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Article

Discovery of Potent Carbonic Anhydrase and Acetylcholinesterase Inhibitors: 2-Aminoindan β-Lactam Derivatives

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Abstract: β-Lactams are pharmacologically important compounds because of their various biological uses, including antibiotic and so on. β-Lactams were synthesized from benzylidene-inden derivatives and acetoxyacetyl chloride. The inhibitory effect of these compounds was also examined for human carbonic anhydrase I and II (hCA I, and II) and acetylcholinesterase (AChE). The results reveal that β-lactams are inhibitors of hCA I, II and AChE. The K_i values of β-lactams (**2a-k)** were 0.44-6.29 nM against hCA I, 0.93-8.34 nM against hCA II, and 0.25-1.13 nM against AChE. Our findings indicate that β-lactams (**2a-k)** inhibit both CA isoenzymes and AChE at low nanomolar concentrations.

Keywords: carbonic anhydrase; acetylcholinesterase; β-Lactam; 2-Azetidinone; enzyme inhibition; enzyme purification

1. Introduction

The β-lactams can be classified into several groups according to their structural characteristics, but their unique structural feature is the presence of the four-membered β-lactam (2-azetidinone) ring [1]. 2-Azetidinone containing antibiotics are known as β-lactam antibiotics and they are the most widely employed family of antibacterial agents [2]. Moreover, they have been reported as having antibacterial, anticancer, antiviral activity and enzyme inhibition effect [3-5]. The investigation of chemistry and biology of β-lactam continue to appeal to synthetic and medicinal organic chemists [6]. They have also been used for the preparation of different heterocyclic compounds of biological significance [7].

Carbonic anhydrases (CAs, E.C. 4.2.1.1) catalyse a very simple but physiologically essential reaction in all life kingdoms, the hydration of carbon dioxide (CO_2) and water (H_2O) to bicarbonate (HCO_3^-) and protons (H^+), with a high efficiency [8-11]. They are metalloenzymes that participate in the control of pH in the body, are encoded by six different independent gene families (α-, β-, γ-, δ -, ζ- and n-CA) and are found in eukaryotic and prokaryotic cells [12-15]. CAs catalyse the reversible of carbon dioxide (CO_2) hydration to yield bicarbonate (HCO_3^-) and protons (H^+), which are essential molecules and ions in many important physiologic processes in all life kingdoms [16-18].

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$$CO_2 + H_2O \overset{CA}{\Leftrightarrow} H_2CO_3 \Leftrightarrow HCO_3^- + H^+$$

Living organisms possess sixteen CA isoenzymes, which differ in their subcellular localization and catalytic activity [19-22]. They were found in various organs and tissues with different kinetic and molecular properties, expression levels, and oligomeric rearrangements as well as various abilities to respond to different inhibitory classes [23-25]. There are very important role of these enzyme in different tissues [26,27]. CA I, II, III, VII, and XIII isoenzymes are cytosolic, CA IV, IX, XII and CA XIV are bound to membrane, CA VA-VB are mitochondrial, and CA VI are in the milk and saliva [28,29]. The erythrocytes contain CA I, and II at high concentrations. The CA inhibitors (CAIs) are used as a class of pharmaceuticals such as; diuretics, anti-glaucoma agents, gastric, duodenal ulcers, neurological disorders, and osteoporosis [30-32]. Up to now the inhibitory effects against different CAs types have been investigated for different sulphonamides derivatives, heavy metal ions, phenols, antibiotics and various drugs [33-35]. β-Lactams are widely used in the treatment of many diseases. However, there is no detailed study on erythrocytes hCA I, and II isoenzymes of β-lactams (**2a-k**).

Acetylcholinesterase (AChE) is a crucial enzyme used for transmission control between neurons [36] when the process is either mediated or modulated by the neurotransmitter acetylcholine (ACh). ACh is released by the axon terminal or varicosities of the transmitter neuron into the extracellular space to interact with the receptors of the other neuron. AChE hydrolysis ACh to choline and acetate [37,38]. If AChE is inhibited in the central nervous system, the concentration of ACh increases in the synaptic cleft, leading to cholinergic crisis, which affords several dangerous effects, such as convulsion and respiratory problems, which could lead to death. AChE inhibitors (AChEIs) have medical applications and are particularly important for the symptomatic treatment of Alzheimer's disease (AD) to enhance central cholinergic transmission [39]. AD is a fatal and chronic neurodegenerative disease that usually starts slowly and gets worse over time [19]. From this perspective, there is a great need for improved AChEIs that show low toxicity, good brain penetration, and high bioavailability. The use of AChEIs to block the cholinergic degradation of acetylcholine (Ach) is therefore considered to be a promising approach for the treatment of AD [40,41].

In the present study, we investigated the inhibition profile of a series of β-lactam derivatives **2a-k** against CA I, and II isoforms from human erythrocytes and acetylcholinesterase (AChE) enzyme.

2.Materials and Methods

2.1.Chemicals

CN-Br-activated Sepharose-4B, p-nitrophenylacetate, and chemicals for electrophoresis were purchased from Sigma-Aldrich Co. All other chemicals were of analytical grade and obtained from Merck.

2.2.General procedure for the synthesis of imines

2-Amino indane (1eq) and benzaldehyde (1eq) were stirred in a beaker for 5 minutes. The resulting crude imine product was recrystallized from dichloromethane/hexane to give target compound in 95-99% yield. General synthesis route of novel β-lactam derivatives (**2a-k**) was given in Figure 1.

(E)-N-Benzylidene-2,3-dihydro-1H-inden-2-amine (1a)

Yield 98%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.14 (dd, J = 16.05, 7.36 Hz, 2H), 3.21 (dd, J = 8.0, 16.3 Hz, 2H), 4.32 (p, J = 7.1 Hz, 1H), 7.16-7.26 (m, 4H), 7.39-7.43 (m, 3H), 7.74-7.77 (m, 2H), 8.39 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.2 (2C), 71.5, 124.7, 126.7 (2C), 128.4, 128.8, 130.83, 136.5, 142.2 (2C), 160.1.

(E)-N-(3-Methoxybenzylidene)-2,3-dihydro-1H-inden-2-amine (1b)

Yield 96%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.12 (dd, J = 15.8, 7.0 Hz, 2H), 3.20 (dd, J = 8.41, 16.4 Hz, 2H), 3.82 (3H, s), 4.26 (p, J = 7.14 Hz, 1H), 6.94 (dt, J = 7.62, 1.66 Hz, 1H), 7.14-7.35 (m, 7H), 8.33 (s, 1H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.1 (2C), 55.6, 71.4, 111.9, 117.5, 121.7, 124.7 (2C), 126.7 (2C), 129.8 (2C), 137.9, 142.3 (2C), 160.1.

(E)-N-(4-Methylbenzylidene)-2,3-dihydro-1H-inden-2-amine (1c)

Yield 97%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ2.36 (3H, s), 3.13 (dd, J = 15.3, 6.9 Hz, 2H), 3.17 (dd, J = 7.3, 15.4 Hz, 2H), 4.27 (p, J = 7.2 Hz, 1H), 7.14-7.23 (m, 6H), 7.63 (d, J = 7.99 Hz, 2H), 8.32 (s, 1H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 21.8, 41.2 (2C), 71.6, 124.7 (2C), 126.7 (2C), 128.4 (2C), 129.5 (2C), 133.8, 141.1, 142.3 (2C), 160.1.

(E)-N-(3-Methylbenzylidene)-2,3-dihydro-1H-inden-2-amine (1d)

Yield 97%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.10 (dd, J = 16.0, 7.4 Hz, 2H), 3.19 (dd, J = 7.6, 15.8 Hz, 2H), 3.81 (3H, s), 4.26 (p, J = 7.20 Hz, 1H), 7.26-7.42 (m, 6H), 7.61-7.63 (d, J = 6.27 Hz, 1H), 7.73 (s, 1H), 8.43 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 21.6, 41.3 (2C), 71.7, 124.8 (2C), 126.2, 126.8 (2C), 128.7, 128.8, 131.8, 136.5, 138.6, 142.3 (2C), 160.5.

(E)-N-(3-Chlorobenzylidene)-2,3-dihydro-1H-inden-2-amine (1e)

Yield 98%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.19 (dd, J = 15.7, 6.4 Hz, 2H), 3.26 (dd, J = 7.3, 15.7 Hz, 2H), 4.36 (p, J = 6.9 Hz, 1H), 7.32-7.44 (m, 6H), 7.63 (d, J = 6.64 Hz, 1H), 7.84 (1H, s), 8.35 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.2 (2C), 71.4, 124.8 (2C), 126.8 (2C), 126.8 (2C), 128.0, 130.1, 130.8, 135.0, 138.3, 142.1, 158.6.

(E)-N-(3,4-Dichlorobenzylidene)-2,3-dihydro-1H-inden-2-amine (1f)

Yield 98%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.11 (dd, J = 15.6, 6.7 Hz, 2H), 3.18 (dd, J = 7.3, 15.6 Hz, 2H), 4.28 (p, J = 7.0 Hz, 1H), 7.15-7.24 (m, 4H), 7.35 (dd, J = 8.50, 2.26 Hz, 2H), 7.66 (dd, J = 8.50, 2.26 Hz, 2H), 8.30 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.2 (2C), 71.5, 124.8 (2C), 126.8 (2C), 129.1 (2C), 129.7 (2C), 134.9, 136.7, 142.1 (2C), 158.8.

(E)-N-(3-Bromobenzylidene)-2,3-dihydro-1H-inden-2-amine (1g)

Yield 95%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.11 (dd, J = 15.6, 6.5 Hz, 2H), 3.19 (dd, J = 7.3, 15.6 Hz, 2H), 4.29 (p, J = 6.9 Hz, 1H), 7.15-7.27 (m, 5H), 7.51 (d, J = 7.67 Hz, 1H), 7,61 (d, J = 7.67 Hz, 1H), 7.93 (1H, s), 8.29 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.1 (2C), 71.4, 123.1, 124.7 (2C), 126.7 (2C), 127.3, 130.3, 130.9, 133.7, 138.5, 142.1 (2C), 158.5.

(E)-N-(2-Bromobenzylidene)-2,3-dihydro-1H-inden-2-amine (1h)

Yield 97%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.17 (dd, J = 15.6, 6.4 Hz, 2H), 3.26 (dd, J = 7.2, 15.7 Hz, 2H), 4.42 (p, J = 6.8 Hz, 1H), 7.13-7.36 (m, 6H), 7.59 (dd, J = 7.67, 1.69 Hz, 1H), 8.08 (dd, J = 7.66, 1.43 Hz, 1H), 8.77 (1H, s). ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.2 (2C), 71.3, 124.7 (2C), 125.2, 126.7 (2C), 127.8, 129.2, 131.9, 133.2, 134.8, 142.1 (2C), 159.1.

(E)-N-(4-Bromobenzylidene)-2,3-dihydro-1H-inden-2-amine (1i)

Yield 98%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.11 (dd, J = 15.6, 6.6 Hz, 2H), 3.19 (dd, J = 7.3, 15.6 Hz, 2H), 4.29 (p, J = 6.9 Hz, 1H), 7.15-7.24 (m, 4H), 7.52 (dd, J = 8.4, 5.0 Hz, 2H), 7.59 (dd, J = 8.6, 4.8 Hz, 2H), 8.30 (1H, s). ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.1 (2C), 71.4, 124.7 (2C), 125.1, 126.7 (2C), 129.8 (2C), 132.0 (2C), 135.3, 142.1 (2C), 158.9.

(E)-N-(2-Nitrobenzylidene)-2,3-dihydro-1H-inden-2-amine (1j)

Yield 97%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.13 (dd, J = 15.7, 5.9 Hz, 2H), 3.25 (dd, J = 7.2, 15.7 Hz, 2H), 4.39 (p, J = 6.6 Hz, 1H), 7.15-7.25 (m, 4H), 7.56 (dt, J = 7.46, 1.54 Hz, 2H), 8.02 (dd, J = 7.71,

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1.39 Hz, 2H), 8.9 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.1 (2C), 71.2, 124.5, 124.8 (2C), 126.8 (2C), 130.1, 130.8, 131.5, 133.7, 141.9 (2C), 149.0, 156.2.

(E)-N-(4-Fluorobenzylidene)-2,3-dihydro-1H-inden-2-amine (1k)

Yield 94%; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 3.11 (dd, J = 15.6, 6.7 Hz, 2H), 3.18 (dd, J = 7.3, 15.6 Hz, 2H), 4.28 (p, J = 7.0 Hz, 1H), 7.03-7.24 (m, 6H), 7.71 (dd, J = 4.9, 5.5 Hz, 2H), 7.59 (dd, J = 8.6, 4.9 Hz, 2H), 8.32 (1H, s); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 41.2 (2C), 71.3, 115.7 (2C), 116.0 (2C), 124.7 (2C), 126.7 (2C), 130.2, 130.3, 142.2 (2C), 158.6.

2.3.General Procedure for The Synthesis of β-Lactams

To a solution of imine (1 eq) and triethylamine (3 eq) in dichloromethane, a solution of acetoxyacetyl chloride (2eq) in dichloromethane was added dropwise over a period of 10 minutes at room temperature. The reaction mixture was then stirred for an additional 1hour at room temperature. The mixture was concentrated, then extracted with Ethyl Acetate and dried over Magnesium Sulphate; the solvent was removed in a vacuum. Obtained product was purified over a silica gel packed column chromatography using Hexane: EtOAc (1:1 v/v). The purified product was dried under vacuo and recrystallized from Ethanol yields β-Lactam derivatives (70-93% yield).

2.4.Spectral Data

(3S*,4R*)-1-(2,3-dihydro-1H-inden-2-yl)-2-oxo-4-phenylazetidin-3-yl acetate (2a)

Yield 91%, m.p. 171-173 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.65 (3H, s), 2.88 (dd, J = 15.8, 6.1 Hz, 1H), 2.89 (dd, J = 15.8, 7.1 Hz, 1H), 3.14-3.28 (m, 2H), 4.54 (p, J = 7.0 Hz, 1H), 4.81 (d, J = 4.6 Hz, 1H), 5.72 (d, J = 4.6 Hz, 1H), 6.93-6.96 (m, 1H), 7.04-7.21 (m, 5H), 7.29-7.31 (m, 3H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.1, 37.3, 37.4, 54.0, 61.7, 76.6, 124.6 (2C), 127.0 (2C), 128.3 (2C), 128.5 (2C), 128.9, 133.7, 140.3, 140.3, 165.3, 169.2; MS: m/z 344.10 $[M + Na]^+$.

(3S*,4R*)-1-(2,3-dihydro-1H-inden-2-yl)-2-(3-methoxyphenyl)-4-oxoazetidin-3-yl acetate (2b)

Yield 70%, m.p. 98-100 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.70 (3H, s), 2.90 (dd, J = 15.9, 6.1 Hz, 1H), 2.99 (dd, J = 15.8, 7.1 Hz, 1H), 3.13-3.27 (m, 2H), 3.75 (s, 3H), 4.54 (p, J = 6.6 Hz, 1H), 4.77 (d, J = 4.7 Hz, 1H), 5.72 (d, J = 4.7 Hz, 1H), 6.66-6.67 (1H, m), 6.77-6.85 (2H, m), 6.96 (d, J = 6.94 Hz, 1H), 7.04-727 (m, 4H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.2, 37.3, 37.4, 54.0, 55.4, 61.6, 76.5, 113.8, 114.6, 120.8, 124.5, 124.6 (2C), 127.0 (2C), 129.4, 135.4, 140.4, 159.5, 165.2, 169.3; MS: m/z 344.16 $[M + Na]^+$.

(3S*,4R*)-1-(2,3-dihydro-1H-inden-2-yl)-2-oxo-4-p-tolylazetidin-3-yl acetate (2c)

Yield 92%, m.p. 113-115 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.68 (3H, s), 2.29 (3H, s), 2.88 (dd, J = 15.8, 6.0 Hz, 1H), 2.98 (dd, J = 15.8, 7.1 Hz, 1H), 3.13-3.27 (m, 2H), 4.53 (p, J = 6.6 Hz, 1H), 4.76 (d, J = 4.6 Hz, 1H), 5.71 (d, J = 4.6 Hz, 1H), 6.92-7.27 (m, 8H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.1, 21.5, 37.3, 37.4, 54.0, 61.7, 76.5, 124.5, 125.6, 127.0 (2C), 128.1, 129.3, 129.6, 133.6, 138.0, 140.4 (2C), 165.3, 169.3; MS: m/z 358.04 $[M + Na]^+$.

(3S*,4R*)-1-(2,3-dihydro-1H-inden-2-yl)-2-oxo-4-m-tolylazetidin-3-yl acetate (2d)

Yield 87%, m.p. 96-98 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.69 (3H, s), 2.34 (3H, s), 2.87 (dd, J = 15.8, 6.4 Hz, 1H), 2.97 (dd, J = 15.7, 7.3 Hz, 1H), 3.18 (m, J = 7.2, 15.3 Hz, 1H), 3.22 (m, J = 6.3, 15.3 Hz, 1H), 3.75 (3H, s), 4.51 (p, J = 6.8 Hz, 1H), 4.79 (d, J = 4.6 Hz, 1H), 6.95-6.97 (m, 1H), 7.02-7.17 (m, 7H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.2, 21.4, 37.2, 37.3, 53.9, 61.6, 76.6, 124.6 (2C), 127.0, 127.0, 128.5 (2C), 129.0 (2C), 130.6, 138.7, 140.4, 140.4, 165.4, 169.4; MS: m/z 358.09 $[M + Na]^+$.

(3S*,4R*)-2-(3-chlorophenyl)-1-(2,3-dihydro-1H-inden-2-yl)-4-oxoazetidin-3-yl acetate (2e)

Yield 98%, m.p. 115-117 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.78 (3H, s), 2.83 (dd, J = 15.9, 7.0 Hz, 1H), 3.00 (dd, J = 15.9, 5.2 Hz, 1H), 3.10-3.24 (m, 2H), 4.57 (p, J = 6.1 Hz, 1H), 4.72 (d, J = 4.6 Hz, 1H), 5.71 (d, J = 4.6 Hz, 1H), 6.89-6.91 (m, 1H), 7.01-7.28 (m, 7H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ

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20.1, 37.5, 37.6, 54.0, 61.1, 76.6, 124.5, 124.6, 126.7, 127.1, 127.2, 128.6, 129.1, 129.5, 134.3, 136.0, 140.2, 164.9, 169.2; MS: m/z 358.20 $[M + 2H]^+$.

(3S*,4R*)-2-(3,4-dichlorophenyl)-1-(2,3-dihydro-1H-inden-2-yl)-4-oxoazetidin-3-yl acetate (2f)

Yield 84%, m.p. 138-140 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.77 (3H, s), 2.79 (dd, J = 4.4, 16.0 Hz, 1H), 3.01 (dd, J = 16.0, 3.8 Hz, 1H), 3.10 (dd, J = 6.6, 17.1 Hz, 1H), 3.18 (dd, J = 4.6, 16.0 Hz, 1H), 4.56 (p, J = 6.2 Hz, 1H), 4.76 (d, J = 4.6 Hz, 1H), 5.70 (d, J = 4.6 Hz, 1H), 6.87-6.90 (m, 1H), 6.97 (d, J = 8.28 Hz, 1H), 7.04-7.14 (m, 4H), 7.33 (d, J = 8.28 Hz, 1H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.3, 37.5, 37.9, 54.0, 60.5, 76.5, 124.5, 124.6, 127.2, 127.3, 127.7, 130.2, 130.5, 132.6, 133.0, 134.3, 140.1, 140.1, 164.8, 169.2; MS: m/z 392.20 $[M + H]^+$.

(3S*,4R*)-2-(3-bromophenyl)-1-(2,3-dihydro-1H-inden-2-yl)-4-oxoazetidin-3-yl acetate (2g)

Yield 78%, m.p. 132-134 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.73 (3H, s), 2.83 (dd, J = 4.9, 16.0 Hz, 1H), 3.00 (dd, J = 15.9, 7.0 Hz, 1H), 3.17 (ddd, J = 5.2, 6.5, 15.6 Hz, 2H), 4.58 (p, J = 6.0 Hz, 1H), 4.70 (d, J = 4.6 Hz, 1H), 5.70 (d, J = 4.6 Hz, 1H), 6.89-6.92 (m, 1H), 7.05-7.17 (m, 4H), 7.26-7.27 (m, 2H), 7.40-7.43 (m, 1H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.1, 37.5, 37.6, 54.0, 61.0, 76.5, 122.3, 124.5, 124.5, 127.1, 127.3, 129.8, 131.5, 132.0, 136.3, 140.2, 140.2, 164.9, 169.2; MS: m/z 424.05 $[M + H + Na]^+$.

(3S*,4R*)-2-(2-Bromophenyl)-1-(2,3-dihydro-1H-inden-2-yl)-4-oxoazetidin-3-yl acetate (2h)

Yield 83%, m.p. 123-125 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.69 (3H, s), 2.92 (dd, J = 6.0, 15.8 Hz, 1H), 3.02 (dd, J = 15.8, 7.0 Hz, 1H), 3.22 (d, J = 6.2 Hz, 2H), 4.53 (p, J = 6.5 Hz, 1H), 5.40 (d, J = 4.7 Hz, 1H), 5.85 (d, J = 4.7 Hz, 1H), 6.99-7.01 (m, 1H), 7.06-7.19 (m, 4H), 7.29-7.38 (m, 2H), 7.47-7.50 (m, 1H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.1, 37.2, 37.5, 54.2, 60.6, 76.0, 124.5, 124.6, 124.7, 126.9, 127.1, 127.2, 129.4, 130.1, 133.0, 133.3, 140.1, 140.2, 165.5, 168.9; MS: m/z 422.03 $[M + Na]^+$.

(3S*,4R*)-2-(4-bromophenyl)-1-(2,3-dihydro-1H-inden-2-yl)-4-oxoazetidin-3-yl acetate (2i)

Yield 76%, m.p. 114-116 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.73 (3H, s), 2.83 (dd, J = 5.3, 15.9 Hz, 1H), 2.99 (dd, J = 15.9, 7.0 Hz, 1H), 3.15 (m, J = 5.7, 13.2 Hz, 1H), 3.18 (m, J = 6.6, 13.2 Hz, 1H), 4.56 (p, J = 6.2 Hz, 1H), 4.74 (d, J = 4.6 Hz, 1H), 6.92 (m, 1H), 7.02 (d, J = 8.28 Hz, 2H), 7.06-7.16 (3H, m), 7.41 (d, J = 8.41 Hz, 2H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.2, 37.5, 37.5, 54.0, 61.1, 76.5, 122.9, 124.5, 124.6, 127.1 (2C), 130.1 (2C), 131.5 (2C), 133.0, 140.2, 140.2, 165.0, 169.2; MS: m/z 421.92 $[M + Na]^+$.

(3S*,4R*)-1-(2,3-dihydro-1H-inden-2-yl)-2-(2-nitrophenyl)-4-oxoazetidin-3-yl acetate (2j)

Yield 93%, m.p. 117-119 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.70 (3H, s), 2.99 (dd, J = 5.6, 15.9 Hz, 1H), 3.08 (dd, J = 15.8, 6.9 Hz, 1H), 3.25 (d, J = 6.1 Hz, 2H), 4.57 (p, J = 6.6 Hz, 1H), 5.59 (d, J = 5.2 Hz, 1H), 6.04 (d, J = 5.2 Hz, 1H), 6.97-6.99 (m, 1H), 7.04-7.12 (m, 3H), 7.46-7.52 (m, 1H), 7.59-7.63 (m, 2H), 7.95-7.98 (m, 1H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.1, 37.2, 37.7, 54.7, 57.4, 76.4, 124.4, 124.6, 125.3, 127.2, 127.4, 129.4, 129.5, 130.3, 132.9, 139.9, 140.1, 149.0, 165.9, 168.6; MS: m/z 388,93 $[M + Na]^+$.

(3S*,4R*)-1-(2,3-dihydro-1H-inden-2-yl)-2-(4-fluorophenyl)-4-oxoazetidin-3-yl acetate (2k)

Yield 91%, m.p. 114-116 ºC; ^{1}H NMR (300 MHz; ppm; $CDCl_3$) δ 1.71 (3H, s), 2.84 (dd, J = 5.5, 15.8 Hz, 1H), 2.99 (dd, J = 15.9, 7.1 Hz, 1H), 3.18 (d, J = 6.2 Hz, 2H), 4.56 (p, J = 6.3 Hz, 1H), 4.77 (d, J = 4.6 Hz, 1H), 5.70 (d, J = 4.6 Hz, 1H), 6.91-6.99 (m, 3H), 7.04-7.16 (m, 5H); ^{13}C NMR (75 MHz; ppm; $CDCl_3$) δ 20.1, 37.4, 37.5, 53.9, 61.0, 76.6, 115.2, 115.5, 124.5, 124.5, 127.1 (2C), 129.6, 129.6, 130.2, 130.3, 140.2 (2C), 165.1, 169.2; MS: m/z 344.13 $[M + Na]^+$.

2.5.Biochemical Assays

Erythrocytes were obtained from the Research Hospital at Atatürk University. The red cells were haemolysed with ice-cold water after washing with 0.9% NaCl. The hemolysate was applied to the prepared Sepharose-4B-tyrosine-sulfanylamide affinity gel [42]. Both CA isoenzymes were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography in a single step [43-45]

Sepharose-4B-L-tyrosine-sulfanilamide was prepared according to a previous method. Thus, homogenate solution acidity was adjusted and supernatant was transferred to the previously prepared Sepharose-4B-L-tyrosine-sulphanilamide affinity column [46,47]. The proteins flow in the column eluates was spectrophotometrically determined at 280 nm. All purification steps were performed at 4 °C. Protein quantity was determined at 595 nm according to the Bradford method [48]. Bovine serum albumin was used as the standard protein [49-51]. To monitor purification of both isoenzymes, sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) was used according to the procedure of Laemmli [52]. In this application, the imaging method was performed out in 10 and 3% acrylamide for the running and the stacking gel, respectively, with 0.1% SDS [53-55].

Enzyme activity was determined hydrolysis of p-nitrophenylacetate (NPA) to p-nitrophenolate at 348 nm according to the method of Verpoorte et al [56] and described previously. The inhibitory effects of β-lactam derivatives **2a-k** were examined. To obtain the half maximal inhibitory concentration (IC_{50}) values, CA I, and II activity was measured in the presence of β-lactam derivatives at different cuvette concentrations. Activity (%)-[β-Lactam] graph was drawn for each β-lactam derivatives **2a-k** [57-59]. To determine Ki values, three different β-lactam derivatives concentrations were tested. In these experiments, different substrate (PNA) concentrations were used and Lineweaver-Burk curves were drawn [60] as previously described [61].

The inhibition effects of β-lactam derivatives **2a-k** on AChE activities were measured according to the Ellman's method [62] described previously [63]. Acetylthiocholine iodide (AChI) and 5,5′-dithio-bis(2-nitro-benzoic) acid (DTNB) were used as substrate. To this end, 100 µL of Tris/HCl buffer (1 M, pH 8.0) and 10 µL of β-lactam derivatives solution at different concentrations and 50 µL AChE ($5.32x10^{-3}$ U) solution were mixed and incubated for 10 min at 25 °C. Then 50 µL of DTNB (0.5 mM) was transferred. Then the reaction was initiated by the addition of 50 µL of AChI. The hydrolysis of AChI was recorded spectrophotometrically by the formation of 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine at a wavelength of 412 nm [64]. The IC_{50} values were determined by spectrophotometric measurement of the effect of increasing test compound (β-lactam derivatives **2a-k**) concentrations on AChE activity. The IC_{50} and Ki values are calculated in the same way as for CA isoenzymes. Tacrine was used as positive control.

3. Results and Discussion

β-Lactam derivatives are drugs that protect against many different gram positive-negative and anaerobic organisms. They are perhaps among the best-studied and most widely used antibiotics in the world [65]. General synthesis route of novel β-lactam derivatives (**2a-k**) was shown in Figure 1.

Compounds	R
a	-H
b	-*m*OMe
c	-*p*Me
d	-*m*Me
e	-*m*Cl
f	-*m*,*p*-diCl
g	-*m*Br
h	-*o*Br
i	-*p*Br
j	-*o*NO_2
k	-*p*F

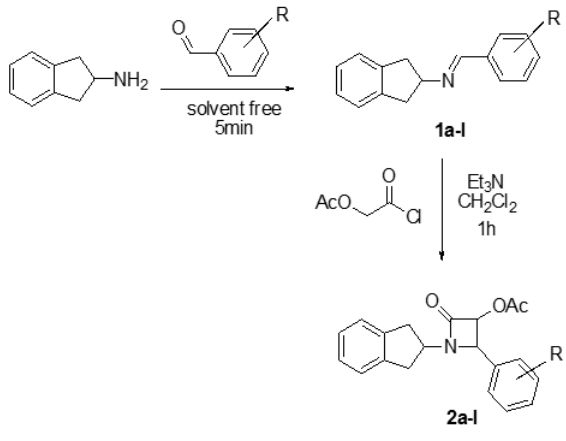

Figure 1. General synthesis route of novel β-lactam derivatives (**2a-k**).

Herein, β-Lactam derivatives (**2a-k)** were prepared from benzylidene-inden derivatives and ketene, and characterized by NMR, and MS. Based on the literature, we assumed that the synthesized compounds were in cis form based on the literature [66-68]. Also, the chemical

structures of **2a-k** were given in Figure 2. The in vitro inhibitory effects of compounds **2a-k** were also examined for purified hCA I, and II isoenzyme activities using the esterase activity method. β-lactams derivatives inhibit growth of sensitive bacteria by inactivating enzymes in the cell membrane. β-lactam derivatives were synthesized and evaluated as inhibitors of the protease, elastase, and the cysteine protease papain. Some drug molecules are enzyme inhibitors, so their discovery is an important in biochemistry research [69]. Inhibitors of CA have several medical applications, such as glaucoma disease, diuretics, the neurological disorders, epilepsy, and Alzheimer's disease. Some research groups are currently working on of the synthesis of new inhibitors of the carbonic anhydrase family for the treatment of some diseases [70-72].

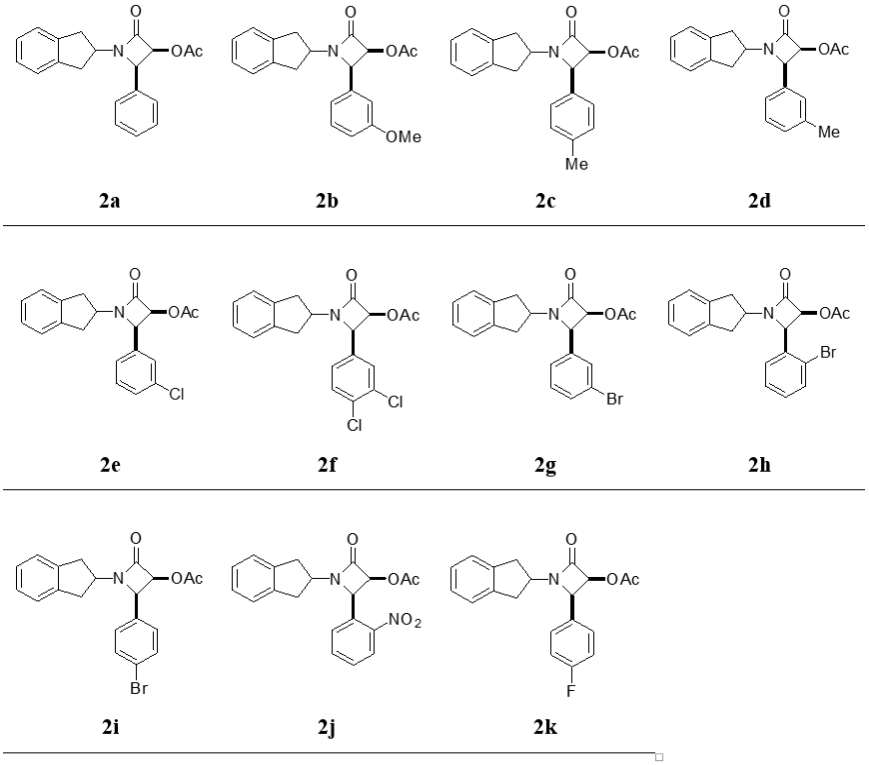

Figure 2. The chemical structures of the investigated β-lactam derivatives (**2a-k**).

Some chemicals at low dosages affect by altering normal enzyme activity and by a specific enzyme inhibition [73,74]. It is well known that β-lactams had inhibition properties on hCA I, and II isoenzymes and used in therapies [75,76]. The inhibition effects of newly synthesized compounds (**2a-k**) were the first time determined against hCA I, and II. For this purpose, as shown in Table 1, hCA I, and II were separately purified from erythrocytes with affinity chromatography. The hCA I was purified 127.9 fold with a specific activity of 1151.4 EU/mg and overall yield of 63.9% and the hCA II enzyme was purified, 788.9-fold with a specific activity of 7100.0 EU/mg and overall yield of 56.4% (Table 1). The purification was monitored by SDS polyacrylamide gel electrophoresis. After this process, a single band was observed for each isoenzyme (Figure 3). For the compounds, the inhibitor concentrations causing up to 50% inhibition (IC_{50} values) were determined from the regression analysis graphs. From in vitro studies, it is understood that hCA I, hCA II, and AChE were inhibited by these β-lactam compounds **2a-k** (Table 2). The inhibition data of β-lactam derivatives **2a-k** reported here are shown in Table 2, and the following comments can be drawn from these data:

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Table 1. Summary of purification procedure for hCA I, and II from fresh human erythrocytes with Sepharose-4B-L-tyrosine -sulphanilamide affinity chromatography

Purification Steps		Volume (mL)	Total enzyme activity (EU)	Total protein (mg)	Specific activity (EU/mg)	Yield (%)	Purification fold
Hemolysate		50	6300	700	9.0	100	1
Sepharose-4B-L-tyrosine -sulphanilamide affinity chromatography	hCA I	10	4030	3.5	1151.4	63.9	127.9
	hCA II	5	3550	0.50	7100.0	56.4	788.9

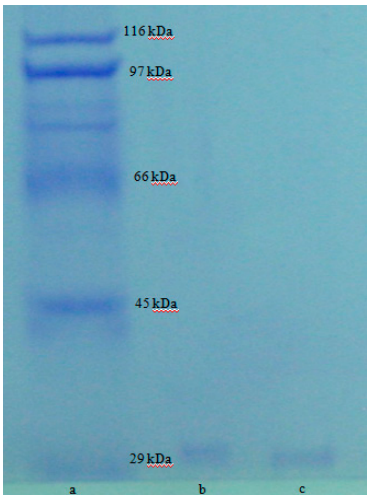

Figure 3. SDS polyacrylamide gel electrophoresis bands of carbonic anhydrase I and II isoenzymes and standard proteins [Lane a: standard proteins; standards: E coli β-galactosidase (116 kDa), rabbit phosphorylate b (97 kDa), bovine albumin (66 kDa), chicken ovalbumin (45 kDa), bovine carbonic anhydrase (29 kDa)], Lane b: hCA I, Lane c: hCA II].

Table 2. Human carbonic anhydrase isoenzymes (hCA I and II) inhibition value with β-lactam derivatives (**2a-k**), by an esterase assay with NPA as a substrat

Compounds	IC_{50} (nM)						K_I (nM)		
	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
2a	0.612	0.9578	5.212	0.9099	0.885	0.9960	0.44±0.115	3.54±0.405	1.13±0.472
2b	2.303	0.9906	6.418	0.9009	0.913	0.9868	1.41±0.547	3.15±1.139	0.39±0.069
2c	2.107	0.9400	3.707	0.9012	0.783	0.9913	1.50±0.657	2.96±0.157	0.55±0.136
2d	0.231	0.9704	4.176	0.9029	0.634	0.9926	1.49±0.290	3.26±0.708	0.77±0.041
2e	3.984	0.9107	5.825	0.9136	0.668	0.9724	6.29±2.068	8.34±3.530	0.42±0.020
2f	1.627	0.9936	5.975	0.9230	0.602	0.9791	1.16±0.514	1.50±0.421	0.46±0.045
2g	0.652	0.9000	3.809	0.9295	0.734	0.9803	0.35±0.105	3.39±1.158	0.44±0.057
2h	2.646	0.9264	5.212	0.9662	0.450	0.9947	0.91±0.143	2.19±0.921	0.25±0.019
2ı	2.548	0.9359	4.030	0.9604	0.705	0.9984	0.97±0.245	0.93±0.295	0.36±0.045
2j	4.814	0.9284	5.023	0.9057	0.704	0.9746	1.09±0.136	2.88±1.168	0.56±0.073
2k	2.502	0.9886	6.863	0.9132	0.859	0.9939	0.94±0.430	1.34±0.539	0.68±0.117
AZA$^{\Psi,*}$	101.19	0.9509	113.75	0.9791	-	-	170.34±2.48	115.43±1.63	-
TAC$^{\text{⌘}}$	-	-	-	-	4.101	0.9951	-	-	3.90±0.792

$^{\Psi}$Acetazolamide (AZA) was used as a standard inhibitor for both CA isoenzymes. $^{\text{⌘}}$ Tacrine (TAC) was used as a standard inhibitor for all AChE. *These values were obtained from reference of 75.

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1. Cytosolic hCA I is expressed in the body and can be found in high concentrations in the blood and gastrointestinal tract. All β-lactam derivatives **2a-k** exhibited effective inhibitory activity against this cytosolic isoenzyme hCA I with a Ki values of 0.35±0.105-6.29±2.068 nM (Table 1). Also, β-lactam derivative **2g** shown the most powerful CA I inhibition effect with Ki value of 0.35±0.105 nM. On the other hand, we found that acetazolamide (AZA), which is used as clinical CAs inhibitor and treatment of glaucoma, cystinuria, epilepsy, altitude sickness, periodic paralysis, dural ectasia, idiopathic intracranial hypertension, and central sleep apnea [75] has a Ki value of 170.34±2.48 nM (Table 2). The results clearly showed that all β-lactam derivatives **2a-k** demonstrated more effective hCA inhibitory activity than that of AZA.

2. With regard to the profiling assay against cytosolic hCA II, β-lactam derivatives **2a-k** have similar inhibition effects; with a Ki values in ranging of 0.93±0.295-8.34±3.530 nM. For comparison, AZA, which was used as clinical CAs inhibitor showed a Ki value 115.43±1.63 nM. This result clearly showed that all β-lactam derivatives **2a-k** are a rather effective inhibition for the cytosolic isoform hCA II. The most powerful CA II inhibition effect was found in β-lactam derivatives of **2i** with Ki value of 0.93±0.295 nM.

3. The compounds or drugs possessing AChE inhibitory effects are used for the treatment of AD. However, these drugs have many undesired side effects. Thus, the development and utilization of new effective AChEIs is highly desired. Currently the most prescribed AChEIs are Galantamine, Rivastigmine and Donepezil. These drugs are used to treat patients with mild-to-moderate AD. Donepezil and Galantamine are short-acting reversible competitive inhibitors, whereas Rivastigmine is actively metabolized by ChE. These agents do not stop disease progression, but clinical studies have shown that they temporarily stabilize cognitive impairment and help to maintain global function, often delaying the need for patient placement in nursing homes by several months [77]. It was reported that in AD, AChE is lost up to 85% in specific brain regions. In the present study, AChE was very effectively inhibited by β-lactam derivatives **1-11**, with Ki value in ranging of 0.25±0.019-1.13±0.472 nM (Table 2). The Ki value of by β-lactam derivatives **1-11** for AChE was calculated from Lineweaver-Burk plots [78]. On the other hand, Tacrine, which is used for the treatment of mild-to-moderate AD and various other memory impairments, had been shown Ki value of 3.90±0.792 nM.

4. Conclusions

The hCA I, and II isoenzymes were inhibited by β-lactams (**2a-k**) at different functional groups (CH_3, NO_2, Br, F, Cl and phenol) in the micromolar range. These compounds have shown nanomolar inhibition against both cytosolic hCA I, and II. These results indicate that the β-lactam ring and derivatives may be new CA inhibitors in addition to the well-known sulphonamides. Also, AChE was potently inhibited by β-lactams (**2a-k**) with Ki values in the range of 0.25±0.019-1.13±0.472 nM.

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Author Contributions: Mustafa Zengin and İlhami Gülçin were responsible for the majority of this work, including the execution of experiments, data analysis, and writing and publication of this report; Hayriye Genç synthesized the 2-aminoindan β-lactam derivatives; Ramazan Kalin and Nastaran Sadeghian performed carbonic anhydrase isoenzymes inhibition assays; Umit M. Kocyigit and Hasan Özdemir analyzed acetylcholinesterase inhibitors data. All authors have read and approved the final manuscript.

Conflicts of Interest: The authors declare no conflict of interest.

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