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RESEARCH ARTICLE

Synthesis of some tetrahydropyrimidine-5-carboxylates, determination of their metal chelating effects and inhibition profiles against acetylcholinesterase, butyrylcholinesterase and carbonic anhydrase

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Abstract

2-(Methacryloyloxy)ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate, is a cyclic urea derivative synthesized from urea, 2-(methacryloyloxy) ethyl acetoacetate and substituted benzaldehyde, and tested in terms of the inhibition of two physiologically relevant carbonic anhydrase (CA) isozymes I and II. Acetylcholinesterase (AChE) is found in high concentrations in the red blood cells and brain. Butyrylcholinesterase (BChE) is another enzyme abundantly present in the liver and released into blood in a soluble form. Also, they were tested for the inhibition of AChE and BChE enzymes and demonstrated effective inhibition profiles with *Ki* values in the range of 429.24–530.80 nM against hCA I, 391.86–530.80 nM against hCA II, 68.48–97.19 nM against AChE and 104.70–214.15 nM against BChE. On the other hand, acetazolamide clinically used as CA inhibitor, showed *Ki* value of 281.33 nM against hCA I, and 202.70 nM against hCA II. Also, Tacrine inhibited AChE and BChE showed *Ki* values of 396.03 and 209.21 nM, respectively.

Keywords

Acetylcholinesterase, butyrylcholinesterase, carbonic anhydrase, urea, X-ray

History

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Introduction

The pyrimidinethiones display many pharmacological properties, as part of our interest in this kind of materials; we report here the synthesis and crystal structure determination of the title compound[1]. Our synthesis is based on the Bidjinelli reaction, which consists of a three-component condensation of an aldehyde, a methylene active compound and a urea derivative in acidic media. This procedure is the most simple and useful for the preparation of 3,4-dihydropyrimidene-2(1H) ones[2–4].

New cyclic ureas were obtained by continuing researches in the field of the synthesis of various classes of organic nitrogen compounds and the study of their transformations. So for the first time 2-(methacryloyloxy)ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate was obtained by us based on the trifluoroacetic acid (TFAA) catalyst.

Transition metal ions are key elements in various biological processes ranging from oxygen formation to hypoxia sensing. Their homeostasis is maintained within strict limits through tightly regulated mechanisms of uptake, storage and secretion[5–7]. The breakdown of metal ion homeostasis can lead to an uncontrolled formation of reactive oxygen species (ROS) via Fenton reaction, which produces hydroxyl radicals (OH·)[8–11].

$$Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^{\cdot} + OH^{-}$$

ROS can cause oxidative damage to biological macromolecules such as DNA, carbohydrate, lipids and proteins. An imbalance between the formation of ROS and their elimination by antioxidant defence systems is termed as oxidative stress[12–14]. Most vulnerable to free radical attack is the cell membrane, which may undergo enhanced lipid peroxidation, finally producing mutagenic and carcinogenic malondialdehyde (MDA), 4-hydroxynonenal and other exocyclic DNA adducts[15–17]. Among the transition metal ions, iron is known as the most important lipid oxidation pro-oxidant due to its high reactivity. Iron is an essential mineral for normal physiology, but excess can result in cellular injury[18–20]. Ferrous iron (Fe^{2+}) can facilitate the production of ROS within animal and human systems and the ability of substances to chelate iron can be valuable for antioxidant property[21–23]. The effective Fe^{2+} chelating may also afford protection against oxidative damage by removing iron that may otherwise participate in HO· generating Fenton-type reactions. Iron, in nature, can be found as either Fe^{2+} or ferric ion (Fe^{3+}),

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with the latter form predominant in foods. Fe^{3+} also produces radicals from peroxides although the rate is tenfold less than that of Fe^{2+}[24–26].

The carbonic anhydrases (CAs, EC 4.2.1.1) are the metalloenzymes containing zinc ions (Zn^{2+}), which classically participate in the maintenance of pH homeostasis. CAs catalyse the reversible hydration of CO_2 in two-step reaction to yield bicarbonate (HCO_3^-) ion and H^+[27,28,29]. The interconversion of these chemical species is shown in following equation, which however is too slow to meet the physiological needs of most biochemical processes[30–32].

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

CAs have six genetically distinct enzyme families: the α-, β-, γ-, δ-, ζ- and η-CA. Mammals generally contain α-CAs, which is the best studied CA family. α-CAs exist in 16 isoforms identified in various tissues and organs and differ in their cellular localization (cytosol, mitochondria or cell membrane), sensibility to inhibitors and catalytic activity[33–35]. Also, this CA family has molecular kinetic and different properties, oligomeric rearrangements and expression levels, as well as various abilities to respond to different inhibitory classes[36–38]. According to the known cellular localization, some of them are cytosolic (CA I, CA II, CA III, CA VII and CA XIII), some other CA isoenzymes are membrane bound (CA IV, CA IX, CA XII and CA XIV), two of CAs are mitochondrial (CA VA and CA VB) and one of CAs is salivary (CA VI)[39–42]. CA XV is not synthesized in humans and other primates and is abundantly found in rodents and other vertebrates as an isoform. Three acatalytic forms are also reported and named as CA related proteins (CARPs), CARP VIII, X and XI, which are found in the cytosol[43–45]. The two important CA isozymes (CA I and CA II) are present at higher concentrations in the cytosol in erythrocytes. hCA I and II have various medical applications and shows optimal activity at physiological pH and temperatures[46–48].

CAs are well established therapeutic targets for treatment of a wide range of disorders through the use of inhibitors or activators, as well as recognized tools for drug delivery purposes[49–51]. CA inhibitors (CAIs) have many clinical usages of major diseases such as diuretics, antiglaucoma, gastroduodenal ulcers, antiobesity drugs, acid-base disequilibria and antiepileptic. CAIs are useful for the treatment of some neurological disorders such as idiopathic intracranial hypertension[52–54]. The design of CAIs as therapeutic agents is related to the large number of isoforms in humans, their rather diffuse localization in many tissues or organs and the lack of isoenzyme selectivity of the presently available inhibitors[55–59].

Acetylcholinesterase (AChE, E.C.3.1.1.7) enzyme, a serine hydrolase, is responsible for the degradation of ACh in the synaptic cleft of cholinergic synapses and neuromuscular junctions into inactive metabolites such as choline and acetate[9,60,61]. It has essential role in regulating many vital functions such as memory, learning, cortical organization of movement and cerebral blood flow control which demonstrates the high degree of importance of ACh as a neurotransmitter target for the study of cerebrovascular diseases associated with hypertension[60,62–64]. On the other hand, butyrylcholinesterase (BChE, E.C. 3.1.1.8) has a higher activity in liver, intestine, heart, lung and kidney. AChE and BChE share 65% amino acid sequence homology and have similar molecular forms and active sites despite being products of different genes on the human chromosomes. Both cholinesterases participate in cholinergic neurotransmission by hydrolysing ACh in the central and peripheral nervous system. Also, they play an important role in the development of Alzheimer's disease (AD)[65]. AD is a neurodegenerative disease of the central nervous system associated with progressive memory loss resulting in dementia[66]. Gradual loss of ACh has been demonstrated to impair memory, especially in the progression of AD[67]. The increased AChE reactivity has been shown to be closely associated with neurofibrillary tangle pathology in AD. Also, AChE inhibitors are thought to be promising therapeutic drugs for the treatment of neurodegenerative disease characterized with ACh deficiency, such as senile dementia or AD[67,68].

The aim of this study is to design and synthesize some novel tetrahydropyrimidine-5-carboxylates (**1–3**) and to investigate their metal chelating, inhibition potential of CA I and II isoenzymes, and to find out the most potent and favourable AChE and BChE inhibition properties of the compounds to give directions to further studies.

Experimental

Chemistry

*Synthesis of 2-(methacryloyloxy)ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate (**1**)*

Urea (1.20 g, 0.02 mol) is dissolved in acetylacetone and ethyl alcohol (3:1 mL:mL). Then, 2-(methacryloyloxy)ethyl acetoacetate (3.82 mL, 0.02 mol) is added on it drop by drop. After being dissolved in magnetic stirrer for 5 min, benzaldehyde (2.03 mL, 0.02 mol) is added. After determining that the reaction has been fully completed, the solvent is evaporated. Processing of the reaction mixture was carried out by washing the reaction mixture with ice water, the precipitate was filtered, washed with 0.5 L of water. Finally, it was dried and recrystallized from ethanol (75 mL). The yield is 2.4 g, mp. 211 °C. Eluent – ethanol:hexane (5:2). ^{1}H NMR (300 MHz, DMSO-d_6) 5 1.35 (s, 3H, CH_3), 6.8–7.1 (m, H, Ar), 7.4 (m, H, Ar), 9.35 (s, 1H, NH). ^{13}C NMR (75 MHz, DMSO-d_6) 5 24, 29, 37, 51, 86, 117, 122, 129, 132, 141, 151, 205 (C=O).

*Synthesis of allyl 1-(3-choloro-2-hydroxypropyl)-4-methyl-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-5-carboxylate (**2**)*

Allyl 6-methyl-4-phenyl-2-thioxo-1,2,3,4-tetrahydropyrimidine-5-carboxylate (0.908 g, 3.3 mmol) is dissolved in 2:1 ratio of acetylacetone and ethyl alcohol (10 mL:5 mL). Then, epichlorohydrin (0.26 mL, 3.3 mmol) is added on it drop by drop. After being dissolved in the stirrer for 25 min, 0.03 g $AlCl_3$ catalyst is added to it and mixed by heating at 65–70 °C. The progress of the reaction is controlled by Sulifol UV 254 plate. After determining the full completion of reaction, solution is evaporated and is cleansed in ethyl alcohol solution. The white crystalline having melting temperature of 168 °C is obtained. Eluent – ethanol:hexane (5:2). ^{1}H NMR (300 MHz, DMSO-d_6) 2.30 (s, 3H, CH_3), 7.06–7.25 (m, H, Ar), 4.81 (H, OH), 9.84 (s, 1H, NH), 3.40, 3.65 (2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) 15.5, 49.5, 56.3, 67.8, 71.2, 74.5, 106, 116.4, 127.1, 128.6, 133.5, 138, 158.8, 167.2, 178.4.

*Synthesis of ethyl 1-(2-hydroxybutyl)-4-methyl-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-5-carboxylate (**3**)*

Ethyl 6-methyl-2-thioxo-4-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1.52 g, 0.02 mol) is dissolved in 2:1 ratio of acetylacetone and ethyl alcohol (12 mL:5 mL) and 1,2-epoxobutane (2.03 mL, 0.02 mol) is added on it drop by drop. After being dissolved in the stirrer for 30 min, 0.02 g $AlCl_3$ catalyst is added on it and mixed by heating at 60–65 °C. The progress of the reaction is controlled by Sulifol UV 254 plate. After determining the full completion of reaction, solution is evaporated and is cleansed in ethyl alcohol solution. The white crystalline having melting temperature of 192 °C is obtained. Eluent – ethanol:hexane (5:2). ^{1}H NMR (300 MHz, DMSO-d_6) 0.96 (s, 3H, CH_3),

4.59–7.25 (m, 6H, Ar), 4.81 (H, OH), 3.45 (1H, CH), 9.84 (s, 1H, NH), 3.40, 1.48 (2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) 14.2, 28.6, 58.9, 61.7, 71.2, 71.6, 104.2, 127.1, 127.9, 128.6, 129.9, 138, 160.3, 167.2, 178.

Biochemical studies

For determination of inhibition effects of tetrahydropyrimidine-5-carboxylates (**1–3**) on CA I and II isoforms, both isoenzymes were purified from fresh human erythrocyte using affinity chromatography technique as the first experimental work[69,70]. To this end, Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography was used for purification of both isoenzymes[71] via a single step described previously[72–74].

CA activity determination was realised spectrophotometric method of Verpoorte et al.[75] as described previously[76]. In this method, changes in absorbance were recorded during 3 min at 25 °C. P-nitrophenylacetate (NPA) was used as substrate and converted by both isoenzymes to p-nitrophenolate ion. These activity determinations are described in detail in our previous studies[77].

For determination of protein quantity, Bradford method was used during the purification steps[78]. This spectrophotometrical protein determination was explained previously[79]. The bovine serum albumin was the standard for this determination which was done at 595 nm[80].

After the purification process of the CA isoenzymes, SDS-polyacrylamide gel electrophoresis (SDS-PAGE) has been carried out. Stacking gel containing (10 and 3%) acrylamide and (0.1%) of SDS[81] was used for running the process using a Minigel system (Mini-PROTEAN Tetra System). The method used for visualization of protein has been explained in detail in previous studies[82]. According to this method, the gel was fixed, then stained with Coomassie Brilliant Blues R-250, later on the gel was de-stained by using standard methods for detecting protein bands that belong to purified CA isoenzymes[83].

The inhibitory effects of tetrahydropyrimidine-5-carboxylates (**1–3**) on AChE/BChE activities were measured according to spectrophotometric method of Ellman et al.[84]. Acetylthiocholine iodide (AChI) or butyrylthiocholine iodide (BChI) was used as substrates for the reaction. 5,5′-Dithio-bis(2-nitro-benzoic)acid (DTNB, D8130-1G, Sigma-Aldrich, Steinheim, Germany) was used for the measurement of the AChE/BChE activities. Briefly, 100 mL of Tris/HCl buffer (1 M, pH 8.0), 10 mL of sample solution dissolved in deionized water at different concentrations and 50 mL AChE/BChE solution were mixed and incubated for 10 min at 25 °C. Then 50 mL of DTNB (0.5 mM) was added. The reaction was then initiated by the addition of 50 mL of AChI/BChI. 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/BChI, with an absorption maximum at a wavelength of 412 nm[85,86].

The effect of novel tetrahydropyrimidine-5-carboxylates (**1–3**) on both CA isoenzymes was examined using the hydratase activity and recorded in triplicate analysis at the each used concentration[87]. For this purpose, different concentrations of tetrahydropyrimidine-5-carboxylates (**1–3**) were determined in preliminary assays. CA isoenzyme activities were measured in the presence of different quantities of them. The control sample activity in the absence of a tetrahydropyrimidine-5-carboxylates (**1–3**) was taken as 100%. For each tetrahydropyrimidine-5-carboxylates (**1–3**), an activity (%) – [Tetrahydropyrimidine-5-carboxylates] was drawn using conventional polynominal regression software. The half maximal inhibitory concentration (IC_{50}) of all tetrahydropyrimidine-5-carboxylates (**1–3**) was calculated from graphs[88]. IC_{50} values are measures of the effectiveness of novel tetrahydropyrimidine-5-carboxylates (**1–3**) in inhibiting both CA isoenzymes. For determination of *Ki* values, three different tetrahydropyrimidine-5-carboxylate (**1–3**) concentrations were used. *Ki* values reflect the binding affinity of the tetrahydropyrimidine-5-carboxylates (**1–3**) to both CA isoenzymes. In this way, the IC_{50} value is converted to an absolute inhibition constant *Ki* value. In this experiment, PNA was used as substrate at five different concentrations. Finally, Lineweaver–Burk curves were drawn for each inhibitor[89].

Fe^{2+} chelating ability of tetrahydropyrimidine-5-carboxylates (**1–3**) was predicted according to Dinis et al.[90] with slight modification[91,92]. Fe^{2+}-binding capacity of tetrahydropyrimidine-5-carboxylates (**1–3**) was spectrophotometrically recorded at 522 nm[93]. In brief, to a mixture of $FeCl_2$ (0.1 mL, 0.6 mM), tetrahydropyrimidine-5-carboxylates (**1–3**) were added at three different concentrations (10–30 μg/mL) in methanol (0.4 mL). The reactions were started by pipyrdyl 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 was measured spectrophotometrically at 522 nm[94].

Results and discussion

Compounds characterization

The synthesis of the new compounds is shown in Scheme 1. The reaction of substituted benzaldehyde with methylene active compounds such as 2-(methacryloyloxy)ethyl acetoacetate and urea in the presence of TFAA led to the desired cyclic urea. At the next stage, we have provided the transformation of obtained compounds. So, by the reaction epichlorohydrin had synthesised allyl 1-(3-choloro-2-hydroxypropyl)-4-metil-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-5-carboxylate. At the same time by the reaction 1,2-epoxobutane synthesised ethyl 1-(2-hydroxybutyl)-4-methyl-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-5-carboxylate.

In the compound, the C8, C9, C11 and C12 atoms of the phenyl ring are disordered over two sets of sites in a 0.60 (3):0.40 (3) ratio. The heterocyclic ring is essentially planar and forms a dihedral angle with the phenyl ring. The crystal packing is stabilized by intermolecular N3—H3N···O1 hydrogen bonds, which link the molecules into chains running parallel to the *b* axis (Figure 1B), with graph-set notation C(6)[95]. Data collection: *APEX2*[96]; cell refinement: *SAINT-Plus*[94]; data reduction: *SAINT-Plus*; program(s) used to solve structure: *SHELXTL*[97]; program(s) used to refine structure: *SHELXTL*; molecular graphics: *SHELXTL*; software used to prepare material for publication: *SHELXTL*.

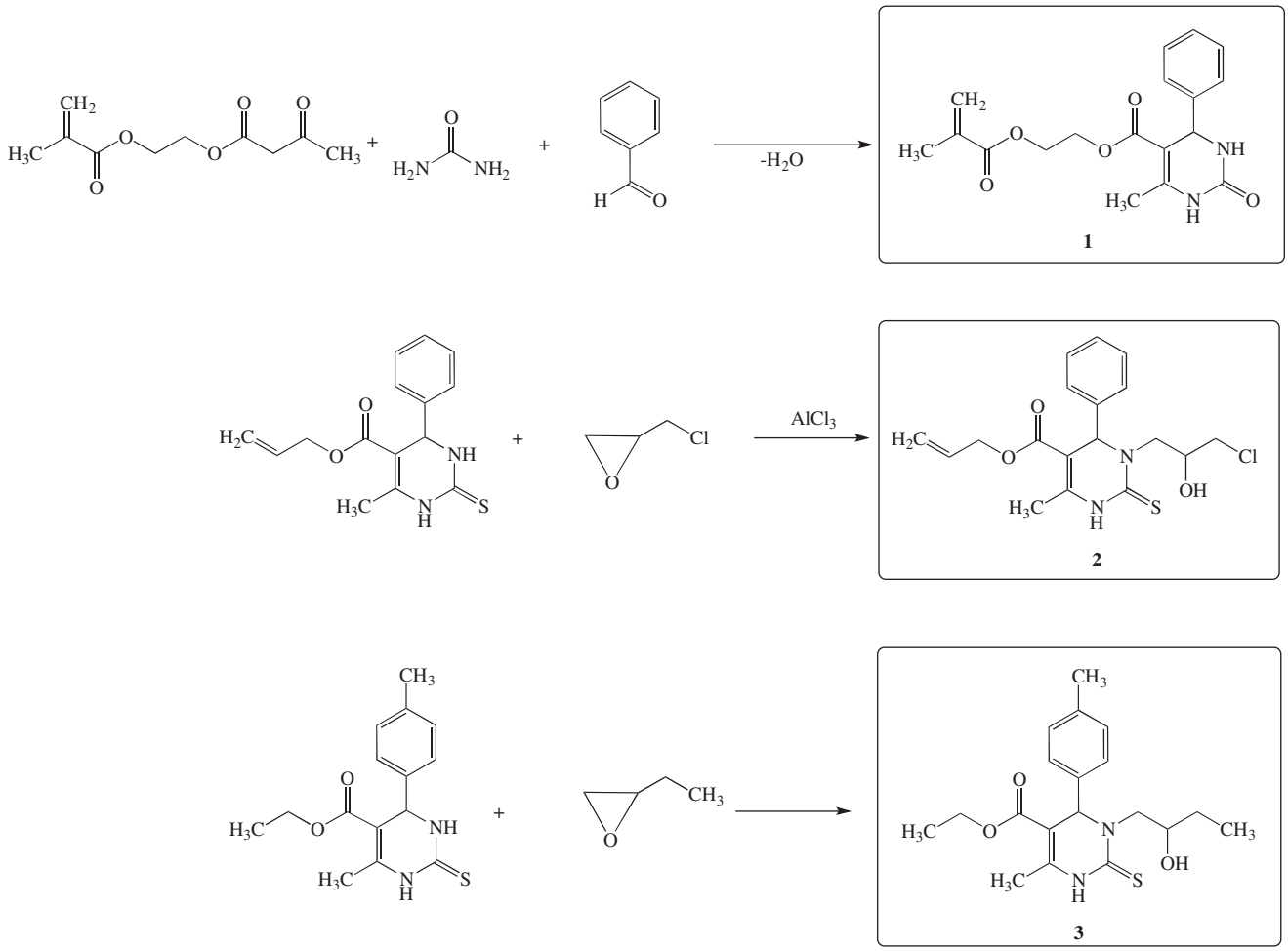
Special details

Geometry

All e.s.d.s (except the e.s.d. in the dihedral angle between two l.s. planes) are estimated using the full covariance matrix. The cell e.s.d.s are taken into account individually in the estimation of e.s.d.s in distances, angles and torsion angles; correlations between e.s.d.s in cell parameters are only used when they are defined by crystal symmetry. An approximate (isotropic) treatment of cell e.s.d.s is used for estimating e.s.d.s involving l.s. planes.

Refinement

Refinement of F^2 against ALL reflections. The weighted *R*-factor *wR* and goodness of fit *S* are based on F^2, conventional *R*-factors *R* are based on *F*, with *F* set to zero for negative F^2. The threshold



Scheme 1. The three-component condensation reactions come to an end within 2.5–3 h at 60–75 °C. The synthesized compounds were crystalline and their structure was confirmed by spectral and physico-chemical methods, among which IQ, ^{1}H, ^{13}C NMR and X-ray spectroscopy: The crystal structure of synthesized 2-(methacryloyloxy)ethyl 6-methyl-2-oxo-4-phenyl-1,2,3,4-tetrahydropyrimidine-5-carboxylate has been determined with X-ray structure analysis method. The structure and crystalline form of new cyclical compound has been shown in Figure 1. Crystalline compound holds two crystallographical independent molecules.

expression of $F^2 > \sigma(F^2)$ is used only for calculating *R*-factors (*gt*) etc. and is not relevant to the choice of reflections for refinement. *R*-factors based on F^2 are statistically about twice as large as those based on *F*, and *R*-factors based on ALL data will be even larger.

Biochemical results

Classical CAIs usually act by complexing the zinc ion (Zn^{2+}) in the active site of CA such as primary sulfonamides and their bioisosters, thiophenols, inorganic anions, hydroxamates and carboxylic acids[98]. It was reported that some derivatives characterized by different substituents including CH_3, CF_3, CN, F, Cl, Br, NO_2, OCH_3 and Ph at the *ortho*-, *meta*- and *para*-positions of the benzylic or benzoylic portion in their skeleton have inhibition profiles against CA isoenzymes[99]. All the synthesized compounds were tested to evaluate their inhibitory activity towards the slower cytosolic isoform (hCA I), the more rapid cytosolic isoenzyme (hCA II) and AChE/BChE enzymes. The chemical formula of novel tetrahydropyrimidine-5-carboxylates (**1**–**3**) is given in Scheme 1 and their CA I and II isoforms inhibition data are summarized in Table 1. Novel tetrahydropyrimidine-5-carboxylates (**1**–**3**) showed effective Fe^{2+} chelating effects and inhibition profiles against CA isoforms, AChE and BChE enzymes. When examining the results, the following structure–activity relationship could be easily observed:

(1) To describe inhibitory effects, researchers often use an IC_{50} value. However, a more suitable measure is the *Ki* constant. *Ki* values were calculated from Lineweaver–Burk graphs[89]. In our study, both *Ki* and IC_{50} parameters of the tetrahydropyrimidine-5-carboxylates (**1**–**3**) were determined and are given in Table 1. For the cytosolic hCA I isoenzyme, tetrahydropyrimidine-5-carboxylates (**1**–**3**) had *Ki* values in the range of 429.24 ± 87.89–539.30 ± 106.70 nM (Table 1). The most significant inhibition effect was observed by novel tetrahydropyrimidine-5-carboxylate **2** (allyl 1-(3-choloro-2-hydroxypropyl)-4-metil-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-5-carboxylate), which possesses biological active groups of $–CH_2$, $–CH_3$, –C=O, –NH, –C=S, Cl and –OH with *Ki* value of 429.24 ± 87.89 nM. It is well known that these biologically active groups inhibit CA isoenzymes. On the other hand, acetazolamide (AZA), used as a CA inhibitor for the medical treatment of epileptic seizure, glaucoma, idiopathic intracranial hypertension, altitude sickness, cystinuria, periodic paralysis, central sleep apnea and dural ectasia, showed *Ki* value of 481.33 ± 55.33 nM. The hCA I is highly abundant in red blood cells and is found in

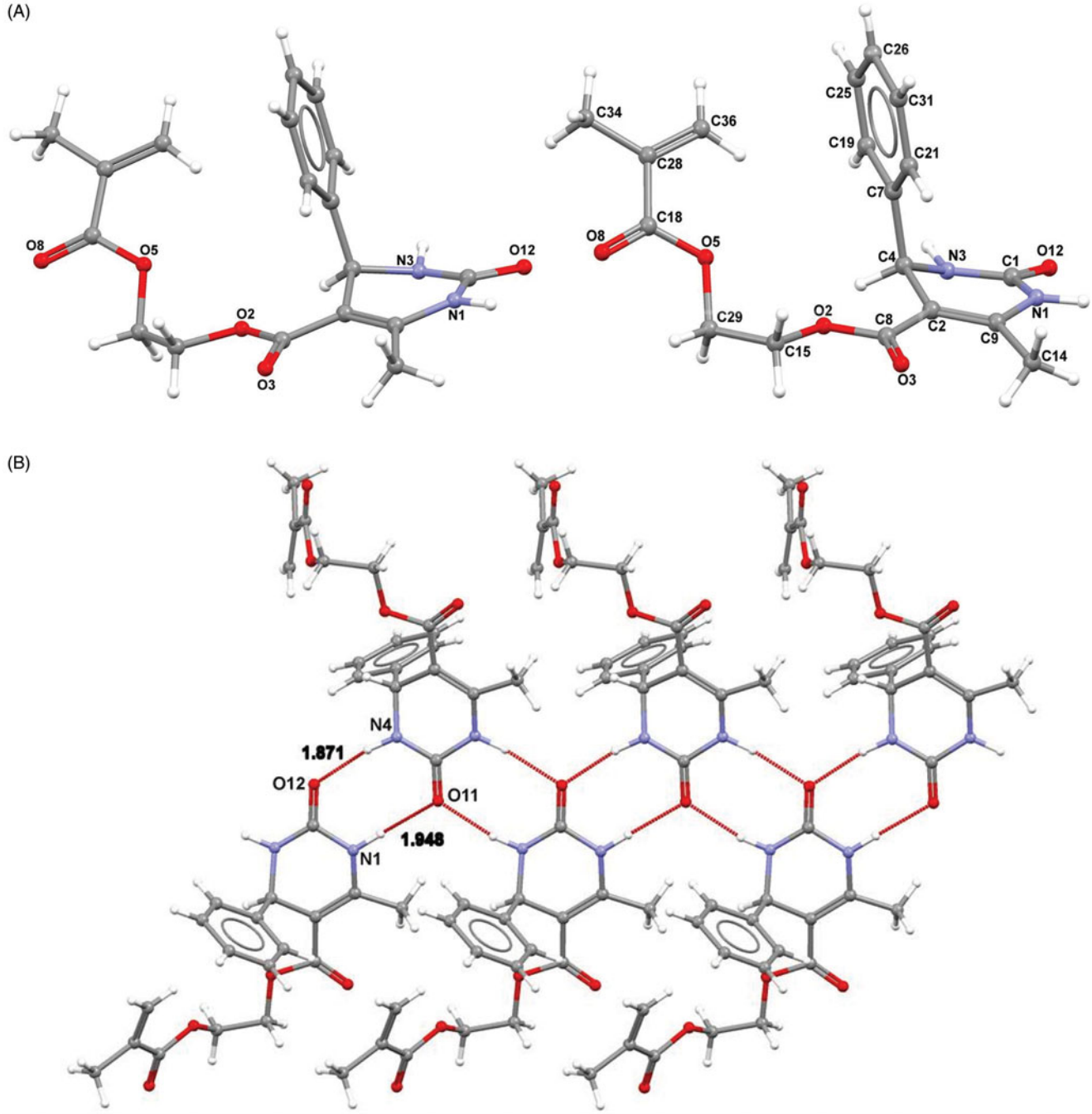


Figure 1. (A) The molecular structure of the new compound. (B) Packing diagram of the title compound. N–H–O hydrogen bonds are shown as dashed lines. For clarity only one of the disordered components of the phenyl ring is shown.

Table 1. The enzyme inhibition values of some tetrahydropyrimidine-5-carboxylates (**1**–**3**) against human carbonic anhydrase isoenzymes I and II (hCA I and II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes.

	IC_{50} (nM)								K_i (nM)			
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	hCA I	hCA II	AChE	BChE
1	473.03	0.9802	596.38	0.9736	186.64	0.9883	228.03	0.9958	539.30 ± 106.70	530.80 ± 103.60	97.19 ± 19.62	117.22 ± 49.37
2	478.92	0.9895	473.36	0.9810	181.41	0.9730	381.40	0.9931	429.24 ± 87.89	391.86 ± 40.16	75.75 ± 16.74	214.15 ± 28.77
3	505.10	0.9792	536.37	0.9552	126.48	0.9922	325.81	0.9942	458.68 ± 48.72	516.08 ± 64.6	68.48 ± 25.07	104.70 ± 8.093
AZA*	522.01	0.9964	987.11	0.9610	–	–	–	–	281.33 ± 55.33	202.7 ± 62.5	–	–
TAC**	–	–	–	–	537.01	0.9977	412.01	0.9962	–	–	396.03 ± 30.66	209.21 ± 15.02

*AZA (acetazolamide) was used as a standard CA I and II isoenzyme inhibitor.
**Tacrine (TAC) was used as a standard acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) inhibitor.

Table 2. Determination of half maximal concentrations (IC_{50}) of Fe^{2+} chelating of some tetrahydropyrimidine-5-carboxylates (**1–3**) and standard compounds.

Compounds	Fe^{2+} chelating*	r^2
BHA	23.89	0.9844
BHT	30.13	0.9933
α-Tocopherol	36.47	0.9636
Trolox	19.80	0.9950
EDTA	16.90	0.9710
1	46.21	0.9837
2	53.30	0.9955
3	45.89	0.9962

*Concentrations were determined in μg/mL.

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 conditions[27].

(2) The ubiquitous and physiologically predominant cytosolic isoform hCA II is associated with several diseases. For hCA II, novel tetrahydropyrimidine-5-carboxylates (**1–3**) had *Ki* values in the range of 391.86 ± 40.16–530.80 ± 103.60 nM. On the other hand, AZA used as a clinical CA inhibitor demonstrated *Ki* value of 202.70 ± 162.5 nM. As can be seen in CA I, similar to CA I, the most significant inhibition effect was observed by tetrahydropyrimidine-5-carboxylates **2** (allyl 1-(3-choloro-2-hydroxypropyl)-4-metil-6-phenyl-2-thioxo-1,2,3,6-tetrahydropyrimidine-5-carboxylate), with *Ki* values of 391.86 ± 40.16 nM.

(3) AChE is a metabolic serine hydrolase that catalyses the hydrolysis of ACh, thus regulating cholinergic neurotransmission. Therefore, in disorders such as AD, where there is diminished cholinergic activity, inhibition of AChE has been employed to treat some of the symptoms attributed to decreased ACh levels[100,101]. Effective AChE and BChE 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 and BChE inhibitors[100–102]. However, during the progression of AD, brain AChE levels decline while BChE activity increases, suggesting that ACh hydrolysis may occur to a greater extent via BChE catalysis. In this regard, it has been reported that highly selective inhibition of BChE is important in raising ACh levels and improving cognition[103]. The inhibition effects of novel tetrahydropyrimidine-5-carboxylates (**1–3**) against AChE/BChE activities were measured according to spectrophotometric method described by Ellman et al.[84] Acetylthiocholine iodide/butyrylthiocholine iodide (AChI/BChI) was used as substrates for the reactions. This method is based on the amount of thiocholine released when the AChE/BChE hydrolyses the substrate AChI/BChI to thiocholine and acetate/butyrate. The product thiocholine reacts with Ellman's reagent (5,5-bisdithionitrobenzoic acid-DTNB) to produce a yellow compound (5-thio-2-nitrobenzoate) anion, which can be detected in a wavelength of 412 nm. AChE and BChE were very effectively inhibited by novel tetrahydropyrimidine-5-carboxylates (**1–3**). It was found that *Ki* values were in the range of 68.48 ± 25.07–97.19 ± 19.62 nM for AChE and 104.70 ± 8.093–214.15 ± 28.77 nM for BChE, respectively (Table 1). On the other hand, Tacrine (TAC) was used as standard AChE and BChE inhibitor it had *Ki* values of 396.03 ± 30.66 and 209.21 ± 15.02 nM, respectively. In addition, donepezil hydrochloride, which is used for the treatment of mild-to-moderate AD and various other memory impairments, had been shown to lower AChE inhibition activity (IC_{50}: 55.0 nM). The *Ki* values of novel tetrahydropyrimidine-5-carboxylates (**1–3**) for AChE and BChE were calculated from Lineweaver–Burk plots[89].

(4) Finally, novel tetrahydropyrimidine-5-carboxylates (**1–3**) had also effective Fe^{2+} chelating effect. The distinction between different concentrations of novel tetrahydropyrimidine-5-carboxylates (**1–3**) (10–30 μg/mL) and the control value was fixed to be statistically important ($p < 0.01$). Furthermore, it is found that IC_{50} values for novel tetrahydropyrimidine-5-carboxylates (**1–3**) were found as 46.21, 53.30 and 45.89 μg/mL, respectively (Table 2). Whereas, IC_{50} values belonging to Fe^{2+} chelating capacity of positive controls like trolox, α-tocopherol, BHT, BHA and EDTA was found to be 19.80, 36.47, 23.89, 30.13 and 16.90 μg/mL, respectively. A lower IC_{50} value reflects a higher Fe^{2+} binding activity. These results clearly introduce that Fe^{2+} chelating effect of novel tetrahydropyrimidine-5-carboxylates (**1–3**) was close to trolox, α-tocopherol, BHT, but lower than that of other standards. Fe^{2+} ions are the most efficient pro-oxidants in pharmacology systems and food[104–106]. Ferrozine can create complexes with Fe^{2+}. In the presence of Fe^{2+} chelating compounds, Ferrozine–Fe^{2+} complex formation is broken down, resulting in a decrease in the red colour of Ferrozine–Fe^{2+} complex[107–109].

Conclusion

Novel tetrahydropyrimidine-5-carboxylates (**1–3**) used in the present study demonstrated effective inhibition profiles against hCA isoforms, AChE and BChE enzymes. Additionally, these compounds demonstrated effective metal chelating properties. Novel tetrahydropyrimidine-5-carboxylates (**1–3**) identified their potential CA isoenzymes, and AChE and BChE enzyme inhibitors. In this study, nanomolar level of *Ki* values was observed for all novel tetrahydropyrimidine-5-carboxylates and these compounds can be selective inhibitor of both cytosolic CA isoenzymes and AChE and BChE enzymes. Also, they can be used as metal chelators in related applications.

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Declaration of interest

The authors declare no conflict of interest.

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