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SYNTHESIS AND ENZYME INHIBITORY PROPERTIES OF QUINOXALINE BRIDGED BIS(IMIDAZOLIUM) SALTS

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Abstract – In this study, a series of new salts containing quinoxaline and imidazole moieties were synthesized in good yield by the reaction of 2,3-bis(bromomethyl)quinoxalines and 1-alkylimidazoles in N,N-dimethylformamide. These salts were characterized by elemental analysis, IR, ^{1}H NMR and ^{13}C NMR spectroscopy, which support the proposed structures. Furthermore, the enzyme inhibition activities of these compounds were investigated. They showed highly potent inhibition effect on acetylcholinesterase (AChE) and carbonic anhydrases (hCAs) (K_i values are in the range of 44.80±14.87 to 288.64±42.68 nM, 21.50±4.76 to 187.30±22.43 nM, and 5.81±0.71 to 164.52±26.0 nM for AChE, hCA I, and hCA II, respectively). Compound **3** showed the best inhibition effect for hCA I and compound **4** showed the best inhibition effect for hCA II and AChE.

INTRODUCTION

Quinoxaline, also called benzopyrazine is an important class of nitrogen contain heterocycles and consist of the fusion of a benzene ring with a pyrazine ring.[1,2] Quinoxaline and its derivatives play an important role as powerful building blocks for the synthesis of a wide variety of compounds with significant biological and pharmacological activities, such as antimicrobial, antiviral, antifungal, anti-inflammatory and antitumor activity.[3-7] In addition to biological and pharmacological properties, quinoxaline derivatives have many important applications in diverse areas such as chemosensors, electroluminescent materials, organic semiconductors, dyes, solar cells and catalysts.[8-13] Some important examples of quinoxaline derivatives are quinoxaline-anellated and quinoxaline bridged imidazolium salts, which used as N-heterocyclic carbene (NHC) precursors.[14-17] Bridged bis(NHC) ligands, derived from bridged bis-azolium salts form an important class of multidentate ligands in coordination chemistry and especially homogeneous catalysis. Symmetrical bis(NHC) ligands are well-known structural motifs and have been used for the synthesis of mono- or di-nuclear complexes that show unique structural and physical properties, and are efficient catalysts in diverse transformations.[18] To date, a wide variety of bridged bis(NHC) ligands have been synthesized, in which between the two NHC moieties have a bridging linker, such as alkane, arene and heteroarene.[19-21] Although heteroarene-bridged bis(NHC) ligands are widely studied in literature, quinoxaline bridged bis(NHC) ligands are very rare. To the best our knowledge, only a few examples of quinoxaline bridged bis(NHC) ligands for catalytic applications have been reported to date.[13,16] However, there is no report on the biological properties of quinoxaline bridged bis(NHC) ligands.

Carbon anhydrase (CA) catalyzes the efficient interconversion of carbon dioxide (CO_2) and water into bicarbonate (HCO_3^-) and protons.[22,23] Among the eight different CA genetic families (α-, β-, γ-, δ-, ζ-, η-, θ-, and t-CAs) known so far, the catalytic and inhibition aspects of α-CAs have been most thoroughly investigated. Human carbonic anhydrases are included in the α-CAs class, which had a highly effective esterase activity.[24,25] Sixteen α-CA isoforms have been defined and divided into many isoforms, depending on the location.[26,27] Also, their cellular and tissue distributions, kinetic and biochemical properties, intracellular localities, susceptibility to inhibitors and activators are also completely different.[28,29] Among the indicated isoenzymes, the cytosolic CA I and CA II are the most commonly studied ones.[30,31] However, CA inhibitors (CAIs) have been linked to variety of global diseases such as obesity, glaucoma and cancer.[32,33] To that end, development of new CAIs had extreme importance.[34,35]

Alzheimer's disease (AD) primarily influences the older people causing loss of memory and behavior.[36,37] Currently, more than 40 million people worldwide suffer from dementia caused by AD. Also, this number is expected to increase one-fold each year.[38-40] Acetylcholinesterase (AChE), which is a crucial component of the central nervous system, is capable of converting ACh to choline and acetate. AChE

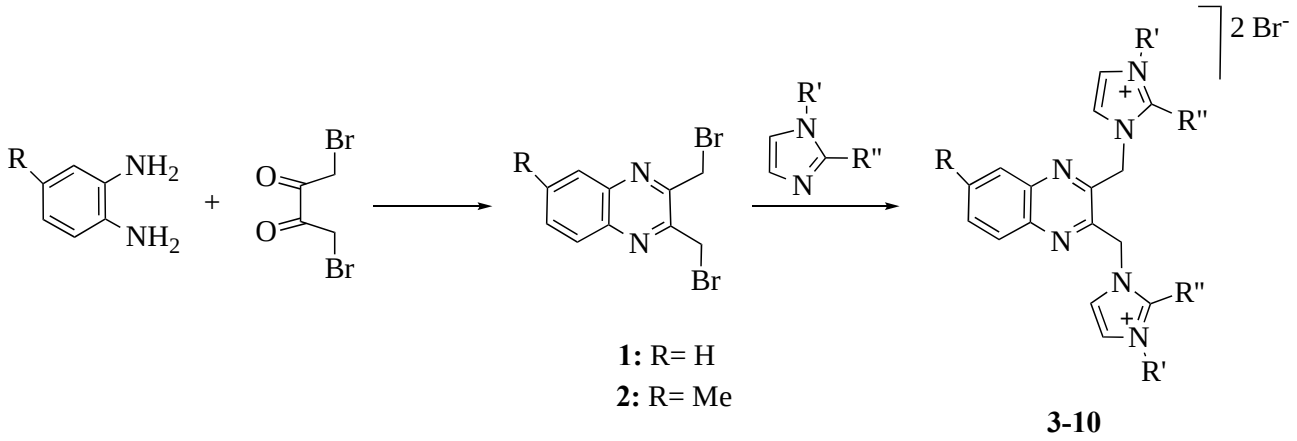
inhibitors (AChEIs) suppress cholinesterase, raising the level and duration of ACh activity.[41,42] In medicine, several AChEIs are widely used for the treatment of AD. Consequently, for the treatment of AD safer AChEIs have been desired.[43,44]

The quinoxaline and imidazole moieties are important structural units in the bioactive compounds. Therefore, in this study, we report the synthesis of quinoxaline bridged bis(imidazolium) salts and investigate on the metabolic enzymes' inhibitory properties of these salts.

RESULTS AND DISCUSSION

Synthesis of bis(imidazolium) salts

In this study, the bridge compounds, 2,3-bis(bromomethyl)quinoxalines **1**, **2** were obtained by the reaction of 1,4-dibromobutane-2,3-dione with appropriate *o*-phenylenediamine previously described in literature.[45] The quinoxaline bridged bis(imidazolium) salts **3-10** were readily prepared by the reaction of N-alkylimidazole with 2,3-bis(bromomethyl)quinoxalines in N,N-dimethylformamide (Scheme 1). After purification, bis(imidazolium) salts **3-10** were isolated as brown solids in good yields ranging between 73% and 86%. These salts are soluble in hot ethanol and dimethyl sulfoxide; insoluble in water, chloroform, dimethylformamide, hexane, toluene, dichloromethane and diethyl ether. They are air- and moisture stable both in the solid state and in solution. All the new bis(imidazolium) salts **4-10** were fully characterized by elemental analysis, ^{1}H NMR, ^{13}C NMR and IR spectroscopy, and their melting points were determined. ^{1}H and ^{13}C NMR spectroscopic characterization of salts **4-10** confirmed the proposed structure (Scheme 1). The ^{1}H NMR spectra of the salts (**4**, **5**, **7-9**) (**6** and **10** have methyl substituents instead of hydrogen) in DMSO-d_6 displayed a characteristic downfield resonance between δ = 10-12 ppm attributed to the resonance of NCHN proton, which indicated the successful formation of desired salts (**4**, **5**, **7-9**). The methylene protons of the bridge for the symmetrical salts (**4-6**) appear as a singlet in the range δ = 4-5 ppm, whereas same signals are observed as two singlets in the range δ = 4-5 ppm for unsymmetrical salts (**7-10**). The aromatic protons of quinoxaline ring were detected as multiplet in the range of 7.10-7.80 ppm. The protons of imidazole ring appear as two singlets in the range of 7.10-7.80 ppm for **4-8**, as mutiplet in the range of 7.10-7.80 ppm for **9**, **10**. In the ^{13}C NMR spectra of the salts **4-10**, the N*C*HN carbon resonance of **4-10** was observed between 180.4 and 187.3 ppm. The appearances of these downfield signals indicate the formation of the salts. The signals of methylene carbons of the bridge for the salts **4-10** were appear in range of 60-70 ppm. The signals for imidazole ring carbons were observed between 119.3 and 125.2 ppm. In addition, in the FT-IR, the ν(CN) stretching frequency peaks of the quinoxaline bridged bis(imidazolium) salts **4-10** were observed between 1543 and 1533 cm^{-1}. These NMR and IR values are in good agreement with previously reported values for quinoxaline bridged salts.[13,16]



3: R = H, R' = Me, R" = H
4: R = H, R' = butyl, R" = H
5: R = H, R' = vinyl, R" = H
6: R = H, R' = Me, R" = Me
7: R = Me, R' = Me, R" = H
8: R = Me, R' = butyl, R" = H
9: R = Me, R' = vinyl, R" = H
10: R = Me, R' = Me, R"= Me

Scheme 1. Synthesis of quinoxaline bridged bis(imidazolium) salts

Enzyme inhibition results

For biochemical pathways, the inhibition of CA isozymes in tissues are important. In the last 10 years, the some several effects of the CAIs have been highly documented.[46] They give rise to the evolution of diuretics in multiple classes among which acetazolamide and thiazides.[47] Many medications, which extensively used clinically, exhibited high CA inhibition effects. These include, chlorthalidone, quinethazone, furosemide, metolazone, indapamide and thiazides.[48] The synthesized a series of quinoxaline compounds, **1-10**, were tested against cytosolic isoforms hCA I, II and AChE by using esterase assay and the Ellman's methods.[49]

It's part of the current study, all the synthesized compounds potent inhibited hCA I with IC_{50} values ranging from 49.50 to 138.60 nM and K_i values ranging from 23±5 to 187±22 nM. Except four compounds, other studied compounds showed the best inhibition, compared to AZA (K_i: 118±13 nM). Compound **3,** showed the best inhibition (K_i: 22±5 nM). As seen in Table 1, the inhibition effects of studied compounds (**1-10**) against hCA I were decreased in the following order: **3** > (K_i: 21.50±4.76 nM) **2** > (K_i: 24±5 nM) **7**> (K_i: 24±1 nM) **6** > (K_i: 54±7 nM) **8** > (K_i: 60±9 nM) **4** > (K_i: 61±10 nM) **9** > (K_i: 122±29 nM) **10** > (K_i: 138±4 nM) **1** > (K_i: 169±28 nM) **5** > (K_i: 187±22 nM). When the groups attached

to the quinoxaline compounds are compared, the inhibition order is as follows: 3-methyl > 2,3-dimethyl >3-butyl > 3-vinyl. When 6-methylquinoxaline structured compounds are compared according to the inhibition order is as follows: 3-methyl > 3-butyl > 3-vinyl > 2,3-dimethyl. The fact that the methyl group is attached at the 3rd position in both groups of compounds increased the inhibition.

For hCA II, all the studied compounds effectively inhibited hCA II with IC_{50} values ranging from 22.68 to 57.75 nM and K_i values ranging from 6±1 to 165±26 nM. Except three compounds (**2**, **6**, and **8**) other studied compounds showed the best inhibition, compared to AZA (K_i: 88±10 nM). Compound **4**, showed the best inhibition (K_i: 6±1 nM). The inhibition effects of studied compounds (**1-10**) against hCA II were decreased in the following order: **4** > (K_i: 6±1 nM) **5** > (K_i: 10±1 nM) **9**> (K_i: 17±3 nM) **7** > (K_i: 27±2 nM) **10** > (K_i: 40±11 nM) **1** > (K_i: 44±2 nM) **3** > (K_i: 46±7 nM) **8** > (K_i: 95±15 nM) **2** > (K_i: 151±23 nM) **6** > (K_i: 165±26 nM) (Table 1). According to results, the binding of the methyl group from the 6th position to the quinoxaline structure in compound **7** caused a decrease in inhibition (**3,** K_i: 45.58±6.82 nM). When the **5** and **9** compounds are compared with each other, the binding of the methyl group at the 6th position increased the inhibition value. Contrary to hCA I, while the butyl group attached to group 3 in quinoxaline compounds and vinyl group attached to group 3 in 6-methylquinoxaline compounds gave the best inhibition effect.

The AChE protein is an important therapeutic target for AD. The loss of neurotransmission and the damage of cholinergic neurons in the brain and are the main causes of the reduced in cognitive function in patients with AD. For this reason, one therapeutic approach requires the use of AChE inhibitors to limit the impairment of Ach.[50] In this study, all the novel synthesized compounds effectively inhibited AChE with IC_{50} values ranging from 69 to 173 nM and K_i values ranging from 45±15 to 289±43 nM. Except compounds **1** and **3**, other studied compounds showed inhibition effect compared to TAC (K_i: 146±17 nM). Like hCA II, compound **4**, exhibited the best inhibition (K_i: 45±15 nM). The inhibition effects of studied compounds (**1-10**) against AChE were decreased in the following order: **4** (K_i: 45±15 nM) > **9** (K_i: 47±6 nM) > **7** (K_i: 90±28 nM) > **8** (K_i: 92±9 nM) > **10** (K_i: 95±24 nM) > **5** (K_i: 99±21 nM) > **6** (K_i: 125±25 nM) > **2** > (K_i: 133±18 nM) > **3** (K_i: 149±10 nM) > **1** (K_i: 289±43 nM) (Table 1). For AChE, when the groups attached to the quinoxaline compounds are compared, the inhibition order is as follows: 3-butyl > 3-vinyl > 2,3-dimethyl > 3-methyl. When 6-methylquinoxaline structured compounds are compared according to the inhibition order is as follows: 3-vinyl > 3-methyl > 3-butyl > 2,3-dimethyl. The values showing the best inhibition for 6-methylquinoxaline and quinoxaline compounds are similar to hCA II isoenzyme.

Table 1. Inhibition data of hCA I and II isoforms and AChE and with a series of quinoxaline bridged bis(imidazolium) salts (**1-10**)

Compounds	IC_{50} (nM)						K_i (nM)		
	hCA I	r^2	hCA II	r^2	AChE	r^2	hCA I	hCA II	AChE
1	139	0.9935	58	0.9782	173	0.9797	169±28	44±2	289±43
2	97	0.9817	42	0.9872	139	0.9944	24±5	151±23	133±18
3	63	0.9882	25	0.9739	116	0.9751	22±5	46±7	149±10
4	69	0.9986	37	0.9931	99	0.9831	61±10	6±1	45±15
5	86	0.9760	29	0.9967	104	0.9735	187±22	19±1	99±21
6	104	0.9828	46	0.9891	119	0.9819	54±7	165±26	125±25
7	99	0.9840	41	0.9712	120	0.9864	24±1	27±2	90±28
8	77	0.9922	39	0.9911	123	0.9922	60±9	95±14	92±9
9	116	0.9919	23	0.9982	77	0.9858	122±29	17±3	47±6
10	50	0.9926	30	0.9725	69	0.9708	138±4	40±11	95±24
AZA*	125	0.9817	99	0.9857	-	-	118±13	88±10	-
TAC*	-	-	-	-	159	0.9891	-	-	146±17

*Acetazolamide (AZA) and Tacrine (TAC) were used as positive control for carbonic anhydrase and acetylcholinesterase enzymes, respectively.

CONCLUSION

In summary, the quinoxaline bridged bis(imidazolium) salts were successfully synthesized and characterized using spectroscopic methods. Especially, the evaluation of the inhibitory efficacy of quinoxaline bridged bis(imidazolium) salts toward acetylcholinesterase enzyme also demonstrates their anti-AD potential. The results were observed to be quite striking and effective due to their strong inhibitory effect when compared with the standards. This study gives insight into several biological abilities of quinoxaline bridged bis(imidazolium) salts and they could be promising novel agents for treatment of different diseases and health applications.

EXPERIMENTAL

All reactions for the preparation of the bis(imidazolium) salts were carried out under air. All commercial chemicals were purchased from Sigma-Aldrich and used as received. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian AS 400 Merkur spectrometer operating at 400 MHz (^{1}H), 100 MHz (^{13}C) in DMSO-d_6 with tetramethylsilane as an internal reference. Coupling constants (*J* values) are given in Hertz. NMR multiplicities are abbreviated as follows: s = singlet, d = doublet, t = triplet, pent = pentet, hext = hextet and m = multiplet signal. FT-IR spectra were recorded on ATR unit in the range 400-4000 cm^{-1} on Perkin Elmer Spectrum 100. Elemental analyses were obtained with a LECO CHNS-932 elemental

analyzer. Melting points were measured in open capillary tubes with Stuart SMP 40 melting point apparatus and uncorrected.

The preparation of 2,3-bis(bromomethyl)quinoxalines, 1-2

2,3-Bis(bromomethyl)quinoxaline and 2,3-bis(bromomethyl)-6-methylquinoxaline were prepared according to the literature.[45]

General procedure for the preparation of the bis(imidazolium) salts, 3-10

To a solution of 2,3-bis(bromomethyl)quinoxalines (3.00 mmol) in DMF (5 mL), 1-alkylimidazole (6 mmol) was added. The reaction mixture was heated at 80 ºC for 18 h, and a brown precipitate was formed. After cooling to room temperature, the product was filtered and washed with Et_2O. Then, the crude products were recrystallized from EtOH.

1,1'-(Quinoxaline-2,3-diyldimethanediyl)bis(3-methyl-1*H*-imidazol-3-ium) dibromide, 3

This compound was prepared according to the literature.[13]

1,1'-(Quinoxaline-2,3-diyldimethanediyl)bis(3-butyl-1*H*-imidazol-3-ium) dibromide, 4

Yield: 1.45 g, 86%; mp 246-248 ºC; IR, $\upsilon_{(NCN)}$ = 1563 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 9.53 (s, 2H, NC*H*N), 7.99 and 7.96 (s, 4H, NC*H*=C*H*N), 7.86-7.90 (m, 4H, quinoxaline-*H*), 6.20 (s, 4H, quinoxaline-CH_2N), 4.37 (t, 4H, *J* = 8.0 Hz, NC$H_2$$CH_2CH_2CH_3$), 1.89 (pent, 4H, *J* = 8.0 Hz, $NCH_2CH_2CH_2CH_3$), 1.36 (hext, 4H, *J* = 8.0 Hz, $NCH_2CH_2CH_2CH_3$), 0.97 (t, 6H, *J* = 8.0 Hz, $NCH_2CH_2CH_2CH_3$). ^{13}C NMR (DMSO-d_6) δ: 138.2 (N*C*HN), 148.5, 140.6, 131.5 and 128.8 (quinoxaline-*C*), 124.4 and 122.8 (N*C*H=*C*HN), 50.9 (quinoxaline-*C*H_2N), 49.2 (N*C*$H_2CH_2CH_2CH_3$), 31.9 ($NCH_2CH_2CH_2CH_3$), 19.2 ($NCH_2CH_2CH_2CH_3$), 13.8 ($NCH_2CH_2CH_2CH_3$). Anal. Calcd for $C_{24}H_{32}N_6Br_2$: C, 51.06; H, 5.67; N, 14.89. Found: C, 51.12; H, 5.71; N, 14.82.

1,1'-(Quinoxaline-2,3-diyldimethanediyl)bis(3-vinyl-1*H*-imidazol-3-ium) dibromide, 5

Yield: 1.24 g, 82%; mp 266-267 ºC; IR, $\upsilon_{(NCN)}$ = 1543 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 9.84 (s, 2H, NC*H*N), 8.42 and 8.12 (s, 4H, NC*H*=C*H*N), 7.99-7.87 (m, 4H, quinoxaline-*H*), 7.55 and 7.51 (dd, 2H, *J* = 8.0 Hz, NC*H*=CH_2), 6.25 (s, 4H, quinoxaline-CH_2N), 6.12 and 5.53 (dd, 4H, *J* = 12 and 8.0 Hz, NCH=CH_2). ^{13}C NMR (DMSO-d_6) δ: 137.4 (N*C*HN), 148.1, 140.6, 131.5 and 129.0 (quinoxaline-*C*), 125.2 and 119.3 (N*C*H=*C*HN), 129.4 (N*C*H=CH_2), 109.6 (NCH=*C*H_2), 51.2 (quinoxaline-*C*H_2N). Anal. Calcd for $C_{20}H_{20}N_6Br_2$: C, 47.61; H, 3.96; N, 16.66. Found: C, 47.65; H, 3.99; N, 16.61.

1,1'-(Quinoxaline-2,3-diyldimethanediyl)bis(2,3-dimethyl-1*H*-imidazol-3-ium) dibromide, 6

Yield: 1.35 g, 89%; mp 323-325 ºC; IR, $\upsilon_{(NCN)}$ = 1545 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 7.93-7.83 (m, 4H, quinoxaline-*H*), 7.81 and 7.75 (s, 4H, NC*H*=C*H*N), 6.22 (s, 4H, quinoxaline-CH_2N), 3.95 (s, 6H, NCH_3), 2.67 (s, 6H, NC(CH_3)N). ^{13}C NMR (DMSO-d_6) δ: 147.0 (N*C*(CH_3)N), 148.0, 140.4, 131.0 and 129.0 (quinoxaline-*C*), 123.0 and 122.5 (N*C*H=*C*HN), 50.1 (quinoxaline-*C*H_2N), 35.5 (N*C*H_3), 10.4 (NC(*C*H_3)N). Anal. Calcd for $C_{20}H_{24}N_6Br_2$: C, 47.24; H, 7.72; N, 16.53. Found: C, 47.19; H, 7.75; N, 16.48.

1,1'-(6-Methylquinoxaline-2,3-diyldimethanediyl)bis(3-methyl-1*H*-imidazol-3-ium) dibromide, 7

Yield: 1.15 g, 78%; mp 236-237 ºC; IR, $\upsilon_{(NCN)}$ = 1567 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 9.44 (s, 2H, NC*H*N), 7.94-7.86 (m, 3H, quinoxaline-*H*), 7.84 and 7.74 (s, 4H, NC*H*=C*H*N), 6.19 and 6.16 (s, 4H, quinoxaline-CH_2N), 4.02 and 4.01 (s, 6H, NCH_3), 2.53 (s, 3H, quinoxaline-CH_3-6). ^{13}C NMR (DMSO-d_6) δ: 138.4 (N*C*HN), 148.2, 147.3, 141.8, 140.7, 139.1, 133.4, 128.6 and 127.8 (quinoxaline-*C*), 124.2 and 124.0 (N*C*H=*C*HN), 50.9 and 50.8 (quinoxaline-*C*H_2N), 36.5 (N*C*H_3), 21.6 (quinoxaline-*C*H_3-6). Anal. Calcd for $C_{19}H_{22}N_6Br_2$: C, 46.15; H, 4.53; N, 17.00. Found: C, 46.11; H, 4.56; N, 17.06.

1,1'-(6-Methylquinoxaline-2,3-diyldimethanediyl)bis(3-butyl-1*H*-imidazol-3-ium) dibromide, 8

Yield: 1.31 g, 76%; mp 259-260 ºC; IR, $\upsilon_{(NCN)}$ = 1561 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 9.46 (s, 2H, NC*H*N), 7.97-7.72 (m, 3H, quinoxaline-*H*), 7.76 and 7.65 (s, 4H, NC*H*=C*H*N), 6.12 and 6.11 (s, 4H, quinoxaline-CH_2N), 4.38-4.34 (m, NC$H_2$$CH_2$$CH_2$$CH_3$), 2.54 (s, 3H, quinoxaline-CH_3-6). 1.92-1.85 (m, 4H, NCH_2C$H_2$$CH_2$$CH_3$), 1.40-1.31 (m, 4H, NCH_2CH_2C$H_2$$CH_3$), 1.00-0.95 (m, 6H, $NCH_2CH_2CH_2$CH_3). ^{13}C NMR (DMSO-d_6) δ: 138.2 (N*C*HN), 148.3, 147.4, 141.8, 140.7, 139.1, 133.5, 128.4 and 127.6 (quinoxaline-*C*), 124.4 and 122.8 (N*C*H=*C*HN), 50.9 and 50.7 (quinoxaline-*C*H_2N), 49.3 and 49.2 (N*C*$H_2CH_2CH_2CH_3$), 31.9 (NCH_2*C*$H_2CH_2CH_3$), 21.7 (quinoxaline-*C*H_3-6), 19.2 (NCH_2CH_2*C*H_2CH_3), 13.8 ($NCH_2CH_2CH_2$*C*H_3). Anal. Calcd for $C_{25}H_{34}N_6Br_2$: C, 51.90; H, 5.88; N, 14.53. Found: C, 51.96; H, 5.90; N, 14.45.

1,1'-(6-Methylquinoxaline-2,3-diyldimethanediyl)bis(3-vinyl-1*H*-imidazol-3-ium) dibromide, 9

Yield: 1.13 g, 73%; mp 261-262 ºC; IR, $\upsilon_{(NCN)}$ = 1546 cm^{-1}. ^1H NMR (DMSO-d_6) δ: 9.82 (s, 2H, NC*H*N), 8.42, 8.41, 8.11 and 8.10 (s, 4H, NC*H*=C*H*N), 7.88-7.71 (m, 3H, quinoxaline-*H*), 7.56-7.49 (m, NC*H*=CH_2), 6.22 and 6.20 (s, 4H, quinoxaline-CH_2N), 6.13-6.09 and 5.54-5.51 (m, 4H, NCH=CH_2), 2.53 (s, 3H, quinoxaline-CH_3-6). ^{13}C NMR (DMSO-d_6) δ: 137.4 (N*C*HN), 147.9, 147.0, 142.0, 140.7, 139.1, 133.6, 128.6 and 127.8 (quinoxaline-*C*), 125.2 and 119.3 (N*C*H=*C*HN), 129.4 (N*C*H=CH_2), 109.5

(NCH=*C*H$_2$), 51.2 and 51.1 (quinoxaline-*C*H$_2$N), 21.6 (quinoxaline-*C*H$_3$-6). Anal. Calcd for $C_{21}H_{22}N_6Br_2$: C, 48.64; H, 4.24; N, 16.21. Found: C, 48.58; H, 4.27; N, 16.15.

1,1'-(6-Methylquinoxaline-2,3-diyldimethanediyl)bis(2,3-dimethyl-1*H*-imidazol-3-ium) dibromide, 10

Yield: 1.33 g, 85%; mp 320-322 ºC; IR, $\upsilon_{(NCN)}$ = 1545 cm^{-1}. ^{1}H NMR (DMSO-d_6) δ: 7.33-7.68 (m, 7H, quinoxaline-*H* and NC*H*=C*H*N), 6.17-6.11 (m, 4H, quinoxaline-C*H*$_2$N), 3.96 and 3.94 (s, 6H, NC*H*$_3$), 2.68 and 2.65 (s, 6H, NC(C*H*$_3$)N), 2.53 (s, 3H, quinoxaline-C*H*$_3$-6). ^{13}C NMR (DMSO-d_6) δ: 147.0 (N*C*(CH$_3$)N), 147.8, 141.4, 140.4, 133.1, 128.5 and 127.8 (quinoxaline-*C*), 123.0 and 122.5 (N*C*H=*C*HN), 49.9 (quinoxaline-*C*H$_2$N), 35.5 (N*C*H$_3$), 21.6 (quinoxaline-*C*H$_3$-6), 10.4 (NC(*C*H$_3$)N). Anal. Calcd for $C_{21}H_{26}N_6Br_2$: C, 48.27; H, 4.98; N, 16.09. Found: C, 48.21; H, 5.01; N, 16.13.

AChE and hCAs activity assay

In the present work, *in vitro* effects on AChE activity of the novel synthesized a series of quinoxaline compounds (**1-10**) evaluated by the method of Ellman et al.[49] Results were performed spectrophotometrically at 412 nm using acetylthiocholine iodide according to previous study.[51] Both hCA isoenzymes were purified via Sepharose-4B-L-Tyrosine-sulfanilamide affinity chromatography.[52,53] After affinity chromatography processes, the CA quantity in tubes was determined at 342 nm.[54] The purity of both isoenzymes was determined by SDS-PAGE.[55] Also, esterase activity of the hCAs were determined by following the change in absorbance at 348 nm according to the assay by Verpoorte et al[56] as defined in previous studies.[57,58]

AChE and hCAs kinetic assay

To investigate the *in vitro* inhibitory mechanisms of quinoxaline bridged bis(imidazolium) salts (**1-10**), kinetic studies were made with the variable compound and substrate concentrations. IC_{50} and Lineweaver-Burk curves[59] as previously reported.[60,61] From the observed data, IC_{50} and K_i values for these derivatives were computed, and the types of inhibition of AChE and hCAs were determined as in previous studies.[62-64]

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