3, 32.2, 30.1, 28.5, 27.4, 24.0, 23.9, 22.3, 18.2. LC/MS (m/z): $C_{25}H_{38}N_2O_2S$ requires 430.64 found $[M + H]^+$: 431.2. Anal. calcd. for $C_{25}H_{38}N_2O_2S$ (430.651) C= 69.73, H= 8.89, N= 6.51. Found C= 70.00, H= 8.92, N= 6.50.

2-[4-(2-Methylpropyl)phenyl]-*N*-(2-methyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (5a)

White powder. mp: 164-166 °C. yield 78%; IR(KBr): ν_{max} 3259 (NH), 1716 (CO), 1681 (NHCO). ^{1}H-NMR (500 MHz): 7.29 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.28 (1H, s, NH), 7.16 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 3.81, 3.77 (1H, 2q, *J* = 7.1 Hz, sp-COCH), 3.72 (1H, q, *J*= 7.1 Hz, COCH), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.89-1.68; 1.61-1.42 (17H, m, sp-H, CH, CH_3 and sp_2-CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3). ^{13}C-NMR(125 MHz): 174.1, 171.6, 141.1, 137.6, 129.7, 127.4, 71.5, 45.0, 38.2, 37.5, 37.4, 37.3, 37.1, 30.1, 24.4, 23.4, 23.3, 23.0, 22.9, 22.3, 19.9, 19.4, 18.3, 18.2. LC/MS (m/z): $C_{22}H_{32}N_2O_2S$ requires 388.56 found $[M + H]^+$: 389.2. Anal. calcd. for $C_{22}H_{32}N_2O_2S$ (388.57) C= 68.00, H= 8.30, N= 7.21. Found C= 67.82, H= 8.21, N= 7.20.

2-[4-(2-Methylpropyl)phenyl]-*N*-(2,8-dimethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (5b)

White powder. mp: 198-203 °C. yield 80%. IR(KBr): ν_{max} 3259 (NH), 1714 (CO), 1681 (NHCO). ^{1}H-NMR (500 MHz): 7.29 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.21, 7.18 (1H, 2s, NH), 7.16 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 3.79, 3.75 (1H, 2q, *J* = 7.1 Hz, sp-COCH), 3.72 (1H, q, *J*= 7.1 Hz, COCH), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.89-1.68; 1.61-1.42 (16H, m, sp-H, CH, CH_3 and sp_2-CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.88 (3H, d, *J*= 7.0 Hz, sp_8-CH_3). ^{13}C-NMR (125 MHz): 174.0, 171.7, 141.0, 137.6, 129.7, 127.4, 71.4, 45.0, 44.9, 37.9, 37.5, 37.3, 37.1, 36.8, 31.9, 31.7, 31.4, 31.3, 31.1, 30.1, 22.3, 21.7, 19.9, 19.4, 18.2, 18.1. LC/MS (m/z): $C_{23}H_{34}N_2O_2S$ requires 402.59 found $[M + H]^+$: 403.2. Anal. calcd. for $C_{23}H_{34}N_2O_2S$ (402.597) C= 68.62, H= 8.51, N= 6.96. Found C= 68.88, H= 8.68, N= 6.90.

2-[4-(2-Methylpropyl)phenyl]-*N*-(2-methyl-8-ethyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (5c)

White crystals. mp: 195-197 °C; yield 86%. IR(KBr): ν_{max} 3257 (NH), 1716 (CO), 1681 (NHCO). ^{1}H-NMR (500 MHz): 7.29 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.16 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 7.13 (1H, s, NH), 3.79, 3.75 (1H, 2q, *J* = 7.1 Hz, sp-COCH), 3.72 (1H, q, *J*= 7.1 Hz, COCH), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.89-1.52; 1.23-1.10 (18H, m, sp-H, CH, CH_3 and sp_2-CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.84 (3H, t, *J*= 7.0 Hz, sp_8-CH_2CH_3). ^{13}C-NMR (125 MHz): 174.0, 171.6, 141.1, 137.7, 129.7, 127.4, 71.7, 45.0, 44.9, 37.9, 37.7, 37.5, 37.3, 37.1, 36.8, 30.1, 29.5, 29.3, 29.0, 28.9, 22.3, 19.9, 19.4, 18.2, 18.1, 11.4. LC/MS (m/z): $C_{24}H_{36}N_2O_2S$ requires 416.61 found $[M + H]^+$: 417.2. Anal. calcd. for $C_{24}H_{36}N_2O_2S$ (416.62) C= 69.19, H= 8.71, N= 6.72. Found C= 69.30, H= 8.64, N= 6.73.

2-[4-(2-Methylpropyl)phenyl]-*N*-(2-methyl-8-propyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (5d)

White crystals. mp: 193-196 °C. yield 80%. IR(KBr): ν_{max} 3257 (NH), 1712 (CO), 1681 (NHCO). ^{1}H-NMR (500 MHz): 7.30 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.17 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 7.10, 7.08 (1H, 2s, NH), 3.79, 3.75 (1H, 2q, *J* = 7.1 Hz, sp-COCH), 3.71 (1H, q, *J*= 7.1 Hz, COCH), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.89-1.52; 1.31-0.99 (20H, m, sp-H, CH, CH_2, CH_3 and sp_2-CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.88 (3H, t, *J*= 7.0 Hz, sp_8-CH_2CH_3). ^{13}C-NMR(125 MHz): 174.0, 171.6, 141.1, 137.6, 129.7, 127.4, 71.7, 45.0, 44.9, 38.6, 37.9, 37.5, 36.9, 35.7, 30.1, 29.8, 29.7, 29.4, 29.3, 22.3, 20.0, 19.9, 18.2, 14.2. LC/MS (m/z): $C_{25}H_{38}N_2O_2S$ requires 430.64 found $[M + H]^+$: 431.3. Anal. calcd. for $C_{25}H_{38}N_2O_2S$ (430.65) C= 69.73, H= 8.89, N= 6.51. Found C= 69.78, H= 8.82, N= 6.50.

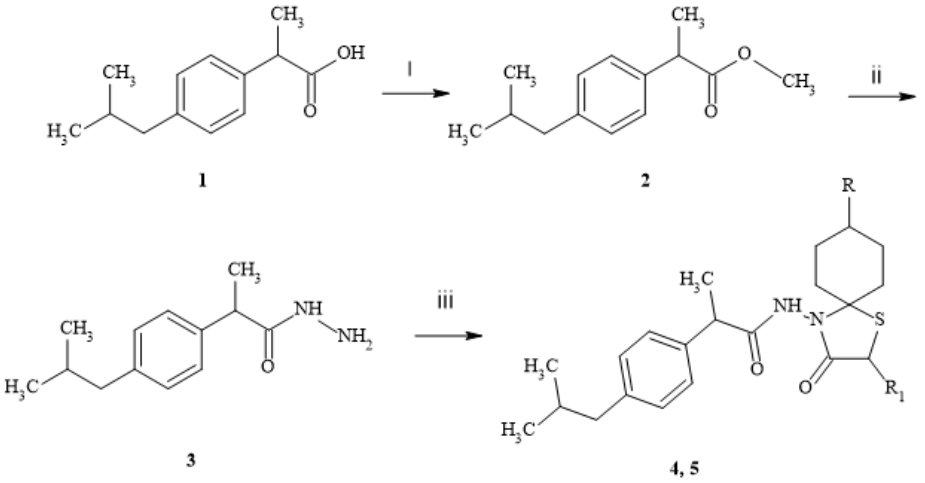
2-[4-(2-Methylpropyl)phenyl]-*N*-(2-methyl-8-*tert*-butyl-3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl) propanamide (5e)

White powder. mp: 203-208 °C. yield 83%. IR(KBr): ν_{max} 3261 (NH), 1716 (CO), 1687 (NHCO). ^{1}H-NMR (500 MHz): 7.30 (2H, d, *J*= 8.1 Hz, phenyl $C_{2,6\text{-H}}$), 7.17 (2H, d, *J*= 8.1 Hz, phenyl $C_{3,5\text{-H}}$), 7.15 (1H, s, NH), 3.81, 3.77 (1H, 2q, *J* = 7.1 Hz, sp-COCH), 3.71 (1H, q, *J*= 7.1 Hz, COCH), 2.48 (2H, d, *J*= 7.2 Hz, CH_2), 1.81-1.51; 1.35-1.17; 0.81-0.71 (16H, m, sp-H, CH, CH_3 and sp_2-CH_3), 0.91 (6H, d, *J*= 7.0 Hz, CH_3), 0.85 (9H, s, *tert*-butyl CH_3). ^{13}C-NMR(125 MHz): 174.0, 168.8, 141.1, 137.7, 129.7, 127.4, 73.2, 45.0, 44.9, 38.6, 37.0, 36.9, 35.7, 30.1, 29.5, 29.4, 28.5, 22.3, 20.0, 18.2, 14.2. LC/MS (m/z): $C_{26}H_{40}N_2O_2S$ requires 444.67 found $[M + H]^+$: 445.2. Anal. calcd. for $C_{26}H_{40}N_2O_2S$ (444.67) C= 70.23, H= 9.07, N= 6.30. Found C= 70.00, H= 9.00, N= 6.22.

Biological Activity Assays

Materials

In the determination of AChE/BuChE inhibitory activity the following reagents were utilized: Acetylcholinesterase (Sigma C3389), acetylthiocholine iodide (ATChI; Sigma A5751), Butyrylcholinesterase (Sigma C7512), butyrylthiocholine iodide (BTChI; Sigma B3253), galantamine (Sigma G1660) and 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; Sigma D8130).



Compound	R	R_1
4a	H	H
4b	CH_3	H
4c	C_2H_5	H
4d	C_3H_7	H
4e	$C(CH_3)_3$	H
5a	H	CH_3
5b	CH_3	CH_3
5c	C_2H_5	CH_3
5d	C_3H_7	CH_3
5e	$C(CH_3)_3$	CH_3

.

Figure 3. Synthesis of compounds 4,5. Reagents and conditions: (i) CH_3OH, conc. H_2SO_4, reflux; (ii), $H_2NNH_2.H_2O$, EtOH, reflux, 8 h; (iii) 4-(R)substituted cyclohexanone, mercaptoacetic acid or 2-mercaptopropionic acid, dry toluene, reflux, 6-8 h

Determination of AChE/BuChE Inhibitory Activity

The method devised by Ellman et al. (1961) was partially modified in order to determine the inhibitory effects of substances on AChE/BuChE. 10 µL of solutions prepared from compounds at different concentrations or the reference inhibitor, galantamine, were mixed with 220 µL of Ellman reagent (phosphate buffer pH 7.5, DTNB and substrate mixture). Following the addition of 20 µL of AChE/BuChE solution the absorbance increase at 412 nm was measured against the blank for 10 minutes at 25°C. Controls were prepared by adding 10 µL of solvent instead of compounds or reference inhibitor. The experiments were conducted in triplicate, and averages were calculated. The following formula was used to determine the samples' AChE/BuChE inhibitory activity (%) [21].

$$\text{Enzyme inhibitory activity (\%)} = \left(1 - \frac{\text{Absorbance change of sample at 412 nm}}{\text{Absorbance change of control at 412 nm}}\right) \text{x } 100$$

RESULT AND DISCUSSION

Chemistry

Spirothiazolidinone compounds (4a-e, 5a-e) were synthesized according to the procedure shown in Figure 3. Refluxing hydrazines and the related cyclohexanone with mercaptoacetic acid or 2-

mercaptopropionic acid in toluene for 6-8 hours using a Dean-Stark apparatus, afforded the targeted spirothiazolidinones. The structures of the compounds were verified by mass spectrometry and nuclear magnetic resonance (^{1}H-NMR, ^{13}C-NMR).

The IR spectra of the newly synthesized compounds (4a-e, 5a-e) revealed that the NH stretching bands of the carboxamide were signaled at between 3433-3257 cm^{-1}. The new lactam carbonyl absorption bands (1726-1705 cm^{-1}) typical for 4-thiazolidinones demonstrated the desired cyclization, in the IR spectra of 4a-e and 5a-e.

A singlet signal at δ 7.30-7.23 ppm in the ^{1}H-NMR spectra of compounds 4a-e was identified as the carboxamide N-H protons. The protons present in the aromatic ring resonated in the region δ 7.29-7.15 ppm as dublets. The protons of 4a-e in the spirodecane C2-H2 resonated at approximately δ 3.54-3.55 and 3.48 ppm as separate doublets, indicative of geminal interaction.

In the ^{1}H NMR spectra of 5a-e, the carboxamide NH protons resonated as a singlet (except for the compounds 5b and 5d which were observed as separate doublets 7.21-7.08 ppm resulting from the chiral centers) at δ 7.28-7.13 ppm. The C2-H protons of spirodecane 5a-e resonated at about δ 3.81-3.75 ppm as two quartets.

The ^{13}C NMR-APT spectra of the new spirothiazolidinone derivatives (4a-e, 5a-e) demonstrated that the carbon signals of the spirocyclic lactam carbonyl and carboxamide carbonyl groups, were observed with the highest shift value, at δ 174.1-174.0 and 171.7-168.8 ppm, respectively. All the new compounds phenyl C4 carbons appeared at δ 141.1-141.0 ppm, whereas phenyl C1 carbons resonated at δ 137.7-137.6 ppm. Moreover, the presence of characteristic C5 resonances (δ 73.2-71.4 ppm) in the APT spectra of 4 and 5, corresponding to the spirothiazolidinone structure, verified the expected spiro ring system's formation.

The mass spectra of the new compounds were acquired utilizing the positive electrospray ionization (ESI^{+}) technique. The determined molecular weights of the compounds were confirmed by the ESI-MS with the detection of protonated $[M+H]^{+}$ ions.

In vitro AChE/BuChE Inhibitory Activities

The modified Ellman's approach was utilized to assess the inhibitory effects of the novel spirothiazolidinone derivatives (4, 5) on AChE and BuChE. Galantamine was used as standard inhibitor. Table 1 outlines the IC_{50} values of compounds 4 and 5 for both AChE and BuChE.

All new compounds (4, 5) inhibited the AChE at the micromolar levels. Among the tested compounds, the *tert*-butyl substituted 4e was found as the most effective inhibitor on AChE (IC_{50}= 39.53±1.94 μM).

Additionally, the BuChE inhibitory activity of compounds 4 and 5 was assessed. The most effective inhibitory action against BuChE was demonstrated by compound 4b (IC_{50}= 95.97±1.79 μM).

The addition of a *tert*-butyl group to the spirothiazolidinone rings enhances the inhibitory effect on AChE (i.e., compounds 4e and 5e). On the other hand, methyl substitution at spirothiazolidinone rings resulted in an increase in the inhibitory activity on BuChE (i.e., compounds 4b and 5b). Besides that, the addition of propyl substitution in spirothiazolidinone rings resulted in no inhibitory activity on BuChE. (i.e., compounds 4d and 5d).

Conclusion

In conclusion, here, we present the chemical synthesis and inhibitory activity against AChE and BuChE of a new series of *N*-(3-oxo-1-thia-4-azaspiro[4.5]decan-4-yl)carboxamides. All tested compounds showed intermediate activity against AChE and BuChE. At the micromolar level, compound 4e was found to be the most effective inhibitor of AChE among the tested derivatives. Structural modifications in the compounds may lead to the generation of new derivatives with increased activity and high selectivity. Consequently, this class of new compounds would be promising in the therapy of Alzheimer's disease.

Table 1. Acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activities of the compounds

Compound	AChE inhibitory activitiy IC_{50} values (µM)	BuChE inhibitory activitiy IC_{50} values (µM)
4a	216.10±3.76	365.10±19.68
4b	106.35±2.32	**95.97±1.79**
4c	92.09±0.87	116.83±0.08
4d	128.65±3.45	-
4e	**39.53±1.94**	287.75±19.25
5a	219.11±8.31	264.39±23.72
5b	198.14±2.46	153.09±0.85
5c	129.91±1.31	215.81±3.69
5d	140.10±2.12	-
5e	124.09±0.87	-
Galantamine	5.01±0.34	36.75±1.00

Values are given as the mean of three replicates±standard deviation
The results were expressed as the IC_{50} value (the sample concentration required to inhibit enzyme activity by 50%)
Through the use of dose-response curve linear regression analysis, IC_{50} values were determined
The compounds numbered 4d, 5d and 5e did not exhibit any BuChE inhibitory activity at the investigated concentrations

AUTHOR CONTRIBUTIONS

Concept: C.B.A.; Design: C.B.A., G.H.C.; Control: C.B.A., T.Y.O.; Sources; C.B.A., G.H.C., T.Y.O.; Materials: C.B.A., G.H.C.; Data Collection and/or Processing: C.B.A., G.H.C.; Analysis and/or Interpretation: C.B.A., G.H.C.; Literature Review: C.B.A., G.H.C., T.Y.O.; Manuscript Writing: C.B.A., G.H.C., T.Y.O.; Critical Review: C.B.A., G.H.C., T.Y.O.; Other: -

CONFLICT OF INTEREST

The authors declare that there is no real, potential, or perceived conflict of interest for this article.

ETHICS COMMITTEE APPROVAL

The authors declare that the ethics committee approval is not required for this study.

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