Received: 17 June 2020 | Revised: 23 July 2020 | Accepted: 29 July 2020

DOI: 10.1002/ardp.202000202

FULL PAPER

DPhG ARCH PHARM Archiv der Pharmazie

Synthesis, characterization, and biological studies of chalcone derivatives containing Schiff bases: Synthetic derivatives for the treatment of epilepsy and Alzheimer's disease

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Funding information
Scientific Research Project Fund of Sivas Cumhuriyet University, Grant/Award Number: SHMYO-013

Abstract

In this study, first, Schiff base-containing chalcone derivatives were synthesized. The human carbonic anhydrase (hCA) isoenzymes I and II were then purified from human erythrocytes using Sepharose-4B-L-tyrosine-sulfanilamide affinity chromatography. In addition, the inhibitory effects of the newly synthesized compounds on the activities of hCA and acetylcholinesterase (AChE) were investigated in vitro, using the esterase and acetylcholine iodide method. The IC_{50} values were determined and the K_i values of AChE and hCA activities were calculated from the Lineweaver–Burk graphs determined in this study. The hCA I isoform was inhibited by these chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) in low nanomolar levels, whose K_i values ranged between 141.88 ± 24.10 and 2,234.47 ± 38.11 nM. Against the physiologically dominant isoform hCA II, the compounds demonstrated K_i values varying from 199.31 ± 40.45 to 602.79 ± 263.22 nM. Also, these compounds effectively inhibited AChE, with K_i values ranging from 20.41 ± 6.04 to 125.94 ± 23.88 nM. According to these results, the newly synthesized molecules were found to be potent inhibitors of these enzymes.

KEYWORDS

acetylcholinesterase, carbonic anhydrase, chalcones, enzyme inhibition, Schiff base

1 | INTRODUCTION

Chalcones are members of the flavonoid family that can be obtained both naturally and synthetically, and they have a wide spectrum of biological activity.[1] Therefore, in recent years, chalcone and its derivatives have received increasing attention, and many studies have been particularly performed on biological activity (Figure 1). Chalcones and chalcone analogs are valuable intermediates in organic synthesis and they have a wide spectrum of biological activity.[2,3]

Studies carried out on chalcones revealed that they showed antibacterial,[4] antimalarial,[5] antihelminitic, amoebicidal, antiulcer, antiviral, insecticidal,[6] antiprotozoal, anticancer,[7] anti-inflammatory,[8] and cytotoxic anti-HIV[9] activities. In addition, the α,β-unsaturated parts in the structures of chalcones make them chemically important and are used as the starting agent in the synthesis of heterocyclic compounds.[10] In this respect, chalcones are ideal compounds for forming carbon–carbon,[11] carbon–sulfur,[12] and carbon–nitrogen bonds. Chalcones are chemically highly active compounds, as they contain both a substituted aromatic ring and an α,β-unsaturated carbonyl unit.[13–16]

Schiff bases were first obtained in 1860 by the German chemist Hugo Schiff. Very weak carbonyl compounds give condensation reactions with primary amine groups, resulting in a carbon–nitrogen double bond.[17] This link is called the link of the imine. If the carbonyl compound is an aldehyde, the bond formed is called aldimine, and when it is a ketone, the bond formed is called the ketimine. Schiff bases are an important class of ligands and have had a very large field of study in coordination chemistry to date. Schiff base derivatives are important compounds used in biological, pharmacological and analytical studies as well as in industrial applications. For example, some Schiff bases act as corrosion inhibitors in steel and dyestuff.[18–20]

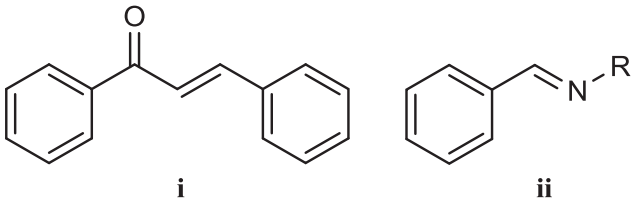


FIGURE 1 Chalcone (**i**) and Schiff base (**ii**)

Carbonic anhydrase (CA; carbonate hydrolysis, EC: 4.2.1.1) is a metalloenzyme that contains zinc (Zn^{2+}) ions in its active area and is very important for biological systems.[21,22] Carbonic hydrase is essentially a very important enzyme that plays a role in many physiological events such as ensuring the dissolution, transport, and removal of CO_2 formed during breathing, as well as acid–base balance, ion exchange, and regulation of the cardiovascular system. The enzyme, first isolated from human erythrocytes, has been studied in many living organisms and tissues.[23,24]

Acetyl CoA is a metabolic product of pyruvate in glycolysis. Alzheimer's disease (AD) occurs as a result of decreased neurotransmitters in the brain. A maximum decrease of neurotransmitters occurs in the case of dementia and neurodegenerative disease. AD was found to be a dysfunction of memory. The decrease in the level of acetylcholine (ACh) in the brain is the biggest biochemical factor of this disease.[25,26] There is no cure for this disease. Currently, the treatments applied are aimed at eliminating the symptoms of this disease. There is no treatment method that eliminates it. Acetylcholinesterase (AChE; EC 3.1.1.7) is a nonspecific enzyme that hydrolyzes lipotropic acetylcholine in tissues that are free or in combination with phospholipids. AChE inhibitors, such as rivastigmine and donepezil, are generally used for this purpose.[27,28] Recent studies have found that the amount of butyrylcholinesterase (BChE) in the brain of Alzheimer's patients is higher than in normal brains.[29,30] Therefore, it is estimated that butyrylcholinesterase inhibition may also be associated with AChE-inhibiting drugs. Examples of these inhibiting drugs are inhibitors such as dichlorovinyl dimethyl phosphate, tetrahydroaminoacridine, galantamine, and rivastigmine.[31–33]

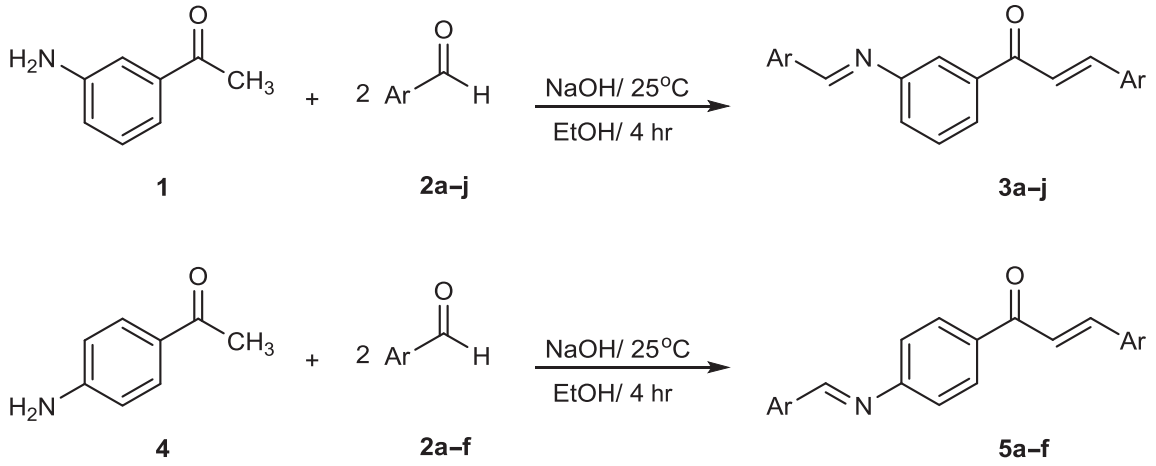
In this study, we first synthesized 12 new and four known (**3e**,[34] **5b**,[35] **5d**,[36] and **5e**[37]) chalcone derivatives containing Schiff bases, which were considered to have a broad biological activity, and performed the characterization of these compounds. To determine the antiepileptic and anticholinergic properties of the synthesized compounds, their inhibitory potentials against carbonic anhydrase I–II isoenzymes and AChE enzymes were investigated in this study.

2 | RESULTS AND DISCUSSION

2.1 | Chemistry

Recently, it was shown that chalcone derivatives are effective antimicrobial and antioxidant additives to self-dispersed oils.[22,23] In this article, we report the synthesis of chalcone derivatives containing Schiff bases **3a–j** and **5a–f**, and their biological activity against carbonic anhydrase I–II isoenzymes and AChE enzymes.

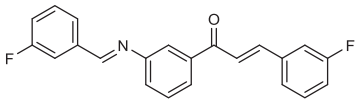
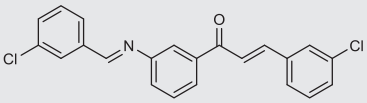
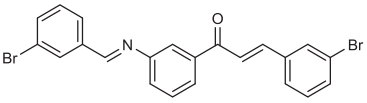
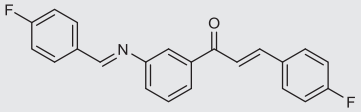
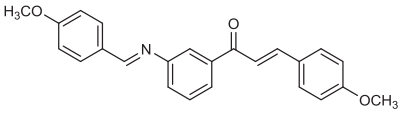
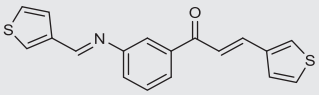
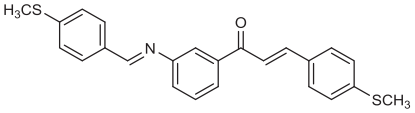
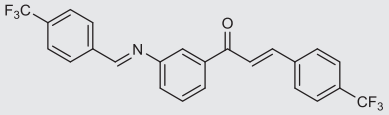
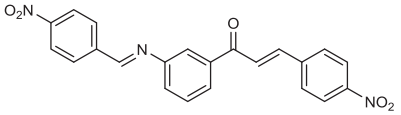
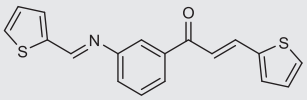
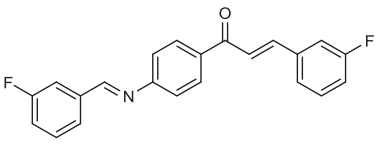
The synthesis of chalcone derivatives containing Schiff bases was carried out in one step by treating 3′-aminoacetophenone (**1**) and 4′-aminoacetophenone (**4**) with aromatic aldehydes (**2a–j**) in 1:2 ratios (Scheme 1). In the reaction, 1 equivalent of aromatic aldehyde reacted with the acetyl part of aminoacetophenone via the Claisen–Schmidt condensation in basic media and the chalcone skeleton was formed. Besides, the remaining aldehyde reacted with the amine group in the acetophenone and formed the Schiff base (aldimine structure). As a result of the studies, it was observed that 3′-aminoacetophenone (**1**) completely reacted with all the aldehydes used and gave the expected product (**3a–j**; Table 1). But in the reaction of 4′-aminoacetophenone (**4**) with 4-(methylthio)benzaldehyde (**2g**), it was observed that the chalcone derivative (**I**), and not the expected product



Ar = **a)** 3-FPh **b)** 3-ClPh **c)** 3-BrPh **d)** 4-FPh **e)** 4-OCH_3Ph **f)** thiophene-3-yl **g)** 4-SCH_3Ph **h)** 4-CF_3Ph **i)** 4-NO_2PH **j)** thiophene-2-yl

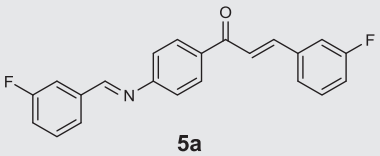
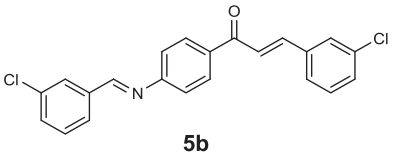
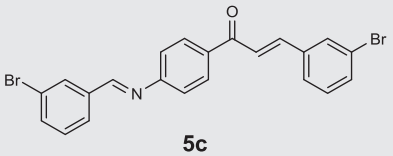
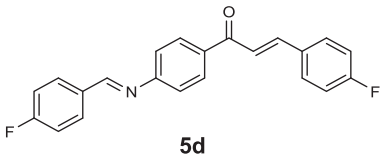
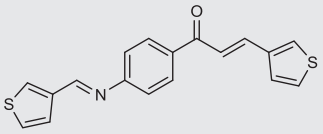
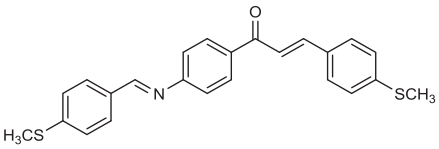
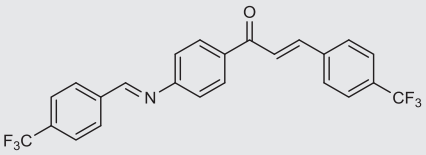
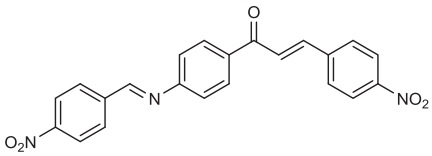
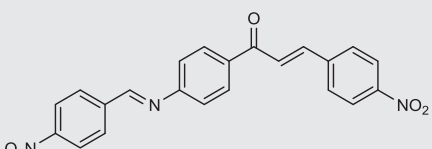
SCHEME 1 The reaction scheme for the synthesis of chalcone derivatives containing Schiff bases

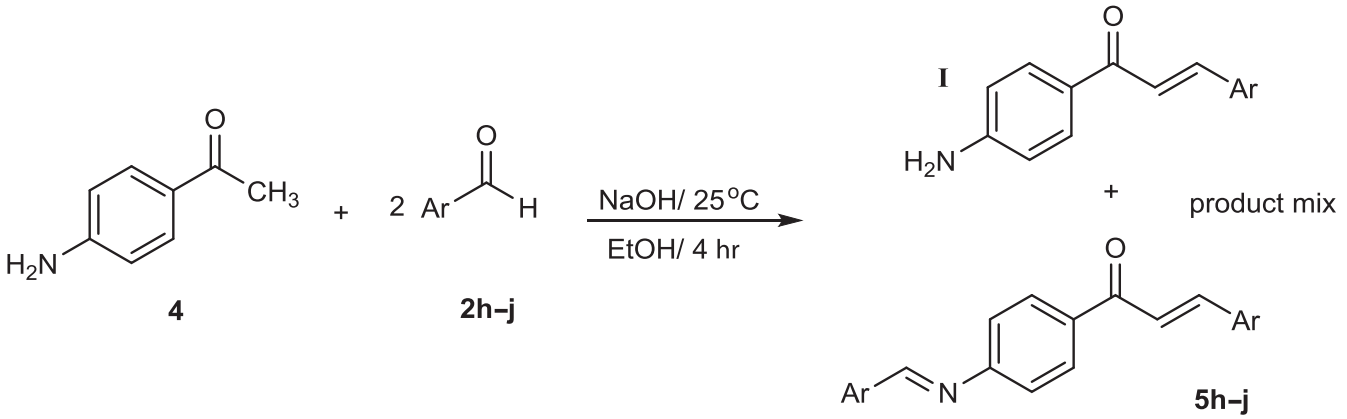
TABLE 1 Synthesized chalcone derivatives containing Schiff bases

Entry	Compounds	Yields (%)
1	 **3a**	82
2	 **3b**	81
3	 **3c**	78
4	 **3d**	84
5	 **3e**	77
6	 **3f**	84
7	 **3g**	70
8	 **3h**	75
9	 **3i**	80
10	 **3j**	79
11	 **5a**	82

(Continues)

TABLE 1 (Continued)

Entry	Compounds	Yields (%)
12	 **5a**	81
13	 **5b**	80
14	 **5c**	78
15	 **5d**	79
16	 **5f**	83
17	 **5g**	Could not be synthesized
18	 **5h**	Could not be obtained pure
19	 **5i**	Could not be obtained pure
20	 **5i**	Could not be obtained pure



Ar = **g)** 4-SCH_3Ph **h)** 4-CF_3Ph **i)** 4-NO_2PH **j)** thiophene-2-yl

SCHEME 2 The reaction scheme for **5h–j**

(**5g**), was formed. In the reactions given by 4′-aminoacetophenone (**4**) with 4-(trifluoromethyl)benzaldehyde (**2h**), 4-nitrobenzaldehyde (**2i**), and thiophene-2-carbaldehyde (**2j**), the product obtained was a mixture of 4′-aminochalcone derivative (**I**) and chalcone derivative containing Schiff bases (**5h–j**; Scheme 2; Table 1). When the reaction time was increased to 24 hr, there was no change in the product composition. The reaction mixture was subjected to column chromatography on silica gel, eluting with AcOEt/hexane (2:8) in order to separate the Schiff base in the medium, but the imine structure was observed to undergo hydrolysis. Targeted compounds (**5h–j**) could not be obtained purely, as the base strength of 4′-aminoacetophenone (**4**) was less than that of 3′-aminoacetophenone (**1**).

The structures of synthesized compounds (**3a–j** and **5a–f**; Table 1) were determined by spectroscopic studies (^{1}H-NMR [nuclear magnetic resonance], ^{13}C-NMR, Fourier transform infrared [FTIR], and quadrupole time-of-flight [Q-TOF] liquid chromatography-mass spectrometry [LC/MS]). In the FTIR spectrum of **3a**, stretching vibrations of C=O and C=N groups were observed at 1,662 and 1,625 cm^{-1}, respectively. In the ^{1}H-NMR spectrum of **3a**, the H-atom of the aldimine group (H–C16) gave a doublet at 8.47 ppm (J = 1.3 Hz). From the aromatic H-atoms, H–C4 gave a doublet of doublets at 7.89 ppm (J = 7.7, 1.7, 1.2 Hz), whereas the H–C6 gave a multiplet at 7.84–7.80 ppm. Aliphatic protons in the structure are resonated by giving the AB system. However, only one side of the system is observed (H–C9) as a doublet at 7.77 ppm in the spectrum (J = 15.7 Hz). The signals of the H–C8 atom, which forms the other side of the AB system, overlapped with the signals of the H–C3 atom and gave a multiplet at 7.56–7.49 ppm. Signals of other aromatic H-atoms appear at 7.67 (ddd, J = 9.4, 2.6, 1.5 Hz, 1H), 7.64 (dt, J = 7.7, 1.2 Hz, 1H), 7.47–7.31 (m, 5H), 7.19 (tdd, J = 8.3, 2.7, 1.0 Hz, 1H), and 7.13–7.06 (m, 1H) ppm, respectively, thus confirming this structure (Figure 2). When the ^{13}C-NMR spectrum of **3a** is analyzed, it is clearly seen that the carbons and coupling constants interact with the fluorine in the structure. Whereas the carbonyl carbon (C7) in the structure resonates at 189.8 ppm, the ipso carbon atoms, to which the fluorides are attached (C14 and C21), are resonated by a doublet at 163.2 (J = 247.2 Hz) and 163.1 (J = 246.9 Hz) ppm, respectively. The carbon atoms of the imine and alkene unit (C16 and C9) interacted with fluorine through the four bonds and gave a doublet at 160.1 (J = 3.2 Hz) and 143.7 (J = 3.0 Hz) ppm. Other carbon atoms (C11 and C18), which are split into a doublet due to the interactions with fluorine through four bonds, gave a signal at 125.4 (J = 3.0 Hz) and 124.7 (J = 3.0 Hz) ppm. Of the carbons that interact with fluorine through three bonds, ipso carbons (C10 and C17) gave a doublet at 138.2 (J = 7.4 Hz) and 137.1 (J = 7.7 Hz) ppm, and also others (C19 and C12) gave a doublet at 130.6 (J = 8.3 Hz) and 130.5 (J = 8.0 Hz) ppm. In addition, the carbon atoms interacting with the fluorines through two bonds (C20, C13, C22, and C15) were split into a doublet, as expected, giving signals at 118.8 (J = 21.7 Hz), 117.6 (J = 21.4 Hz), 114.9 (J = 22.4 Hz), and 114.6 (J = 21.5 Hz) ppm, respectively. The remaining signals in the ^{13}C-NMR spectrum are also in harmony with the structure.

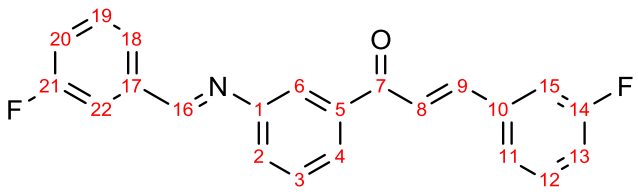


FIGURE 2 Numbering carbon atoms of **3a**

2.2 | Biochemical studies

The inhibitors of some CA isoenzymes (like CA I and CA II) are utilized to design novel classes of drugs for glaucoma and epilepsy. Additionally, novel CA inhibitors have been required to expand as therapeutic factors. Various groups have studied the inhibition of hCAs with catecholamines, thiourea derivatives, bromophenols, anions, uracil derivatives, and sulfonamides. In addition, chalcones and pyrazoles have also been studied to inhibit hCAs as well.[22,23] The results presented in Table 2 reveal that chalcone derivatives containing Schiff bases had an effective inhibition against hCA I isoform. The hCA I isoform was inhibited by **3a–j** and **5a–f** in nanomolar levels, whose K_i value ranged between 141.88 ± 24.10 and

TABLE 2 Inhibition results of **3a–j** and **5a–f** against carbonic anhydrase isoenzymes (I/II) and acetylcholinesterase enzymes

	IC_{50} (nM)						K_i (nM)		
Compounds	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
3a	395.13	0.9820	401.37	0.9740	107.66	0.9726	355.23 ± 76.25	279.22 ± 64.12	52.93 ± 10.25
3b	354.11	0.9760	295.28	0.9677	97.13	0.9924	2,234.47 ± 38.11	199.31 ± 40.45	82.27 ± 34.02
3c	327.99	0.9799	401.37	0.9649	141.14	0.9432	312.44 ± 37.13	443.21 ± 199.21	82.77 ± 14.18
3d	456.98	0.9819	471.87	0.9673	115.14	0.9660	381.14 ± 21.22	450.27 ± 197.46	50.11 ± 22.46
3e	388.37	0.9500	369.24	0.9783	79.22	0.9661	324.46 ± 81.25	602.79 ± 263.22	24.03 ± 11.01
3f	311.97	0.9790	368.22	0.9930	81.00	0.9692	325.14 ± 146.10	359.84 ± 148.30	28.07 ± 12.33
3g	429.25	0.9740	459.02	0.9637	117.18	0.9808	306.13 ± 23.13	569.44 ± 249.14	87.11 ± 39.01
3h	425.73	0.9813	445.37	0.9427	95.16	0.9700	288.47 ± 36.85	479.52 ± 176.22	46. 70 ± 12.52
3i	370.22	0.9934	449.21	0.9528	91.31	0.9804	281.76 ± 38.13	398.35 ± 81.32	61.93 ± 19.72
3j	369.57	0.9723	387.08	0.9756	128.55	0.9812	281.46 ± 79.03	302.44 ± 81.25	110.26 ± 5.57
5a	342.40	0.9913	437.50	0.9598	150.98	0.9884	358.03 ± 49.75	459.66 ± 104.77	125.94 ± 23.88
5b	340.15	0.9969	341.01	0.9894	111.23	0.9774	368.21 ± 67.83	337.31 ± 91.55	97.20 ± 30.71
5c	331.88	0.9776	452.07	0.9812	72.77	0.9759	352.11 ± 78.05	494.40 ± 124.28	20.41 ± 6.04
5d	307.93	0.9804	358.84	0.9635	67.33	0.9880	256.24 ± 11.21	421.05 ± 184.18	37.40 ± 176.01
5e	226.10	0.9947	340.13	0.9691	92.50	0.9784	141.88 ± 24.10	363.50 ± 143.27	30.90 ± 12.11
5f	366.27	0.9812	303.94	0.9943	131.77	0.9932	384.94 ± 94.06	350.03 ± 54.82	98.04 ± 30.71
AZA[a]	1,023.10	0.9758	1,168.22	0.9630	–	–	859.07 ± 219.25	1,022.20 ± 279.30	–
Tacrine[a]	–	–	–	–	436.01	0.9924	–	–	231.55 ± 32.30

[a]Acetazolamide (AZA) was used as a standard inhibitor for all human carbonic anhydrase (hCA) I and II and tacrine (TAC) was used as a standard inhibitor for acetylcholinesterase (AChE) enzyme.

2,234.47 ± 38.11 nM. Indeed, acetazolamide (AZA), as a broad-specificity CA inhibitor, showed a K_i value of 859.07 ± 219.25 nM against hCA I. Among the inhibitor compounds, the **5e** and **5d** were obtained to be as excellent hCA I inhibitors with K_i values of 141.88 ± 24.10 and 256.24 ± 11.21 nM, respectively. The hCA I inhibition effects of chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) were found to be greater than AZA. For hCA I, half-maximal inhibitor concentrations (IC_{50}) values of AZA as a positive control and some chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) exhibit the following order: **5e** (226.10 nM, r^2: .9947) < **5d** (307.93 nM, r^2: .9804) < **3f** (311.97 nM, r^2: .9790) < **3c** (327.99 nM, r^2: .9799) < AZA (1,023.10 nM, r^2: .9758). Against the hCA II isoform, the chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) demonstrated K_i values varying from 199.31 ± 40.45 to 602.79 ± 2,633.22 nM (Table 2). These chalcones containing Schiff bases (**3a–j** and **5a–f**) were observed to have high inhibition effects against hCA II. Additionally, AZA showed a K_i value of 1,022.20 ± 279.30 nM against hCA II. **3b** and **3a** had shown the maximum inhibition effect, with K_i values of 199.31 ± 40.45 and 279.22 ± 64.12 nM, respectively. For hCA II, IC_{50} values of AZA and some synthesized compounds (**3a–j** and **5a–f**) exhibit the following order: **3b** (295.28 nM, r^2: .9677) < **5f** (303.94 nM, r^2: .9943) < **5e** (340.13 nM, r^2: .9691) < **5b** (341.01 nM, r^2: .9894) < AZA (1,168.22 nM, r^2: .9630).

The enzyme can be inactivated by various inhibitors, leading to ACh accumulation and also disrupted neurotransmission caused by hyperstimulation of muscarinic and nicotinic receptors. Reversible AChE inhibitors are extensively utilized for the treatment of neurodegenerative diseases, whereas irreversible inhibitor compounds are associated with toxic effects. Indeed, irreversible AChE inhibitors (AChEIs) include chemical warfare factors and many organophosphorus molecules used as insecticides and pesticides. All chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) exhibited a significantly higher AChE inhibitory activity than tacrine as control molecule. Furthermore, the K_i values of chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) and tacrine are summarized in Table 2. As can be observed from the results recorded in Table 2 and Figure 3, compounds **3a–j** and **5a–f** effectively inhibited AChE, with K_i values ranging from 20.41 ± 6.04 to 125.94 ± 23.88 nM. Thus, all of these compounds had almost similar inhibition profiles. The most active compounds, **5h** and **3d**, showed K_i values of 20.41 ± 6.04 and 24.03 ± 11.01 nM. For AChE, IC_{50} values of TAC and some chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) exhibit the following order: **5d** (67.33 nM, r^2: .9880) < **5c** (72.77 nM, r^2: .9759) < **3e** (79.22 nM, r^2: .9661) < **3f** (81.00 nM, r^2: .9692) < TAC (436.01 nM, r^2: .9924). AChEIs like donepezil are approved by the United States Food and Drug Administration for the treatment of AD, and they are supported by clinical trial data to be utilized in the treatment of Parkinson's disease and vascular dementia.

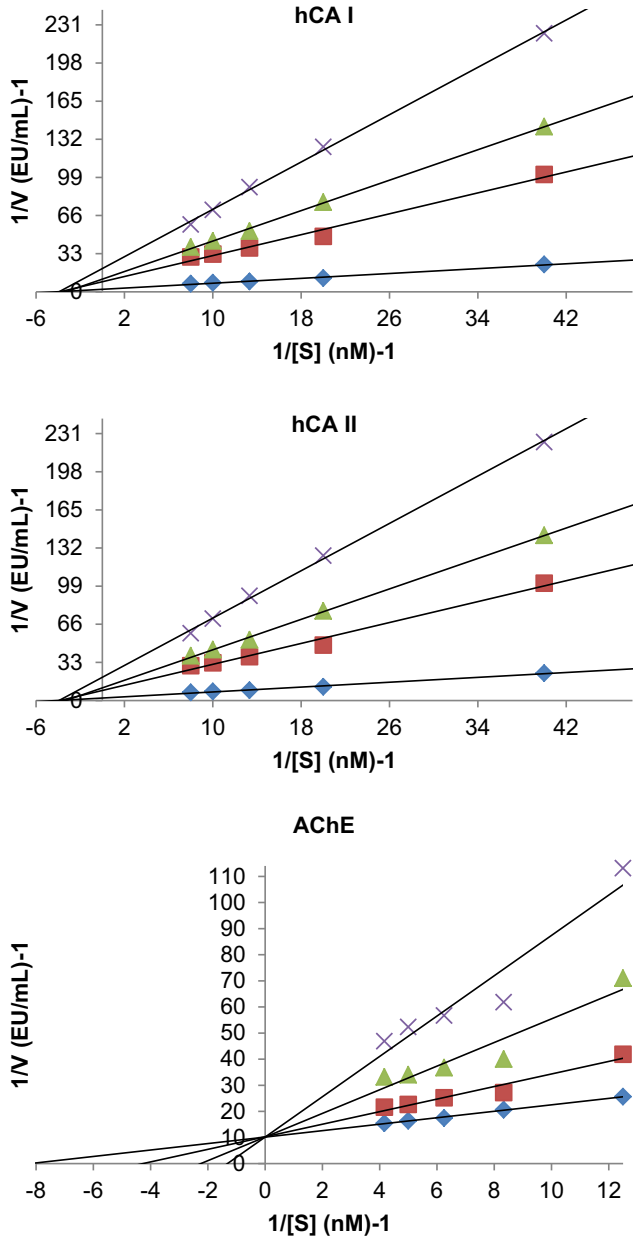


FIGURE 3 Determination of Lineweaver–Burk graphs for excellent inhibitors of human carbonic anhydrase (hCA)-I, hCA-II, and acetylcholinesterase (AChE) enzymes

3 | CONCLUSIONS

In conclusion, in this study, a series of chalcone derivatives containing Schiff bases (3**a–j** and **5a–f**) was synthesized in high yields (70–84%) and investigated in vitro for their inhibition properties against AChE and the hCA I and II isoforms. Synthesized compounds demonstrated remarkable inhibition activities as compared with standard drugs (Table 2). In addition, compound **5e** with *p*-OCH_3 substituent showed the best inhibition activity ($K_{iAZA}/K_{i\mathbf{5e}}$ = 6.05) against hCA I, compound **3b** with *m*-Cl substituent showed the best inhibition activity ($K_{iAZA}/K_{i\mathbf{3b}}$ = 5.14) against hCA II, and compound **5c** with *m*-Br substituent showed the best inhibition activity ($K_{iTAC}/K_{i\mathbf{5c}}$ = 11.34) against AChE. The findings could be useful in developing and synthesizing new metabolic enzyme inhibitors as possible candidates for the design of new drugs for the future treatment of certain diseases including epilepsy and Alzheimer's disease.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

Anhydrous sodium sulfate was used as a drying agent for the organic phase. Column chromatography was performed on silica gel (SiO_2, 60–230 mesh; Merck). Melting points were measured on an Electrothermal 9100 apparatus. IR spectra (KBr disc) were recorded on a Jasco FT/IR-430 spectrometer. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a JEOL (400 MHz) JNM-ECZ400S/L1 NMR instrument in $CDCl_3$ and (d_6) dimethyl sulfoxide (DMSO); δ in ppm relative to Me_4Si (δ: 0.00) for ^{1}H-NMR; $CDCl_3$ (δ: 77.0), and (d_6)DMSO (δ: 39.5) for ^{13}C-NMR spectra as internal standards; *J* in Hz. Agilent Technology Inc. of 1260 Infinity HPLC System was coupled with 6530 Q-TOF LC/MS detector and ZORBAX SB-C18 (2.1 × 50 mm, 1.8 μm) column.

The original spectra of the investigated compounds are provided as Supporting Information, together with their InChI codes and some biological activity data.

4.1.2 | General procedure the synthesis of Schiff base-containing chalcone derivatives

2.5 M NaOH (5 ml) was added to a solution of 3′-aminoacetophenone (**1**) or 4′-aminoacetophenone (**4**) (7.5 mmol) in EtOH (50 ml). The corresponding benzaldehyde derivative, which was then weighed in a separate container and dissolved in 30 ml of EtOH, was added to this mixture. The corresponding aromatic aldehyde (**2a–j**) (15 mmol), which was then weighed in a beaker and dissolved in 30 ml of EtOH, was added onto the mixture. An additional 20 ml of EtOH was added to the mixture and stirred at room temperature for 4 hr. At the end of the reaction, the mixture, which was precipitated, was transferred to a beaker containing 250 ml of water and stirred for a while. It was then dried by vacuum filtration. The obtained products (**3a–j** and **5a–f**) were purified by crystallization from EtOH.

*(2E)-3-(3-Fluorophenyl)-1-(3-{[(1E)-(3-fluorophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**3a**)*

Yellow solid. Yield: 82%, m.p.: 136–138°C. IR (KBr, cm^{-1}): 3,066, 3,042, 2,911, 1,662, 1,625, 1,605, 1,581, 1,483, 1,447, 1,366, 1,326, 1,261, 1,239, 1,189, 1,162, 1,144, 1,046, 974, 903, 850, 771, 684, 672, 518, and 445. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (d, *J* = 1.3 Hz, 1H), 7.89 (ddd, *J* = 7.7, 1.7, 1.2 Hz, 1H), 7.84–7.80 (m, 1H), 7.77 (d, *J* = 15.7 Hz, 1H), 7.67 (ddd, *J* = 9.4, 2.6, 1.5 Hz, 1H), 7.64 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.56–7.49 (m, 2H), 7.47–7.31 (m, 5H), 7.19 (tdd, *J* = 8.3,

2.7, 1.0 Hz, 1H), and 7.13–7.06 (m, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.8, 163.2 (d, J = 247.2 Hz), 163.1 (d, J = 246.9 Hz), 160.1 (d, J = 3.2 Hz), 152.1, 143.7 (d, J = 3.0 Hz), 139.1, 138.2 (d, J = 7.4 Hz), 137.1 (d, J = 7.7 Hz), 130.6 (d, J = 8.3 Hz), 130.5 (d, J = 8.0 Hz), 129.7, 126.4, 125.8, 125.4 (d, J = 3.0 Hz), 124.7 (d, J = 3.0 Hz), 123.1, 120.7, 118.8 (d, J = 21.7 Hz), 117.6 (d, J = 21.4 Hz), 114.9 (d, J = 22.4 Hz), and 114.6 (d, J = 21.5 Hz). Q-TOF LC/MS: 348.1339 ([M+H]$^+$, $C_{22}H_{15}F_2NO^+$; calc. 348.1194).

*(2E)-3-(3-Chlorophenyl)-1-(3-{[(1E)-(3-chlorophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**3b**)*

Yellow solid. Yield: 81%, m.p.: 116–119°C. IR (KBr, cm^{-1}): 3,064, 2,980, 2,921, 2,865, 1,662, 1,625, 1,596, 1,569, 1,471, 1,433, 1,364, 1,320, 1,268, 1,211, 1,186, 1,153, 1,077, 1,047, 985, 977, 895, 776, 684, and 565. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.95 (t, J = 1.8 Hz, 1H), 7.92–7.86 (m, 1H), 7.82 (t, J = 1.8 Hz, 1H), 7.78–7.72 (m, 2H), 7.63 (t, J = 1.7 Hz, 1H), 7.57–7.53 (m, 1H), 7.52–7.46 (m, 2H), 7.46–7.38 (m, 3H), and 7.37–7.30 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.7, 159.9, 152.0, 143.5, 139.1, 137.7, 136.7, 135.2, 135.1, 131.8, 130.6, 130.3, 130.2, 129.7, 128.5, 128.0, 127.5, 126.9, 126.4, 125.8, 123.1, and 120.7. Q-TOF LC/MS: 381.3118 ([M+H]$^+$, $C_{22}H_{15}Cl_2NO^+$; calc. 380.0604).

*(2E)-3-(3-Bromophenyl)-1-(3-{[(1E)-(3-bromophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**3c**)*

Yellow solid. Yield: 78%, m.p.: 115–118°C. IR (KBr, cm^{-1}): 3,062, 3,929, 3,884, 1,661, 1,626, 1,591, 1,569, 1,470, 1,440, 1,429, 1,311, 1,241, 1,208, 1,183, 1,151, 1,089, 1,042, 986, 879, 780, 773, 683, 675, and 575. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.45 (s, 1H), 8.12 (t, J = 1.7 Hz, 1H), 7.92–7.87 (m, 1H), 7.83–7.77 (m, 3H), 7.74 (d, J = 15.7 Hz, 1H), 7.62 (ddd, J = 8.0, 2.0, 1.0 Hz, 1H), 7.56–7.50 (m, 4H), 7.43 (ddd, J = 7.9, 2.1, 1.1 Hz, 1H), 7.35 (t, J = 7.8 Hz, 1H), and 7.29 (t, J = 7.8 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.7, 159.8, 152.0, 143.4, 139.1, 137.9, 137.0, 134.7, 133.5, 131.5, 130.9, 130.6, 130.4, 129.7, 127.9, 127.4, 126.4, 125.8, 123.3, 123.2, 123.1, and 120.7. Q-TOF LC/MS: 468.4174 ([M+H]$^+$, $C_{22}H_{15}Br_2NO^+$; calc. 467.9593).

*(2E)-3-(4-Fluorophenyl)-1-(3-{[(1E)-(4-fluorophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**3d**)*

Yellow solid. Yield: 84%, m.p.: 126–128°C. IR (KBr, cm^{-1}): 3,063, 3,049, 3,004, 1,663, 1,626, 1,598, 1,584, 1,572, 1,506, 1,442, 1,411, 1,324, 1,313, 1,294, 1,241, 1,221, 1,151, 1,045, 975, 901, 823, 785, 676, and 500. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.91 (ddd, J = 8.3, 5.2, 2.5 Hz, 2H), 7.87 (dt, J = 7.7, 1.3 Hz, 1H), 7.83–7.76 (m, 2H), 7.63 (ddd, J = 8.3, 5.2, 2.5 Hz, 2H), 7.55–7.44 (m, 2H), 7.41 (ddd, J = 7.8, 2.0, 0.9 Hz, 1H), 7.20–7.14 (m, 2H), and 7.13–7.06 (m, 2H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.0, 164.9 (d, J = 252.9 Hz), 164.1 (d, J = 252.0 Hz), 159.5, 152.3, 143.6, 139.2, 132.2 (d, J = 2.9 Hz), 131.1, 131.0 (d, J = 8.9 Hz, 2 C), 130.4 (d, J = 8.5 Hz, 2 C), 129.5, 125.9, 125.5, 121.6 (d, J = 1.6 Hz), 120.5, 116.2 (d, J = 21.9 Hz, 2C), and 116.1 (d, J = 22.0 Hz, 2C). Q-TOF LC/MS: 348.1328 ([M+H]$^+$, $C_{22}H_{15}F_2NO^+$; calc. 348.1194).

*(2E)-3-(4-Methoxyphenyl)-1-(3-{[(1E)-(4-methoxyphenyl)methylene]-amino}phenyl)prop-2-en-1-one (**3e**)*[34]

Yellow solid. Yield: 77% (Lit: 76%),[34] m.p.: 123–126°C (Lit: 132°C).[34] IR (KBr, cm^{-1}): 3,019, 2,964, 2,901, 2,839, 1,651, 1,627, 1,577, 1,509, 1,490, 1,453, 1,418, 1,320, 1,301, 1256, 1184, 1,167, 1,105, 1,021, 989, 875, 824, 787, 678, 528, and 512. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.47 (s, 1H), 7.92–7.80 (m, 5H), 7.63 (d, J = 8.7 Hz, 2H), 7.53 (t, J = 7.7 Hz, 1H), 7.47 (d, J = 15.6 Hz, 1H), 7.42 (d, J = 7.9 Hz, 1H), 7.02 (d, J = 8.7 Hz, 2H), 6.96 (d, J = 8.7 Hz, 2H), 3.90 (s, 3H), and 3.87 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 190.2, 162.5, 161.7, 160.6, 152.7, 144.8, 139.6, 130.7 (2C), 130.3 (2C), 129.3, 129.1, 127.6, 125.5, 125.3, 120.5, 119.8, 114.4 (2C), 114.3 (2C), 55.5, and 55.4. Q-TOF LC/MS: 254.1274 ([M+H]$^+$, [$C_{24}H_{21}NO_3^+$–$C_8H_8O_2$]; calc. 372.159) The expected mass could not be observed due to the hydrolysis of the imine structure in the HPLC column.

*(2E)-3-(3-Thienyl)-1-(3-{[(1E)-3-thienylmethylene]amino}phenyl)-prop-2-en-1-one (**3f**)*

Yellow solid. Yield: 84%, m.p.: 106–109°C. IR (KBr, cm^{-1}): 3,085, 2,967, 2,882, 1,651, 1,615, 1,578, 1,567, 1,517, 1,436, 1,404, 1,322, 1,283, 1,231, 1,167, 1,141, 1,046, 978, 913, 862, 773, 682, 645, 606, and 582. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.46 (s, 1H), 7.86–7.76 (m, 4H), 7.67 (d, J = 5.1 Hz, 1H), 7.59–7.54 (m, 1H), 7.46 (td, J = 7.7, 1.5 Hz, 1H), and 7.41–7.31 (m, 5H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 190.4, 155.5, 152.5, 140.6, 139.3, 138.5, 138.2, 131.2, 129.6, 129.5, 127.2, 127.0, 126.0, 125.9, 125.5, 125.4, 121.7, and 120.6. Q-TOF LC/MS: 324.066 ([M+H]$^+$, $C_{18}H_{13}NOS_2^+$; calc. 324.051).

*(2E)-3-[4-(Methylthio)phenyl]-1-[3-({(1E)-[4-(methylthio)phenyl]-methylene}amino)phenyl]prop-2-en-1-one (**3g**)*

Yellow solid. Yield: 70%, m.p.: 114–116°C. IR (KBr, cm^{-1}): 3,164, 3,051, 2,980, 2,918, 2,863, 1,653, 1,619, 1,606, 1,576, 1,551, 1,491, 1,437, 1,404, 1,327, 1,300, 1,220, 1,187, 1,149, 1,090, 1,047, 1,012, 981, 919, 815, 788, 680, 510, and 493. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.89 (d, J = 7.7 Hz, 1H), 7.83 (t, J = 12.6 Hz, 4H), 7.59 (d, J = 8.4 Hz, 2H), 7.56–7.50 (m, 2H), 7.44 (d, J = 6.5 Hz, 1H), 7.34 (d, J = 8.3 Hz, 2H), 7.28 (dd, J = 5.4, 2.9 Hz, 2H), 2.56 (s, 3H), and 2.54 (s, 3H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 190.1, 160.6, 152.5, 144.5, 143.8, 142.5, 139.4, 132.6, 131.4, 129.4, 129.3 (2C), 128.9 (2C), 126.0 (2C), 125.8, 125.7 (2C), 125.4, 120.9, 120.6, 15.2, and 15.1. Q-TOF LC/MS: 404.1334 ([M+H]$^+$, $C_{24}H_{21}NOS_2^+$; calc. 404.1137).

*(2E)-3-[4-(Trifluoromethyl)phenyl]-1-[3-({(1E)-[4-(trifluoromethyl)-phenyl]methylene}amino)phenyl]prop-2-en-1-one (**3h**)*

Yellow solid. Yield: 75%, m.p.: 103–105°C. IR (KBr, cm^{-1}): 2,980, 2,972, 2,938, 2,865, 2,825, 1,663, 1,625, 1,607, 1,572, 1,455, 1,438, 1,413, 1,318, 1,287, 1,216, 1,166, 1,104, 1,059, 1,032, 1,014, 958, 918, 907, 829, 790, 687, 673, 591, and 499. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.56 (s, 1H), 8.03 (d, J = 8.1 Hz, 2H), 7.92 (dt, J = 7.7, 1.4 Hz, 1H), 7.87–7.80 (m, 2H), 7.74 (d, J = 8.1 Hz, 4H), 7.66 (d, J = 8.3 Hz, 2H), 7.62 (d, J = 15.7 Hz, 1H), 7.56 (t, J = 7.8 Hz, 1H), and 7.47 (ddd, J = 7.9, 2.1, 1.1 Hz, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.62, 159.92, 151.98, 143.20, 139.02, 138.99, 138.98, 138.94, 138.92,

138.91, 138.22, 133.36, 133.03, 132.24, 131.92, 129.79, 129.23 (2C), 128.68 (2C), 126.62, 126.09, 126.04, 126.01, 125.97, 125.97, 125.94, 125.90, 125.89, 125.86, 125.25, 124.03, 122.54, and 120.76. Q-TOF LC/MS: 448.1362 ($[M+H]^+$, $C_{24}H_{15}F_6NO^+$; calc. 448.1130).

*(2E)-3-(4-Nitrophenyl)-1-(3-{[(1E)-(4-nitrophenyl)methylene]amino}-phenyl)prop-2-en-1-one (**3i**)*

Yellow solid. Yield: 80%, m.p.: 213–216°C. IR (KBr, cm^{-1}): 3,101, 3,075, 2,960, 2,884, 2,851, 1,662, 1,625, 1,610, 1,593, 1,572, 1,513, 1,489, 1,430, 1,333, 1,316, 1,290, 1,245, 1,206, 1,151, 1,103, 988, 850, 838, 754, 688, 677, 621, and 483. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.93 (s, 1H), 8.41 (d, J = 8.7 Hz, 2H), 8.30 (d, J = 8.8 Hz, 2H), 8.27–8.20 (m, 5H), 8.16–8.11 (m, 2H), 7.88 (d, J = 15.6 Hz, 1H), and 7.70–7.66 (m, 2H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 189.1, 161.0, 151.8, 149.5, 148.6, 142.0, 141.8, 141.6, 138.8, 130.6 (2C), 130.5, 130.3 (2C), 126.9, 126.4, 124.6 (2C), 124.4 (2C), 122.4, and 121.7. Q-TOF LC/MS: 402.3735 ($[M+H]^+$, $C_{24}H_{15}F_6NO^+$; calc. 402.1084).

*(2E)-3-(2-Thienyl)-1-(3-{[(1E)-2-thienylmethylene]amino}phenyl)-prop-2-en-1-one (**3j**)*

Brown viscous oil. Yield: 79%. IR (KBr, cm^{-1}): 3,098, 3,071, 2,960, 2,885, 1,654, 1,610, 1,568, 1,509, 1,417, 1,362, 1,304, 1,277, 1,209, 1,152, 1,039, 1,025, 963, 916, 822, 793, 698, 582, and 482. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.60 (s, 1H), 7.94 (d, J = 15.3 Hz, 1H), 7.86–7.78 (m, 2H), 7.54–7.48 (m, 3H), 7.42–7.38 (m, 2H), 7.35–7.29 (m, 2H), 7.14–7.10 (m, 1H), and 7.07–7.04 (m, 1H). ^{13}C-NMR (101 MHz, $CDCl_3$) δ 189.5, 154.1, 151.7, 142.3, 140.3, 139.1, 137.4, 133.0, 132.3, 130.9, 129.5, 129.1, 128.4, 127.9, 125.9, 125.6, 120.7, and 120.5. Q-TOF LC/MS: 324.064 ($[M+H]^+$, $C_{18}H_{13}NOS_2^+$; calc. 324.051).

*(2E)-3-(3-Fluorophenyl)-1-(4-{[(1E)-(3-fluorophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**5a**)*

Yellow solid. Yield: 82%, m.p.: 132–135°C. IR (KBr, cm^{-1}): 3,062, 3,048, 2,968, 1,659, 1,628, 1,603, 1,579, 1,557, 1,485, 1,443, 1,412, 1,338, 1,296, 1,243, 1,207, 1,172, 1,139, 1,031, 971, 943, 876, 828, 775, 737, 680, 661, 518, and 443. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.72 (d, J = 0.9 Hz, 1H), 8.29 (d, J = 8.6 Hz, 2H), 8.10 (d, J = 15.6 Hz, 1H), 7.94–7.87 (m, 1H), 7.86–7.81 (m, 1H), 7.80–7.70 (m, 3H), 7.62 (td, J = 8.0, 5.9 Hz, 1H), 7.52 (td, J = 8.0, 6.2 Hz, 1H), 7.48–7.40 (m, 3H), and 7.36–7.26 (m, 1H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.4, 163.0 (d, J = 243.4 Hz), 162.9 (d, J = 244.5 Hz), 162.1 (d, J = 3.0 Hz), 155.8, 142.9 (d, J = 2.8 Hz), 138.6 (d, J = 7.4 Hz), 137.8 (d, J = 8.0 Hz), 135.5, 131.6 (d, J = 8.2 Hz), 131.4 (d, J = 8.4 Hz), 130.7 (2 C), 126.2 (d, J = 2.6 Hz), 126.0 (d, J = 2.8 Hz), 123.9, 121.9 (2C), 119.4 (d, J = 21.5 Hz), 117.8 (d, J = 21.4 Hz), 115.2 (d, J = 22.2 Hz), and 115.2 (d, J = 22.0 Hz). Q-TOF LC/MS: 348.1342 ($[M+H]^+$, $C_{22}H_{15}F_2NO^+$; calc. 348.1194).

*(2E)-3-(3-Chlorophenyl)-1-(4-{[(1E)-(3-chlorophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**5b**)*[35]

Yellow solid. Yield: 81%, m.p.: 128–130°C. IR (KBr, cm^{-1}): 3,046, 2,894, 1,659, 1,601, 1,590, 1,565, 1,473, 1,420, 1,411, 1,365, 1,333, 1,308, 1,220, 1,202, 1,194, 1,167, 1,098, 1,073, 978, 906, 832, 780, 724, 679, 665, 563, and 430. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.71 (s, 1H), 8.30 (d, J = 8.6 Hz, 2H), 8.17–8.07 (m, 2H), 8.05–8.00 (m, 1H), 7.95 (dd, J = 6.4, 1.2 Hz, 1H), 7.86 (dt, J = 6.5, 1.6 Hz, 1H), 7.75 (d, J = 15.6 Hz, 1H), 7.66 (ddd, J = 8.0, 2.1, 1.2 Hz, 1H), 7.59 (t, J = 7.8 Hz, 1H), 7.54–7.47 (m, 2H), and 7.43 (d, J = 8.5 Hz, 2H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.4, 161.9, 155.8, 142.6, 138.2, 137.5, 135.5, 134.3, 134.2, 132.2, 131.4, 131.2, 130.8 (2C), 130.7, 128.9, 128.5 (2C), 128.1, 123.9, and 121.9 (2C). Q-TOF LC/MS: 381.3080 ($[M+H]^+$, $C_{22}H_{15}Cl_2NO^+$; calc. 380.0604).

*(2E)-3-(3-Bromophenyl)-1-(4-{[(1E)-(3-bromophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**5c**)*

Yellow solid. Yield: 80%, m.p.: 130–132°C. IR (KBr, cm^{-1}): 3,058, 3,027, 2,903, 1,655, 1,625, 1,585, 1,559, 1,515, 1,468, 1,416, 1,356, 1,324, 1,265, 1,217, 1,167, 1,068, 1,024, 975, 969, 956, 889, 826, 778, 670, 661, 592, and 566. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.65 (s, 1H), 8.25 (d, J = 8.6 Hz, 2H), 8.21 (t, J = 1.7 Hz, 1H), 8.13–8.10 (m, 1H), 8.06 (d, J = 15.6 Hz, 1H), 7.96–7.92 (m, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.74 (ddd, J = 8.0, 2.1, 1.0 Hz, 1H), 7.68 (d, J = 15.6 Hz, 1H), 7.60 (ddd, J = 8.0, 1.9, 0.8 Hz, 1H), 7.48 (t, J = 7.8 Hz, 1H), and 7.38 (dt, J = 4.1, 3.6 Hz, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.4, 161.8, 155.8, 142.6, 138.5, 137.8, 135.5, 135.0, 133.6, 131.8, 131.7, 131.5, 131.3, 130.8 (2C), 128.9, 128.5, 123.9, 122.9, 122.7, and 121.9 (2C). Q-TOF LC/MS: 468.4180 ($[M+H]^+$, $C_{22}H_{15}Br_2NO^+$; calc. 467.9593).

*(2E)-3-(4-Fluorophenyl)-1-(4-{[(1E)-(4-fluorophenyl)methylene]-amino}phenyl)prop-2-en-1-one (**5d**)*[36]

Yellow solid. Yield: 78%, m.p.: 141–143°C (Lit: 156.37°C).[36] IR (KBr, cm^{-1}): 3,043, 2,981, 2,921, 2,878, 1,656, 1,624, 1,581, 1,562, 1,506, 1,414, 1,335, 1,321, 1,291, 1,228, 1,213, 1,159, 1,089, 1,022, 1,008, 987, 889, 830, 815, 783, 672, 649, 535, 512, and 497. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.69 (s, 1H), 8.26 (d, J = 8.6 Hz, 2H), 8.09–7.94 (m, 5H), 7.78 (d, J = 15.6 Hz, 1H), 7.47–7.37 (m, 4H), and 7.33 (t, J = 8.9 Hz, 2H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.4, 164.8 (d, J = 250.3 Hz), 163.9 (d, J = 249.0 Hz), 161.8, 156.1, 143.1, 132.9 (d, J = 2.9 Hz), 132.0 (d, J = 3.2 Hz), 131.9 (d, J = 9.1 Hz, 2 C), 131.8 (d, J = 8.6 Hz, 2 C), 135.4, 130.6 (2C), 122.37 (d, J = 2.3 Hz), 121.8 (2C), 116.6 (d, J = 22.0 Hz, 2C), and 116.4 (d, J = 21.7 Hz, 2 C). Q-TOF LC/MS: 348.1317 ($[M+H]^+$, $C_{22}H_{15}F_2NO^+$; calc. 348.1194).

*(2E)-3-(4-Methoxyphenyl)-1-(4-{[(1E)-(4-methoxyphenyl)methylene]-amino}phenyl)prop-2-en-1-one (**5e**)*[37]

Yellow solid. Yield: 79%, m.p.: 140–142°C (Lit: 148–149°C).[37] IR (KBr, cm^{-1}): 3,061, 3,013, 2,959, 2,911, 2,839, 1,650, 1,625, 1,600, 1,587, 1,571, 1,508, 1,439, 1,415, 1,363, 1,303, 1,244, 1,165, 1,109, 1,075, 1,026, 990, 855, 834, 818, 721, 671, 583, 543, and 531. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.60 (s, 1H), 8.22 (d, J = 8.3 Hz, 2H), 7.98–7.82 (m, 5H), 7.75 (d, J = 15.5 Hz, 1H), 7.36 (d, J = 8.3 Hz, 2H), 7.10 (d, J = 8.6 Hz, 2H), 7.03 (d, J = 8.6 Hz, 2H), 3.86 (s, 3H), and 3.83 (s, 3H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.3, 162.8, 162.1, 161.8, 156.4, 144.2, 135.3, 131.4 (2C), 131.3 (2C), 130.5 (2C), 129.2, 127.9, 121.7 (2C), 119.9, 114.9 (2C), 114.8 (2C), 56.0, and 55.9. Q-TOF LC/MS: 254.1303 ($[M+H]^+$, $[C_{24}H_{21}NO_3^+ - C_8H_8O_2]$; calc. 372.159)

The expected mass could not be observed due to the hydrolysis of the imine structure in the HPLC column.

*(2E)-3-(3-Thienyl)-1-(4-{[(1E)-3-thienylmethylene]amino}phenyl)-prop-2-en-1-one (**5f**)*

Yellow solid. Yield: 83% m.p.: 120–123°C. IR (KBr, cm^{-1}): 3,096, 3,075, 2,975, 2,884, 1,653, 1,632, 1,598, 1,577, 1,533, 1,510, 1,439, 1,336, 1,318, 1,279, 1,242, 1,215, 1,172, 1,157, 1,132, 1,026, 1,007, 970, 864, 830, 781, 691, 617, 599, and 575. ^{1}H-NMR (400 MHz, DMSO-d_6) δ 8.66 (s, 1H), 8.32–8.25 (m, 1H), 8.22 (d, J = 8.4 Hz, 2H), 8.13 (d, J = 2.7 Hz, 1H), 7.88–7.76 (m, 3H), 7.73–7.64 (m, 3H), and 7.37 (d, J = 8.4 Hz, 2H). ^{13}C-NMR (101 MHz, DMSO-d_6) δ 188.7, 157.3, 156.3, 140.8, 138.9, 138.1, 135.3, 133.8, 131.1, 130.5, 128.5, 128.2, 126.8, 125.9, 122.0, and 121.7. Q-TOF LC/MS: 324.069 ([M+H]$^+$, $C_{18}H_{13}NOS_2^+$; calc. 324.051).

4.2 | Carbonic anhydrases and acetylcholinesterase inhibition

The inhibitory effect of chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) on AChE activity was performed according to the spectrophotometric method of Ellman,[38] as described previously.[39] In the present work, hCA I and II isoenzymes were purified by Sepharose-4B-L-tyrosine-sulfanilamide affinity column chromatography, and CA isoenzymes' activity was determined according to the spectrophotometric method of Verpoorte et al.[40] as described in our previous studies in detail. *p*-Nitrophenylacetate (*p*-NPA) was used as a substrate for the enzymatic reaction. One CA enzyme unit is adopted as the amount of CA that had an absorbance difference at 348 nm for 3 min at 25°C. For determination of inhibition kinetics of chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**), an activity (%) and [the derivatives] graph was drawn. From these graphs, IC_{50} for compounds (**3a–j** and **5a–f**) were determined. Also, for K_{is}, three different concentrations of the compounds (**3a–j** and **5a–f**) were used.[41] Then, Lineweaver–Burk graphs were drawn according to these measurements. K_i values of chalcone derivatives containing Schiff bases (**3a–j** and **5a–f**) were determined from Lineweaver–Burk graphs, as previously described. To determine the quantity of protein during the purification processing, Bradford's technique was utilized, and bovine serum albumin was used as the standard protein. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis was employed for visualizing the image of isoenzymes.

ACKNOWLEDGMENTS

This study is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number SHMYO-013. ^{1}H-NMR, ^{13}C-NMR, and Q-TOF LC/MS analyses of the compounds were carried out at the Advanced Technology Application and Research Center (CUTAM) of Sivas Cumhuriyet University.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest.

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How to cite this article: Koçyiğit ÜM, Gezegen H, Taslimi P. Synthesis, characterization, and biological studies of chalcone derivatives containing Schiff bases: Synthetic derivatives for the treatment of epilepsy and Alzheimer's disease. *Arch Pharm.* 2020;e2000202. https://doi.org/10.1002/ardp.202000202