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RESEARCH ARTICLE

The synthesis of some β -lactams and investigation of their metal-chelating activity, carbonic anhydrase and acetylcholinesterase inhibition profiles

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Abstract

β -Lactam antibiotics are a broad class of antibiotics, consisting of all antibiotic agents that contain a β -lactam ring in their molecular structures. Synthesis of β -lactam analogs, which are containing dichloride atoms and *N*-methyl, *N*-aromatic rings, was achieved by Schiff bases and dichloroketene compounds. All the synthesized imines and β -lactam analogs were tested against two physiologically relevant carbonic anhydrase isozymes (hCA I and II) and acetylcholinesterase (AChE). They demonstrated effective inhibitory profiles with K_i values in ranging of 3.22–11.18 nM against hCA I, 3.74–10.41 nM against hCA II, and 0.50–1.57 nM against AChE. On the other hand, acetazolamide and dorzolamide clinically used as CA inhibitors, showed K_i value of 170.34 and 129.26 nM against hCA I, and 115.43 and 135.67 nM against hCA II, respectively. Also, tacrine used as standard AChE inhibitor showed K_i value of 5.70 nM against AChE.

Keywords

Acetylcholinesterase, β -lactams, carbonic anhydrase, enzyme inhibition, enzyme purification, Schiff bases

History

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Introduction

The effect of a substituent such as halogen on the biological activity of a potential drug structure or bioactive organic compound still has to be planned for experimentally in biological studies designed to detect intended activity. In some position on molecule skeleton, the presence of active atoms such as a chlorine atom is very important for the specific activity of a compound obtained by organic reactions. Sometimes chlorine atom demonstrates to be the optimum substituent of a chemical molecule for developed activity. As it is known that chlorinated organic compounds are not generally hazardous and toxic, the chlorine atom is used as a useful substituent in synthetic organic chemistry by chemists. Nowadays, it remains an important tool for investigation between structure and activity relationships in biochemistry researches and as a substituent in organic molecular structures in commercialized compounds¹. Especially, chloro

atom containing β -lactam derivatives are interesting compounds in terms of pharmaceutical and synthetic organic chemistry. Due to bioactivity properties, β -lactam molecules have widely large area in drug chemistry; new synthetic methods developed for these molecules are an precious issue in organic chemistry. As known, the β -lactam structure is part of the moiety of several antibiotic molecules such as carbapenems (1), penicillins (2), and cephalosporins (3), therefore, also called β -lactam antibiotics¹. Particularly, all of β -lactam antibiotics inhibit bacteria cell wall biosynthesis. Up to now, more than thousand different β -lactamase enzymes have been reported in several species of bacteria. These enzymes influence widely β -lactam structure and catalytic efficiencies².

The carbonic anhydrases (CAs, EC 4.2.1.1) are widespread metalloenzymes family and catalyze the reversible hydration of carbon dioxide (CO_2) and water (H_2O) to (HCO_3^-) and proton (H^+)^{3–7}. CAs have six genetically and distinct enzyme families, including the α -, β -, γ -, δ -, ζ -, and η -CA^{8–10}. This regulatory reaction supports many physiological processes associated with pH control, fluid secretion, ion transport, and several biosynthetic processes^{11–15}. The inhibition of CAs has been exploited clinically for many decades, as most CA isoforms of the 16 known in humans are therapeutic targets with the potential to be inhibited or activated^{16–20}. The two of them (hCA I and II) are cytosolic ones. The catalytic activity of hCA I, which shows blood enzyme, is much lower compared to that of hCA II, and in addition hCA I is

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inhibited by the chloride and bicarbonate present in the plasma, raising a lot of questions regarding the physiologic function of this isoform^{15,21–24}. The physiologically dominant isoform is the red blood cell CA II, which present in many other tissues and its inhibitors have widespread use in a variety of applications as diuretics, antiglaucoma, and antiepileptic agents^{25–30}. The discovery of new classes of CA inhibitors (CAIs), which possessing different inhibition mechanisms when compared with the classical inhibitors^{15,31–35}. For such reasons, the design of novel classes of potent, possibly isoform-selective inhibitors targeting other human CA (hCA) isoforms, may lead to clinical applications for treating a multitude of diseases such as edema, epilepsy, obesity, neuropathic pain, and other neurological disorders^{36–40}.

Alzheimer's disease (AD) is a progressive neuro-degenerative disorder and the fourth leading cause of death in people over 65-year-old worldwide. AD is characterized by the atrophy of cholinergic neurons, behavioral abnormalities, deterioration of cognition, memory loss, loss of speech, and, eventually, death^{41–44}. Although the etiology of this disease is still elusive, several hallmarks, such as low levels of acetylcholine, oxidative stress, β -amyloid deposits, γ -protein aggregation, and the dyshomeostasis of biometals, are thought to play crucial roles in the development of AD^{41,45–47}. Because of the complex pathogenesis of AD, there is no ideal drug for the prevention or treatment of AD up to now. Acetylcholinesterase (AChE, EC 3.1.1.7), a serine protease, is responsible for acetylcholine (ACh) hydrolysis and plays a fundamental role in impulse transmission by terminating the action of the neurotransmitter ACh at the cholinergic synapses and neuromuscular junction^{48,49}. One of the best primary pharmacological strategies for the treatment of AD is to improve cholinergic neurotransmission by decreasing the rate of decomposition of ACh at synapses in the brain with the use of acetylcholinesterase inhibitors (AChEIs)⁵⁰.

The aim of this study is to design and synthesize some imines (**10–14**) and β -lactam analogs (**15–19**) and to investigate their potential carbonic anhydrase isoenzymes I and II (CA I and II) and acetylcholinesterase (AChE) inhibition properties.

Materials and methods

The synthesis of Schiff bases from 2-naphthalene aldehyde and amine compounds

Aryl or methyl amines (**5–9**) (1.0 mmol) were dissolved in 30-mL ethyl alcohol and added for 10 min in droplets to 0.05% 3 mL H₂SO₄ acid and aldehyde (**4**) (1.0 mmol) solution dissolved in 20 mL ethyl alcohol. After that, reaction was refluxed at 80–90 °C for 24 h. At the end of the reaction followed by TLC, reaction mixture was taken to a 250 mL beaker glass and added aqueous Na₂CO₃ solution, so H₂SO₄ was removed from the reaction medium. Obtained Schiff base mixture was taken to organic phase by extracting and using ethyl acetate (3 \times 100 mL). The water in organic phase was removed by drier Na₂SO₄ after organic phase was washed off in 100 mL saturated NaHCO₃. Organic solvent was removed in vacuum (20 °C, 20 mm Hg). Remnant crude product was purified using silica gel through column chromatography in ethylacetate:methanol:*n*-hexane (7:1:2) solvent mixture.

Typical procedure for dichloroketene cycloadditions

A solution of 0.025 mol of freshly distilled trichloroacetyl chloride in 250 mL of dry ether was added over 4 h to a stirred, refluxing mixture of 0.025 mol of Schiff base (imine) in 250 mL of dry ether and 5 g of activated zinc under a nitrogen atmosphere. The reaction mixture was stirred at reflux for an additional 10 h after the addition was complete. The excess zinc was filtered,

washed, and dried. The reaction solution was concentrated to about 50 mL and then stirred with 100 mL of *n*-hexane. The hexane solution was decanted from the zinc salts and evaporated, and the residue was purified on silica-jel column chromatography to yield the cycloadduct.

Chemistry

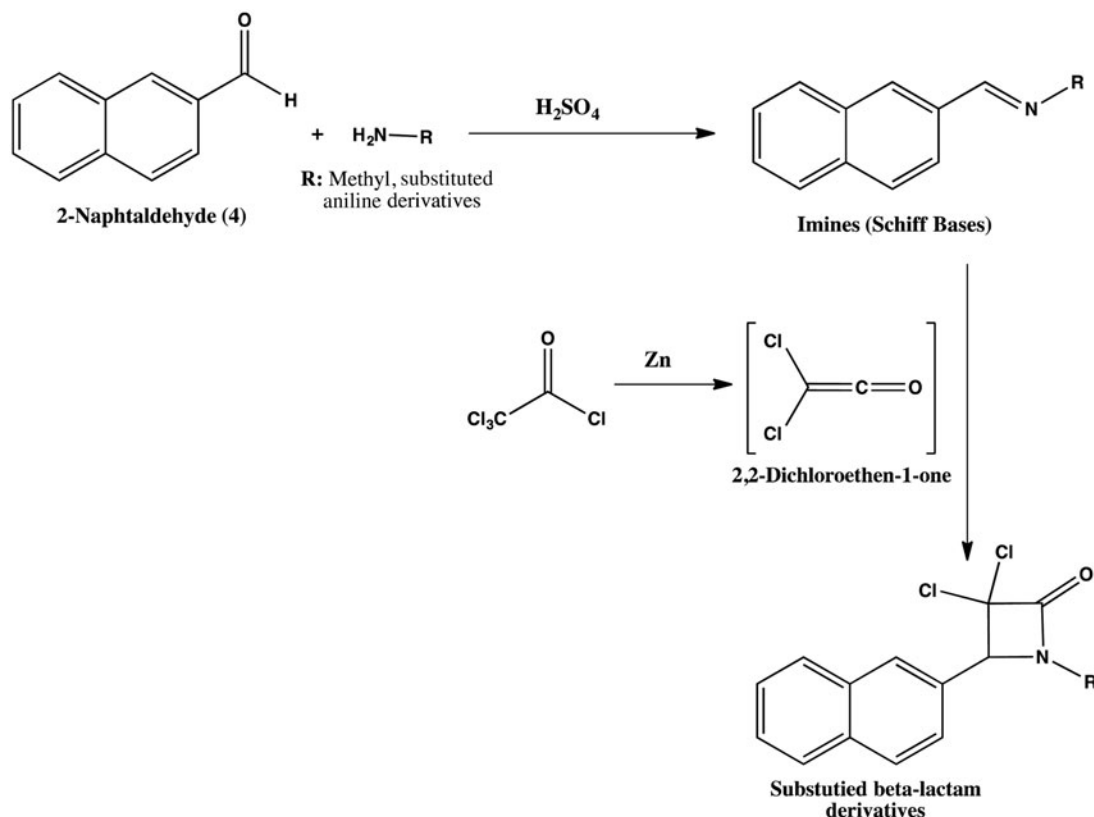
Anhydrous solvents and all reagents were purchased from Sigma-Aldrich and Alfa Aesar Nuclear magnetic resonance (¹H-NMR and ¹³C-NMR); spectra were recorded using a Varian 400 MHz or Bruker Advance 400 DPX MHz spectrometer (Erzurum, Turkey) in CDCl₃; Chemical shifts are reported in parts per million (ppm), and the coupling constants (*J*) are expressed in Hertz (Hz). Splitting patterns are designated as follows: s, singlet; d, doublet; sept, septet; t, triplet; q, quartet; m, multiplet; brs, broad singlet; brm, broad multiplet; dd, double of double; td, triplet of double; tt, triplet of triplet; appd, apparent double; appt, apparent triplet. Analytical thin-layer chromatography (TLC) was carried out on Merck silica gel F-254 plates.

We synthesized the β -lactam analogs containing dichloride atoms and N-methyl, N-aromatic rings as substituents, by Schiff bases and dichloroketene compounds. Synthesis of molecules comprising Schiff base has long been attracting attentions due to their biological activities such as anticancer, antibacterial, antifungal, antidepressant, analgesic, and cytotoxic effects in addition to their regular use in electronic industry, cosmetic, and polymer industry. Schiff bases are frequently used in addition reactions, Staudinger Reactions, Hetero-Diels-Alder reactions, and several ligand complexes, thanks to their structural characteristics^{51,52}.

In this study, first, imine groups known as Schiff bases in literature, 1-(naphthalene-2-yl)-*N*-phenylmethanimine (**10**)⁵³, *N*-(4-ethylphenyl)-1-(naphthalene-2-yl)methanimine (**11**), *N*-mesityl-1-(naphthalene-2-yl)methanimine (**12**), 1-(naphthalene-2-yl)-*N*-(*p*-tolyl) methan-imine (**13**)⁵⁴, *N*-methyl-1-(naphthalene-2-yl) methanimine (**14**)⁵⁵, were synthesized from corresponding aromatic aldehyde (**4**) and amine derivatives aniline (**5**), 4-ethylaniline (**6**), 2,4,6-trimethylaniline (**7**), *p*-toluidine (**8**), methanamine (**9**). Some Schiff bases (**10**, **13**, **14**) as known in literature and other Schiff bases were synthesized in this study. After characterization of imine compounds by NMR spectrum, dichloroketene (2,2-dichloroethene-1-one), compounds were obtained with Zn from trichloride acetyl chloride and active zinc metal in basic media in high yields (Scheme 1).

These imines were purified by easy methods such as crystallization method or silica-gel column chromatography. The yields and reaction conditions of synthesized imines were shown in Table 1.

In second step, the β -lactam derivatives 3,3-dichloro-4-(naphthalene-2-yl)-1-phenylazetidin-2-one (**15**), 3,3-dichloro-1-(4-ethylphenyl)-4-(naphthalene-2-yl)azetidin-2-one (**16**), 3,3-dichloro-1-mesityl-4-(naphthalene-2-yl)azetidin-2-one (**17**), 3,3-dichloro-4-(naphthalene-2-yl)-1-(*p*-tolyl) azetidin-2-one (**18**), 3,3-dichloro-1-methyl-4-(naphthalene-2-yl)azetidin-2-one (**19**) was synthesized with [2 + 2] ketene addition reaction from dichloroketene and corresponding imine derivatives obtained from **10–14** amines and 2-naphthalaldehyde (**4**) in acidic reaction condition (Scheme 1). All of the β -lactam structures **15–19** were synthesized first time in literature in this study. The dichloroketene (2,2-dichloroethene-1-one) was also obtained from Zn and trichloroacetyl chloride at 40 °C in 10 h (Table 2). The yields of addition reaction were changed according to the structure of imine compounds. All of the compounds were purified by column chromatography and characterized by NMR spectroscopy.

Scheme 1. The synthesis route of β -lactam derivatives.

NMR spectral data of first synthesized imines and β -lactam molecules

N-(4-Ethylphenyl)-1-(naphthalene-2-yl)methanimine (**11**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 8.46 (s, 1H), 8.19 (m, 2H), 7.92 (m, 3H), 7.56 (m, 2H), 7.28 (m, 4H), 2.71 (q, J = 7.4, 7.6 Hz, 2H), 1.3 (t, J = 7.7 Hz, 3H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 159.8, 149.9, 142.6, 135.2, 134.4, 133.4, 131.3, 129.0, 128.9, 128.2, 127.7, 126.8, 124.2, 121.2, 28.7, 15.9.

N-Mesityl-1-(naphthalene-2-yl)methanimine (**12**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 8.39 (s, 1H), 8.25 (d, J = 1.5 Hz, 8.4 Hz, 1H), 8.18 (s, 1H), 7.95 (m, 3H), 7.57 (m, 2H), 6.9 (s, 2H), 2.3 (s, 3H), 2.2 (s, 6H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 162.9, 149.1, 135.3, 134.1, 133.6, 133.3, 131.1, 129.1, 129.0, 128.2, 127.7, 127.3, 126.9, 123.9, 21.0, 18.5.

3,3-Dichloro-4-(naphthalene-2-yl)-1-phenylazetidin-2-one (**15**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 7.1–7.9 (m, 12H), 5.6 (s, 1H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 158.6, 133.7, 136.1, 134.1, 133.2, 129.3, 128.7, 128.4, 128.1, 127.9, 127.6, 127.4, 127.1, 125.8, 124.5, 118.3, 74.4.

3,3-Dichloro-1-(4-ethylphenyl)-4-(naphthalene-2-yl)azetidin-2-one (**16**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 7.3–7.9 (m, 7H), 7.2 (d, J = 8.4 Hz, 2H), 7.1 (d, J = 8.4 Hz, 2H), 5.6 (s, 1H), 2.5 (q, J = 7.3, 7.5 Hz, 2H), 1.1 ppm (t, J = 7.7 Hz, 3H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 165.1, 142.0, 134.1, 133.8, 133.2, 129.9, 129.5, 129.1, 128.9, 128.4, 128.1, 127.8, 127.3, 127.0, 124.6, 118.2, 74.3, 31.8, 15.7.

3,3-Dichloro-1-mesityl-4-(naphthalene-2-yl)azetidin-2-one (**17**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 7.4–7.8 (m, 7H), 6.8 (bs, 2H), 5.9 (s, 1H), 2.4 (bs, 6H), 2.2 (s, 3H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 160.2, 138.6, 133.9, 133.0, 130.4, 130.2, 129.8, 128.7, 128.4, 128.0, 127.8, 127.3, 126.9, 126.4, 125.2, 124.9, 77.9, 21.0, 19.7.

3,3-Dichloro-4-(naphthalene-2-yl)-1-(*p*-tolyl)azetidin-2-one (**18**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 7.0–7.8 (m, 11H), 5.6 (s, 1H), 2.2 (s, 3H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 167.5, 135.7, 130.1, 129.1, 128.8, 128.4, 127.9, 127.7, 127.5, 127.3, 127.0, 126.9, 126.8, 126.7, 126.5, 118.2, 74.3, 21.2.

3,3-Dichloro-1-methyl-4-(naphthalene-2-yl)azetidin-2-one (**19**): $^1\text{H-NMR}$ (400 MHz, CDCl_3 , ppm): δ = 7.3–7.9 (m, 7H), 5.2 (s, 1H), 3.0 (s, 3H). $^{13}\text{C-NMR}$ (100 MHz, CDCl_3 , ppm): δ = 162.1, 134.1, 133.2, 129.4, 129.3, 129.0, 128.7, 128.3, 128.1, 127.4, 127.1, 124.7, 75.9, 28.0.

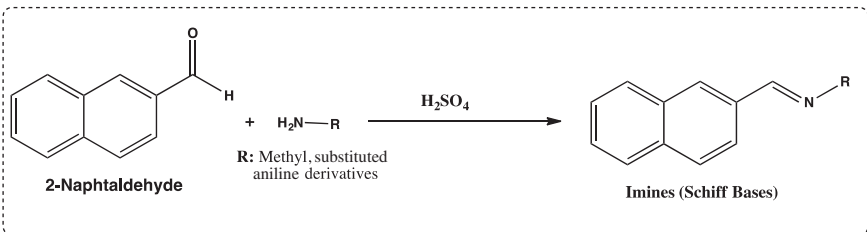
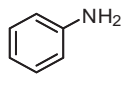
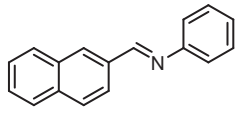
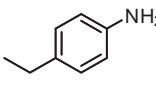
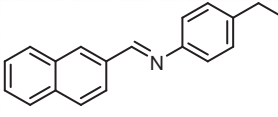
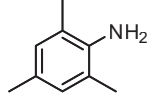
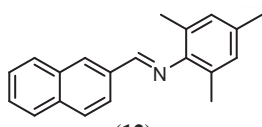
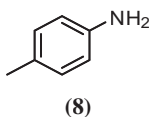
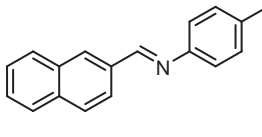
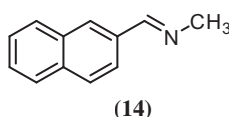
Biochemical studies

For the determination of inhibition effects of imines (**10–14**) and β -lactam analogs (**15–19**) on CA isoforms I and II. Both isoenzymes were purified from fresh human erythrocyte using affinity chromatography technique^{56–60}. Chromatographic separation technique was widely used for the purification of biomolecules, including protein and enzymes. To this end, sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography was used for the purification of both isoenzymes^{61–63} as described previously^{64–66}.

CA activity determination was realized spectrophotometrically according to Verpoorte et al.⁶⁷ as described previously⁴⁹. In this method, absorbance change was recorded during 3 min at 25 °C. *p*-Nitrophenylacetate (NPA) was used as substrate for both isoenzymes and enzymatically converted to *p*-nitrophenolate ion⁶⁸. These activity determinations are described in detail in our previous studies⁶⁹.

Bradford method was used for the determination of protein quantity during the purification steps⁷⁰. This spectrophotometrical protein assay was explained as previously⁷¹. The bovine serum

Table 1. The synthesis of imine derivatives (10–14).

 <p>2-Naphtaldehyde + $\text{H}_2\text{N}-\text{R} \xrightarrow{\text{H}_2\text{SO}_4}$ Imines (Schiff Bases)</p> <p>R: Methyl, substituted aniline derivatives</p>				
Entry	Amine	Reaction Condition	Schiff bases	Yields (%)
1	 (5)	2-Naphtaldehyde (4) + H_2SO_4 (cat.), EtOH Reflux, 12 h	 (10)	95
2	 (6)		 (11)	90
3	 (7)		 (12)	85
4	 (8)		 (13)	90
5	$\text{H}_3\text{C}-\text{NH}_2$ (9)		 (14)	95

albumin was used as standard protein which demonstrated maximum activity at 595 nm^{71–73}.

After the purification process of the CA isoenzymes, SDS-PAGE has been carried out^{74–76}. Stacking gel containing (10 and 3%) acrylamide and (0.1%) SDS^{77,78} was used for running the process using a Minigel system (Mini-PROTEAN Tetra System, China). The method used for visualization of protein has been explained in detail at previous studies⁷⁹. Briefly, the gel was fixed and then stained with Coomassie Brilliant Blue R-250 later on the gel destained by using standard methods for detecting protein bands that are belong to purified CA isoenzymes^{80,81}.

The inhibitory effects of imines (10–14) and β -lactam analogs (15–19) on AChE activity were measured according to the spectrophotometric method of Ellman et al.⁸² Acetylthiocholine iodide (AChI) was used as substrate for the enzymatic reaction. 5,5'-Dithio-bis(2-nitro-benzoic) acid (DTNB) was used for the measurement of the AChE activity. Briefly, 100 μL of Tris/HCl buffer (1 M, pH 8.0), 10 μL of sample solution dissolved in deionized water at different concentrations, and 50 μL of AChE solution were mixed and incubated for 10 min at 25 °C. Then, 50 μL of DTNB (0.5 mM) was added. Then reaction was initiated

by the addition of 50 μL of AChI. The hydrolysis of these substrates was monitored spectrophotometrically by formation of the yellow 5-thio-2-nitrobenzoate anion as the result of the reaction of DTNB with thiocholine, released by enzymatic hydrolysis of AChI, with an absorption maximum at a wavelength of 412 nm⁵⁰.

The effect of imines (10–14) and β -lactam analogs (15–19) on both CA isoenzymes was examined using the hydratase activity and recorded in triplicate analysis at the each concentration used⁸³. For this purpose, different concentrations of imines (10–14) and β -lactam analogs (15–19) were determined in preliminary assays. CA isoenzyme activities were measured in the presence of different quantity of them. The control sample activity in the absence of a imines (10–14) and β -lactam analogs (15–19) was taken as 100%. For each imines (10–14) and β -lactam analogs (15–19), an activity (%)=[imines or β -lactams] was drawn using conventional polynomial regression software. The half maximal inhibitory concentration (IC_{50}) of each imines (10–14) and β -lactam analogs (15–19) was calculated from graphs⁵⁰. IC_{50} values are measure of the effectiveness of imines (10–14) and β -lactam analogs (15–19) in inhibiting both CA

Table 2. The addition reactions of imines and dichloroketene compounds.

Entry	Schiff bases	Reaction Condition	β -Lactams	Yields (%)
1	 (10)	Zn, 40°C, 10 h	 (15)	20
2	 (11)		 (16)	85
3	 (12)		 (17)	40
4	 (13)		 (18)	50
5	 (14)		 (19)	40

isoenzymes. For the determination of K_i values, three different imines (**10–14**) and β -lactam analogs (**15–19**) concentrations were used. K_i values reflect the binding affinity of imines (**10–14**) and β -lactam analogs (**15–19**) to both CA isoenzymes. In this way, The IC_{50} value is converted to an absolute inhibition constant K_i value. In this experiment, PNA was used as substrate at five different concentrations. Finally, Lineweaver–Burk curves were drawn for each imine (**10–14**) and β -lactam analog (**15–19**)⁵⁰.

Metal-chelating study

Metal-chelating ability of imines (**10–14**) and β -lactam analogs (**15–19**) was performed according to Dinis et al.⁸⁴ with slight modification^{85–88}. Fe^{2+} -binding capacity of imines (**10–14**) and β -lactam analogs (**15–19**) was spectrophotometrically recorded at

562 nm^{88–91}. For this purpose, to a mixture of $FeCl_2$ (0.1 mL, 0.6 mM), three different concentrations (10–30 μ g/mL) of imines (**10–14**) and β -lactam analogs (**15–19**) in methanol (0.4 mL) were added. The reactions were started by ferrozine solution addition (0.1 mL, 5 mM). After that, the solution was mixed and incubated at room temperature for 10 min. Finally, absorbance value of the mixture of imines (**10–14**) and β -lactam analogs (**15–19**) was determined spectrophotometrically at 562 nm^{92–95}.

Result and discussion

Chemistry

Many chlorinated organic compounds are known for their detrimental effects as a toxicological properties and environmentally harmful chemicals. However, chlorinated organic molecules,

Table 3. The enzyme inhibition values of some imines (**10–14**) and β -lactam analogs (**15–19**) against human carbonic anhydrase isoenzymes I and II (hCA I and II) and acetylcholinesterase (AChE) enzyme.

Compounds	IC ₅₀ (nM)						K _i (nM)		
	hCA I	r ²	hCA II	r ²	AChE	r ²	hCA I	hCA II	AChE
10	7.79	0.9816	10.21	0.9516	3.21	0.9695	5.31 ± 0.39	7.27 ± 0.91	1.57 ± 0.55
11	6.30	0.9829	7.79	0.9611	2.76	0.9933	4.67 ± 0.54	9.93 ± 4.19	1.48 ± 0.06
12	6.93	0.9646	8.66	0.9655	3.01	0.9714	9.09 ± 4.06	8.98 ± 1.80	1.01 ± 0.46
13	8.25	0.9783	9.12	0.9644	2.44	0.9776	7.45 ± 1.42	10.29 ± 3.55	0.82 ± 0.34
14	10.83	0.9727	14.74	0.9339	3.03	0.9434	11.18 ± 1.98	10.41 ± 2.69	1.36 ± 0.54
15	4.59	0.9688	7.37	0.9561	1.35	0.9632	4.81 ± 1.78	6.59 ± 2.51	0.82 ± 0.39
16	3.59	0.9911	5.29	0.9709	1.18	0.9760	3.22 ± 0.22	3.74 ± 0.39	0.50 ± 0.20
17	4.15	0.9856	5.02	0.9606	1.39	0.9631	3.40 ± 0.82	4.37 ± 1.74	0.89 ± 0.37
18	5.09	0.9831	6.13	0.9616	1.52	0.9496	3.74 ± 0.71	5.88 ± 2.32	0.61 ± 0.15
19	7.37	0.9727	7.88	0.9382	1.97	0.9593	4.99 ± 0.69	7.08 ± 1.46	0.94 ± 0.35
AZA*	101.19	0.9509	113.75	0.9791	–	–	170.34 ± 2.48	115.43 ± 1.63	–
DZA*	88.45	0.9760	93.01	0.9910	–	–	129.26 ± 0.21	135.67 ± 0.32	–
TAC**	–	–	–	–	9.76	0.9463	–	–	5.70 ± 1.09

*Acetazolamide (AZA) and dorzolamide (DZA) were used as a standard inhibitor for all hCA I and II.

**Tacrine (TAC) was used as a standard inhibitor for AChE enzyme.

molecular weight range from 200 to 600, are used as pharmaceutical or biologically active substances in drug chemistry and plant protection agents, which are important and essential chemicals in green chemistry. As a result of the scientific investigation, it has been experimentally found that the introduction of a chlorine atom into several specific positions of a biologically active molecule may be essential to improve the basic biological activity.

Biochemistry

The discovery of new classes of CAIs, possessing different inhibition mechanisms compared with the classical inhibitors of the sulfonamide and anion type, has also seen important developments ultimately⁹⁶. So far, five different CA inhibition mechanisms were reported: (i) the zinc ion (Zn²⁺) binders are the inhibitors which coordinate to the catalytically crucial Zn²⁺ from the enzyme active site. In this inhibition type, Zn²⁺ may be in a tetrahedral or trigonal bipyramidal geometries, with the sulfonamides and sulfamides, sulfamates, most anions, dithiocarbamates, carboxylates, and hydroxamates binding in this way⁹⁷; (ii) the inhibitors that anchor to the Zn²⁺-coordinated water molecule (H₂O)/hydroxide ion (–OH), represented by the phenols, some carboxylates, the polyamines, 2-thioxocoumarins, and sulfocoumarins^{96,98}; (iii) the inhibitors-like coumarins and their isosteres which occlude the entrance to the active site cavity, this binding site coinciding with that where CA activators bind⁹⁹; (iv) the compounds which bind out of the active site cavity, and (v) compounds for which the inhibition mechanism is not known, among which the secondary or tertiary sulfonamides are the most investigated examples¹⁰⁰.

The hCA I and II isoenzyme inhibitory activity of the imines (**10–14**) and β -lactam analogs (**15–19**) was shown in Table 3. CA II isoform was chosen because of its antiglaucoma drug targets, however CA I, due to its diffuse distribution in the blood and gastrointestinal tract is one of the main off-targets for such pharmacologic agents¹⁰¹. On the other hand, AChE was chosen for its significant contribution in drug discovery and development for AD. All the synthesized imines (**10–14**) and β -lactam analogs (**15–19**) were tested for the evaluation of their inhibitory activity toward the slower cytosolic hCA I isoform, the more rapid cytosolic hCA II isoenzyme and AChE enzyme. Also, these novel derivatives showed effective ferrous ions (Fe²⁺)-chelating effects. The following results can be observed from the inhibition data of Table 3:

- (1) Cytosolic hCA I isoenzyme was potently inhibited by imines (**10–14**) and β -lactam analogs (**15–19**). K_i values were found in ranging between 3.22 ± 0.22 and 11.18 ± 1.98 nM (Table 3). The best inhibition for this isoform was observed by novel β -lactam analog **16** (3,3-dichloro-1-(4-ethylphenyl)-4-(naphthalene-2-yl)azetidin-2-one), which possess biological active groups of –C=O, –CH₃, and Cl with K_i value of 3.22 ± 0.22 nM. It is well-known that CA isoenzymes easily inhibited by the compounds, which had these biologically active groups. On the other hand, as can seen in Figure 1 acetazolamide (AZA, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide) and dorzolamide (DZA, N-(5-sulfamoyl-1,3,4-thiadiazol-2-yl)acetamide) used as CAI for the medical treatment of some diseases including idiopathic intracranial hypertension, epileptic seizure, glaucoma, altitude sickness, periodic paralysis, cystinuria, central sleep apnea, and dural ectasia, showed K_i values of 170.34 ± 2.48 and 129.26 ± 0.21 nM, respectively. The hCA I is highly abundant in red blood cells and is found in many tissues but its precise physiological function is unknown. CA I is associated with cerebral and retinal edema, and the inhibition of CA I may be a valuable tool for fighting these conditions⁶.
- (2) The ubiquitous and physiologically predominant cytosolic isoform hCA II is associated with several diseases. For hCA II, β -lactam analogs (**15–19**) had K_i values ranging in 3.74 ± 0.39–10.29 ± 3.55 nm. On the other hand, acetazolamide (AZA, 5-acetamido-1,3,4-thiadiazole-2-sulfonamide), a clinically used compound, was a medium potency CA II inhibition for this isoform, with an inhibition constant of 115.43 ± 1.63 nM. Also, dorzolamide (DZA), a carbonic anhydrase inhibitor, demonstrated K_i value of 135.67 ± 0.32 nM. DZA was used as antiglaucoma agent and acts by decreasing the production of aqueous humour¹⁰². All of the derivatives showed highly effective hCA II inhibitory activity, comparable to that of AZA and DZA. As can be seen in CA I, the most inhibition effect of CA II was observed by β -lactam analog **16** (3,3-dichloro-1-(4-ethylphenyl)-4-(naphthalene-2-yl)azetidin-2-one) with K_i values of 3.74 ± 0.39 nM.
- (3) Effective AChE inhibitors can be used for AD treatment. Most of the currently available drugs on the market including tacrine, rivastigmine, donepezil, and galantamine intended to treat AD are AChE inhibitors¹⁰³. The inhibition effects of some imines (**10–14**) and β -lactam analogs (**15–19**) against AChE activities were measured according to

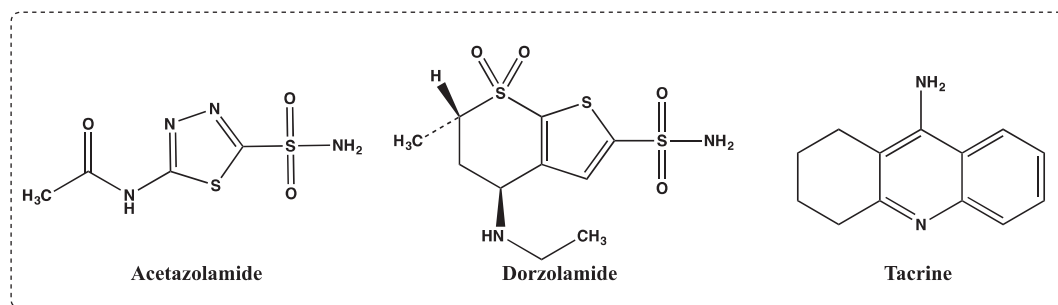


Figure 1. Standard compounds used for carbonic anhydrase I and II isoenzymes (acetazolamide and dorzolamide) and acetylcholinesterase (tacrine) inhibitors.

spectrophotometric Ellman method⁸². AChI was used as substrates of the reaction. This method is based on the amount of thiocholine released when the enzyme AChE hydrolyzes the AChI to thiocholine and acetate. The product thiocholine reacts with DTNB to produce a yellow compound (5-thio-2-nitrobenzoate) anion, which can be detected at a wavelength of 412 nm. AChE was very effectively inhibited by imines (**10–14**) and β -lactam analogs (**15–19**). It was found that K_i values were ranging between 0.50 ± 0.20 and 1.57 ± 0.55 nM (Table 3). All imines (**10–14**) and β -lactam analogs (**15–19**) demonstrated similar inhibition profile against AChE. On the other hand, tacrine, a first centrally acting cholinesterase inhibitor approved for the treatment of AD, was used as a standard AChE with K_i values of 5.70 ± 1.09 nM. Imines **10** (1-(naphthalene-2-yl)-*N*-phenyl-methanimine), which shown the weakest AChE inhibition, had 3.63-times AChE inhibition effects than that of Tacrine. The K_i values of imines (**10–14**) and β -lactam analogs (**15–19**) for AChE were calculated from Lineweaver–Burk plots⁸³.

- (4) Metal-chelating measurement is an antioxidant method, which based on the absorbance measurement of ferrous ion (Fe^{2+})-ferrozine complex after prior treatment of a Fe^{2+} solution with test material^{104–106}. Ferrozine forms a complex with free Fe^{2+} , but not with Fe^{2+} bound to other chelators; thus, a decrease in the amount of Fe^{2+} -ferrozine complex formed after treatment indicates the presence of antioxidant chelators^{107,108}. The ferrozine- Fe^{2+} complex produced a red chromophore with absorbance that can be measured at 562 nm^{109,110}. A significant drawback of this complexation reaction in measuring the presence of antioxidant chelator is that the reaction is affected by both the antioxidant- Fe^{2+} and ferrozine- Fe^{2+} complex formation constants, and the competition between the two chelators for binding to iron. Thus, a weak antioxidant iron chelator would be seriously underestimated in quantitative determination^{111,112}. The metal-chelating capacity was significant since it reduced the concentration of the catalyzing transition metal in lipid peroxidation. It was reported that chelating agents are effective as secondary antioxidants because they reduce the redox potential, and, thereby, stabilize the oxidized form of the metal ion^{113,114}. EDTA is a strong metal chelator; hence, it was used as standard metal chelator agent in this study. The data obtained from Table 4 reveal that imines (**10–14**) and β -lactam analogs (**15–19**) possess marked iron-binding capacity, suggesting that their main action as a peroxidation inhibitor may be related to their iron-binding capacity. The distinction between different concentrations (10–30 $\mu\text{g/mL}$) of imines (**10–14**) and β -lactam analogs (**15–19**) and the control value was fixed to be statistically important ($p < 0.01$). Furthermore, it is found that IC_{50} values for imines (**10–14**) and β -lactam analogs (**15–19**)

Table 4. Determination of half maximal concentrations (IC_{50} , $\mu\text{g/mL}$) of Fe^{2+} chelating of some imines (**10–14**) and β -lactam analogs (**15–19**) and standard compounds.

Compounds	Fe^{2+} chelating	r^2
BHA	36.47	0.9837
BHT	57.75	0.9922
α-Tocopherol	18.73	0.9861
Trolox	28.88	0.9828
EDTA	12.83	0.9924
10	76.15	0.9855
11	113.60	0.9944
12	85.56	0.9813
13	49.50	0.9737
14	57.75	0.9983
15	138.60	0.9889
16	230.01	0.9783
17	98.89	0.9828
18	77.01	0.9911
19	115.50	0.9728

were found in the range of 49.50–230.01 $\mu\text{g/mL}$ (Table 4). Whereas, IC_{50} values belonging to Fe^{2+} ion-chelating capacity of positive controls like α -tocopherol, trolox, BHT, BHA, and EDTA were found to be in range of 12.83–57.75 $\mu\text{g/mL}$. A lower IC_{50} value reflects a higher Fe^{2+} ions-binding activity. These results clearly show that Fe^{2+} ion-chelating effect of imines (**10–14**) and β -lactam analogs (**15–19**) had effective Fe^{2+} ions chelating; however, these values were lower than that of standard metal chelators. Fe^{2+} ions are the most efficient pro-oxidants in pharmacology systems and food^{115,116}. Ferrozine can create complexes with Fe^{2+} . In the presence of Fe^{2+} -chelating compounds, ferrozine- Fe^{2+} complex formation is a broken down, resulting in a decrease in the red color formation of Ferrozine- Fe^{2+} complex^{117,118}.

Conclusion

In the present study, practical and efficient method has been developed for *N*-aryl β -lactams the first time in literature from Schiff bases and simple dichloroketene compound. Schiff base reactions are widely used in chemistry due to their mild reaction conditions and high reaction rates. The synthesis of β -lactams derived **15–19** from these Schiff bases and the dichloroketene **4** by using [2 + 2] addition reaction. It is evident from the results of the investigation that a effective method has been obtained, by EtOH solvent under acidic conditions, for the synthesis of Schiff bases from corresponding amines **10–14** and 2-naphtalaldehyde (**4**) in high yields in our study. The imines (**10–14**) and β -lactam analogs

(15–19) used in the present study showed effective inhibition profiles against AChE enzyme and both hCA isoenzymes. Also, these compounds demonstrated effective ferrous ion (Fe^{2+})-chelating activity. In this study, nanomolar level of K_i values was observed for each novel imines (10–14) and β -lactam analogs (15–19), and these compounds can be selective inhibitor of both cytosolic CA isoenzymes and AChE enzyme. Also, they can be used as metal chelators in related applications.

Declaration of interest

The authors declare no conflict of interest.

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