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Synthesis, characterization, crystal structure of novel bis-thiomethylcyclohexanone derivatives and their inhibitory properties against some metabolic enzymes

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ABSTRACT

In this study, a series of novel bis-thiomethylcyclohexanone compounds (**3a–3j**) were synthesized by the addition of thio-Michael to the bis-chalcones under mild reaction conditions. The bis-thiomethylcyclohexanone derivatives (bis-sulfides) were characterized by ^{1}H NMR, ^{13}C NMR, FTIR and elemental analysis techniques. Furthermore, the molecular and crystal structures of **3h, 3i** and **3j** compounds were determined by single crystal X-ray diffraction studies. In this study, X-ray crystallography provided an alternative and often-complementary means for elucidating functional groups at the enzyme inhibitory site. Acetylcholinesterase (AChE) is a member of the hydrolase protein super family and has a significant role in acetylcholine-mediated neurotransmission. Here, we report the synthesis and determining of novel bis-thiomethylcyclohexanone compounds based hybrid scaffold of AChE inhibitors. The newly synthesized bis-thiomethylcyclohexanone compounds showed K_i values of in range of 39.14–183.23 nM against human carbonic anhydrase I isoenzyme (hCA I), 46.03–194.02 nM against human carbonic anhydrase II isoenzyme (hCA II), 4.55–32.64 nM against AChE and 12.77–37.38 nM against butyrylcholinesterase (BChE). As a result, novel bis-thiomethylcyclohexanone compounds can have promising anti Alzheimer drug potential and record novel hCA I, and hCA II enzymes inhibitor.

1. Introduction

Carbon-sulfur bond formation is an important methodology used in the synthesis of sulfur-containing natural and pharmaceutical products [1–5]. The thio-Michael addition reaction is one of the most important processes for C—S bond formation [6]. Generally, C—S bond formation is performed with the addition of a thiol, 1,4-conjugate on the acceptor α,β-unsaturated carbonyl compounds by Lewis acid or deprotonation of thiol [7,8]. It is known that this method plays an important role in the synthesis of bioactive compounds [8–11]. Sulfide and bis-sulfide compounds have been reported to possess important biological activities in organic, pharmaceutical and biological applications (Scheme 1) [4,12–14].

It is well known that the studies related to enzyme inhibition and activation is one of the most common biological activities. Carbonic anhydrases (CAs) are extensively distributed zinc-comprising metalloenzymes available in all life phyla, which hold pH homeostasis factor in the human body by catalyzing the carbon dioxide (CO_2) hydration action to bicarbonate (HCO_3^-) and proton (H^+) plus other hydrolytic reactions [15–18]. Also, depending on their localization in diverse organisms, capacity, and catalytic activity to various classes of inhibitors, CAs are divided in seven genetically separate classes, α-, β-, δ-, γ-, ζ-, η- and θ-CAs [19,20]. Indeed, only the α-class is recorded to be present in human bodies, in which sixteen α-CA isoenzymes were explained which differ in their distribution in tissues, subcellular localization, and kinetic and molecular properties [21–23]. The CA isoenzymes are involved in multiple physiological and biochemical processes like calcification, acid-base regulation, ureagenesis, bone resorption, tumorigenicity, and gluconeogenesis, hence representing interesting biochemical aims for the design of CA inhibitors (CAIs) with

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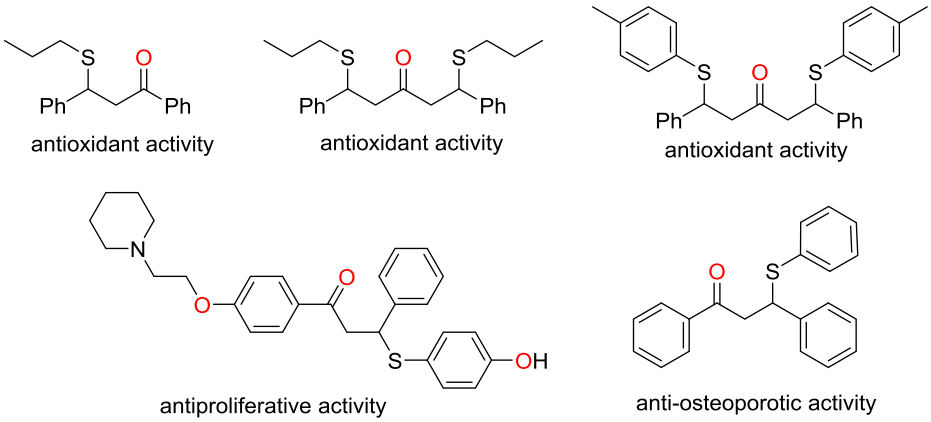
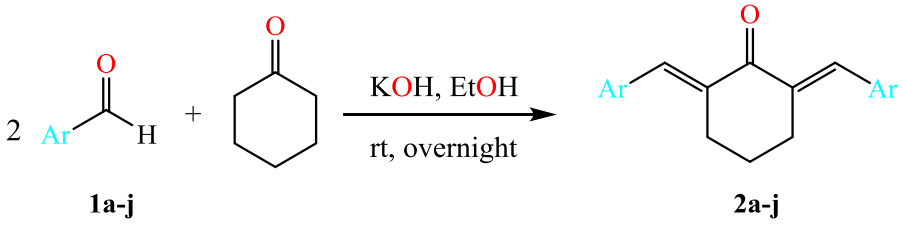


Scheme 1. Biological properties of sulfide and bis-sulfide compounds.



a Ar =C_6H_5-, **b** Ar=2-Naphthyl-, **c** Ar=3-$NO_2C_6H_4$-, **d** Ar= 4-$NO_2C_6H_4$-, **e** Ar=3-FC_6H_4-
f Ar=4-FC_6H_4-, **g** Ar=3-ClC_6H_4-, **h** Ar=4-ClC_6H_4-, **i** Ar= 3-BrC_6H_4-, **j** Ar=4-BrC_6H_4-

Scheme 2. Synthesis of bis-chalcone derivatives (**2a–j**).

many biological applications [24,25]. The ubiquitous hCA I isoenzyme is involved in cerebral edema, and retinal, and its inhibition action can be a valuable factor in fighting these situations. The cytosolic hCA II isozyme is involved in epilepsy, edema, glaucoma, and among others [26,27]. The clinical usage of CAIs has been established as anti-glaucoma agents, antiepileptics and diuretics. Also, they frequently used in the treatment of mountain sickness, duodenal and gastric ulcers, neurological disorders, idiopathic intracranial hypertension and osteoporosis [28,29].

On the other hand, Alzheimer's disease (AD) causes an advanced harm on the central neural mechanism, such as the decline in language, behavioral disorders, and memory loss [30,31]. Various anomalies in the brain cells including inflammation, oxidative stress, neuronal cell death and protein aggregates are relevant to the effects of the AD [32]. The heterogeneous etiology and complexity of AD play a key role in novel drug development to avoid the progress of AD [33–35]. One significant therapeutic palliative-process to minimize the AD effects has been utilized of AChE inhibitors (AChEIs) such as rivastigmine, donepezil, tacrine, and galantamine. Recently, it was reported that some identified AChEIs as promising molecules to treat dementia, withdrawn from the clinical trials due to their toxicity and poor central nervous system stability [36,37]. Therefore, the design, discovery and development of novel AChEIs are still a big challenge for medicinal chemist. Thus, the selectivity of the inhibition has a great importance and presents a significant consideration for developing novel kinds of AChEIs [38-41].

In this study, we synthesized new bis-sulfide derivatives using bis-benzylidenecyclohexanone (bis-chalcone) and evaluated their biological activities. Also, we aimed to investigate the inhibitory effect of these novel bis-thiomethylcyclohexanone derivatives (**3a–j**) using bis-benzylidenecyclohexanone (**2a–j**) derivatives on the hCA I, hCA II, AChE and BChE enzymes for alternative and compared to standard and clinically used inhibitors.

2. Result and discussion

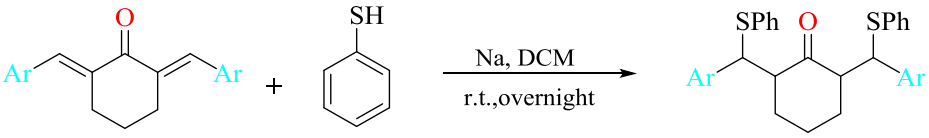
2.1. Chemistry

Firstly, the bis-chalcone compounds were synthesized according to previous studies [42,43]. For this purpose, the substituted aromatic aldehydes and cyclohexanones were subjected to the Claisen-Schmidt condensation reaction in ethanol with potassium hydroxide (Scheme 2). Then, suitable reaction conditions for the synthesis of bis-sulfide compounds were determined. For this, the reaction of addition of thiophenol to bis-chalcone using different Lewis acids and bases was studied and the most suitable reaction condition was found to be metallic Na in DCM (Table 1).

TLC detected the product mixture in experiments with iodine. However, any products were not isolated from this mixture. Also, in the

Table 1
Optimization of reaction conditions for addition of thiophenol to bis-chalcones.

Entry	Catalyzer	Temperature	Solvent	Time	Yield
1	I_2 (%10 mol)	Room temperature	DCM	1 day	Isomer mixture
2	I_2 (%10 mol)	Reflux	DCM	1 day	Isomer mixture
3	$FeCl_3$ (%2 mol)	Room temperature	DCM	1 day	No reaction
4	$AlCl_3$ (%2 mol)	Room temperature	DCM	1 day	No reaction
5	Al_2O_3 (5 mol)	Reflux	EtOH	1 day	No reaction
6	*t*-BuOK (2 mol)	Room temperature	BuOH	1 day	No reaction
7	NEt_3 (5 mol)	Reflux	DCM	1 day	No reaction
8	Na (1 mol)	Room temperature	DCM	1 day	Product observed



2a-j **3a-j**

a Ar =C_6H_5-, **b** Ar=2-Naphthyl-, **c** Ar=3-$NO_2C_6H_4$-, **d** Ar= 4-$NO_2C_6H_4$-, **e** Ar=3-FC_6H_4-
f Ar=4-FC_6H_4-, **g** Ar=3-ClC_6H_4-, **h** Ar=4-ClC_6H_4-, **i** Ar= 3-BrC_6H_4-, **j** Ar=4-BrC_6H_4-

Scheme 3. Synthesis of the novel bis-sulfide derivatives (**3a-j**).

Table 2
Inhibition parameters including half maximal inhibitory concentration (IC_{50}) and inhibition constants (Ki) of a dousen of novel bis-sulfide compounds (**3a-j**) against human carbonic anhydrase I, and II isoenzymes (hCA I, and II), acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes.

Compounds	IC_{50} (nM)								K_i (nM)				
	hCA I	r^2	hCA II	r^2	AChE	r^2	BChE	r^2	hCA I	hCA II	AChE	BChE	AChE/BChE
3a	109.43	0.9736	138.55	0.9308	12.73	0.9563	28.93	0.9892	87.53 ± 14.07	127.44 ± 31.84	8.63 ± 1.83	19.73 ± 3.64	0.43
3b	147.05	0.9927	170.82	0.9673	10.03	0.9604	31.53	0.9722	183.23 ± 28.18	157.99 ± 50.72	7.14 ± 1.05	22.84 ± 4.93	0.31
3c	180.34	0.9598	201.58	0.9902	19.39	0.9912	42.04	0.9831	167.34 ± 22.72	194.02 ± 28.55	12.74 ± 2.95	20.51 ± 3.02	0.62
3d	117.03	0.9819	143.84	0.9819	41.77	0.9732	61.38	0.9682	137.03 ± 43.94	154.93 ± 36.04	32.64 ± 6.86	37.38 ± 11.53	0.87
3e	71.66	0.9816	80.03	0.9772	7.18	0.9208	17.90	0.9801	80.48 ± 17.11	88.32 ± 18.13	4.55 ± 0.94	12.77 ± 2.20	0.35
3f	55.08	0.9743	74.22	0.9609	15.71	0.9714	24.83	0.9492	50.66 ± 9.17	80.12 ± 21.98	11.42 ± 1.98	18.53 ± 5.94	0.61
3 g	61.38	0.9891	68.52	0.9923	15.02	0.9843	20.28	0.9618	64.83 ± 19.33	61.34 ± 13.81	13.56 ± 2.64	14.82 ± 4.08	0.91
3 h	38.63	0.9812	42.84	0.9904	23.83	0.9672	28.11	0.9910	47.92 ± 7.20	48.18 ± 19.10	17.81 ± 4.66	19.73 ± 3.98	0.90
3i	44.28	0.9590	64.03	0.9891	9.83	0.9840	29.43	0.9733	39.14 ± 9.94	46.03 ± 10.47	6.06 ± 2.01	17.43 ± 2.25	0.34
3j	69.43	0.9711	72.71	0.9690	17.47	0.9531	31.47	0.9562	54.37 ± 11.10	60.52 ± 18.52	14.37 ± 3.33	20.28 ± 4.90	0.70
AZA*	265.41	0.9954	248.54	0.9714	–	–	–	–	273.61 ± 76.86	229.08 ± 55.14	–	–	–
TAC$^{\Psi}$	–	–	–	–	92.20	0.9796	105.16	0.9711	–	–	56.37 ± 15.10	63.40 ± 13.62	0.88

* Acetazolamide (AZA) was used as a standard inhibitor for both carbonic anhydrases I, and II (hCA I and II) isoenzymes.
$^{\Psi}$ Tacrine (TAC) was used as a standard inhibitor for both acetylcholinesterase (AChE), and butyrylcholinesterase (BChE) enzymes.

experiments with other catalysts (Table 1, entry 3–7), product formation was not observed. A single product was formed in the experiments with sodium at room temperature in DCM. This compound was detected by TLC and purified by crystallization (Table 1). The bis-chalcone based sulfides were synthesized by reacting bis-chalcones with thiophenol in the presence of sodium-metal in dichloromethane at room temperature via thio-Michael addition reaction (Scheme 3). Spectrometric data (^{1}H NMR, ^{13}C NMR, FTIR and elemental analysis) and physical properties (color and melting point) of these products have been confirmed by comparison with similar studies reported in the literature [44,45].

2.2. Enzyme inhibition studies

Evaluation of the effects of novel bis-sulfide derivatives (**3a–j**) on both cholinergic enzymes of AChE and BChE, and both hCA isoenzymes was the main objective of this study. The inhibition results are summarised in Table 2 and related Figs. 1 and 2. The cytosolic hCA I, and II isoenzymes are present in the human body and are involved in the secretion of electrolytes in a plenty of tissue cells, like the HCO_3^- rich aqueous humor in the anterior chamber of the cerebrospinal fluid or the eyes, as well as hold CO_2 homeostasis and pH all the body [46,47]. Indeed, dysregulation of hCA I, and II isoenzymes in tissue cells lead to some pathologic conditions including edema, glaucoma and epilepsy [48,49]. For hCA I isozyme, the Ki values were found in range of 39.14 ± 9.94–183.23 ± 28.18 nM. In this study, the Ki for the positive control CA inhibitor acetazolamide (AZA), a recorded hCA I inhibitor, was 273.61 ± 76.86 nM against hCA I isoenzyme (Table 2). All novel bis-sulfide compounds (**3a–j**) had effective inhibition effects against hCA I isoenzyme. In addition, between these novel molecules, 2,6-bis((4-chlorophenyl)(phenylthio)methyl)cyclohexanone (**3h**) and 2,6-bis((3-bromophenyl)(phenylthio)methyl)cyclohexanone (**3i**), which possessed chlorophenyl and bromophenyl groups demonstrated the best hCA I inhibitor (K_is of 39.14 ± 9.94 and 47.92 ± 7.20 nM, respectively) (Fig. 1). It is well known that the molecules comprising chlorophenyl and bromophenyl groups are efficient CA inhibitors [50]. As seen in Table 2, IC_{50} values were found in the range of 38.63–180.34 nM towards hCA I, and 42.84–201.58 nM for hCA II. Novel bis-sulfide derivatives (**3a–j**) synthesized in this study significantly inhibited the slow cytosolic hCA II isozyme with K_i in the low nanomolar levels. Ki values were obtained between 46.03 ± 10.47 and 194.02 ± 28.55 nM (Table 2). Also, the compounds of **3h** and **3i** are, in fact, the best inhibitor among these molecules (Ki 46.03 ± 10.47 and 48.18 ± 19.10 nM, respectively) compared to the AZA, which is known as specific inhibitor of CA isoenzymes as a standard (Ki: 229.08 ± 55.14 nM). For hCA I isoform, its excellent inhibitors were (**3h**) and (**3i**), which were the best hCA I inhibitor with IC_{50}: 38.63 nM (r^2: 0.9812) and IC_{50}: 44.28 nM (r^2: 0.9590), respectively. 2,6-Bis((3-nitrophenyl)(phenylthio)methyl) cyclohexanone (**3c**) compound had relatively weak inhibition effects when compared to other compounds for this isoenzyme IC_{50}: 180.34 nM (r^2: 0.9598) for hCA I, IC_{50}: 201.58 nM (r^2: 0.9902) for hCA II isoenzyme.

In this work, AChE and BChE were also extremely inhibited by novel

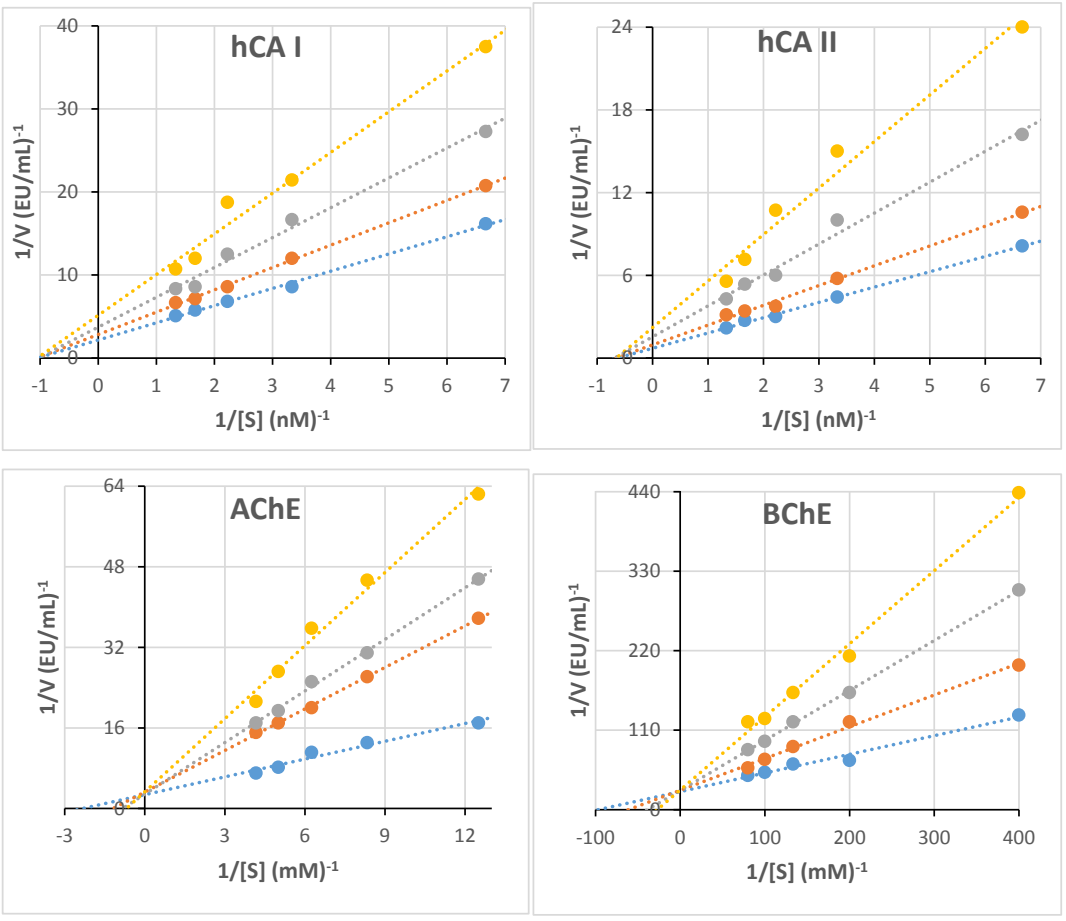


Fig. 1. Determination of inhibition constant values (Ki) of novel bis-sulfide compounds (**3a–j**) obtained from Lineweaver-Burk graphs against human carbonic anhydrases isoenzyme I and II (hCA I and II) (**3i**), and acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes (**3e**).

bis-sulfide compounds (**3a–j**) at the nanomolar range inhibition. Ki values of this novel compounds were found in the range of 4.55 ± 0.94–32.64 ± 6.86 nM for AChE and 12.77 ± 2.20 –37.38 ± 11.53 nM for AChE (Table 2). This inhibition results clearly designated that novel synthesized compounds had efficient both cholinergic enzymes inhibition properties. Indeed, the most potent AChE and BChE inhibition were obtained by novel compound 2,6-bis((3-fluorophenyl)(phenylthio)methyl)cyclohexanone (**3e**) with Ki values of 4.55 ± 0.94 and 12.77 ± 2.20 nM, respectively (Fig. 1). Also, tacrine (1,2,3,4-tetrahydroacridin-9-amine), which the first centrally acting cholinesterase inhibitor approved for the treatment of AD, possessed Ki values of 56.37 ± 15.10 and 63.40 ± 13.62 nM against both cholinergic AChE and BChE enzymes, respectively. Eventually, the K_i values of these derivatives for AChE and BChE were calculated from Lineweaver-Burk plots. Additionally, as seen in Table 2, IC_{50} values are in the range of 7.18–41.77 nM towards AChE and in the range of 17.90–61.38 nM for BChE. 2,6-Bis((4-nitrophenyl)(phenylthio)methyl) cyclohexanone (**3d**) is weak inhibitor compared to other compounds for these enzymes. IC_{50} were found as 41.77 nM (r^2:0.9732) for AChE and 61.38 nM for BChE (r^2:0.9682).

CA inhibitors targeting hCAs are clinically used in recent years for the management of diverse diseases among which obesity, glaucoma, intracranial hypertension epilepsy, and as diuretics [51]. Recently, they started to be utilized for the therapy of hypoxic tumours, were also evaluated as possible drugs for cerebral ischemia, neuropathic pain, and arthritis. Additionally, plenty of work has been done on the synthesis and characterization of CAIs belonging to diverse groups, such as coumarins, carboxylic acids, sulfonamides, phenols, dithiocarbamates, heterocyclic derivatives, etc [52]. Indeed, sulfonamides and their bioisosteres such as the sulfamides and the sulfamates are powerful active site coordinating CAIs, which in deprotonated form, bind to the Zn^{2+} ion present within the active site of enzyme [53]. Many sulfonamide-based drugs, such as methazolamide, acetazolamide, dorzolamide, ethoxzolamide, brinzolamide and celecoxib are recorded. Some of them are in clinical use as antiepileptics (targeting hCAs VII and XIV), diuretics (targeting hCAs II, IV, XII and XIV), antiglaucoma (targeting hCAs II, IV and XII), or in clinical trials as antitumor or antimetastatic agents (targeting hCAs IX and XII) [54,55].

Many researches have recorded that by raising ACh level through inhibiting AChE and BChE enzymes in the body offer a potential treatment for therapy AD and hence improve both memory and mental symptoms in that disease [56]. Control AChEIs such as galantamine, donepezil, tacrine, and rivastigmine are involved in improving symptoms for most patients by enhancing cholinergic neurotransmission range in the human body [57]. Thus, these inhibitors are expensive as well as they are long-term utilize give rise to diverse harmful side effects and adverse symptoms. Recently, naturally occurring BChE and AChE inhibitors have been isolated from the animals and plants and

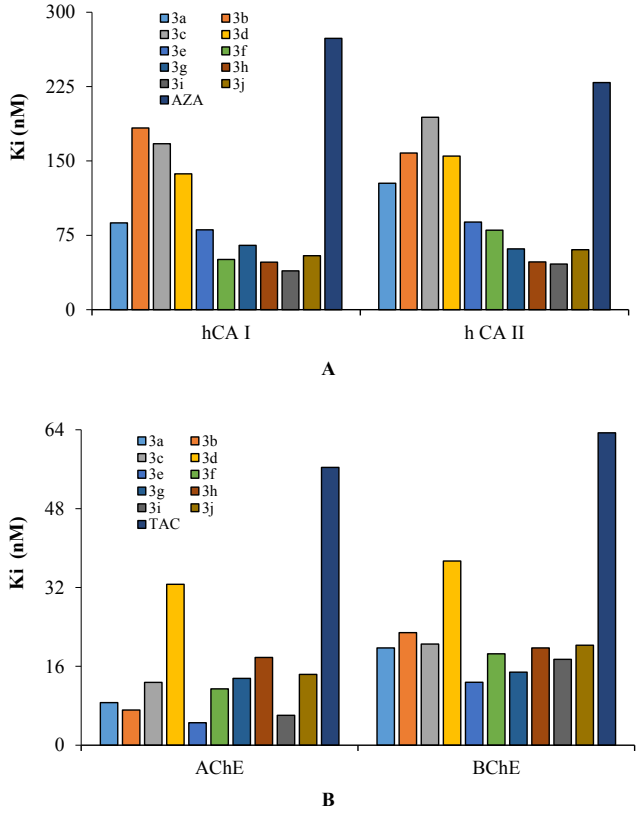


Fig. 2. Inhibition constant values (Ki) of novel bis-sulfide compounds (**3a–j**) for human carbonic anhydrases isoenzyme I, and II (hCA I, and II) (**A**) and acetylcholinesterase (AChE) and butrylcholinesterase (BChE) enzymes (**B**).

utilized as the natural treatment for the AD [58–60]. In this study, novel bis-sulfide compounds (**3a-j**) have been reported as potent both cholinesterase enzymes inhibitors.

2.3. Crystallographic studies

Thermal ellipsoid views of the **3h, 3i** and **3j** are shown in Fig. 3. Selected bond lengths and angles are illustrated in Table 3, mostly consistent with the similar molecules in the literature [61–64].

As depicted in Fig. 3, the asymmetric unit of the molecule **3h** has one-half-molecule and it is completed with a twofold symmetry axis [symmetry code: x, 1 − y, z]. Compound **3h** crystallizes in a monoclinic system with space group *lm* (Table 4). The dihedral angle between the phenylthio ring and cyclohexanone ring is 60.0 (3)° while the phenylthio rings are inclined to chlorophenyl ring by 34.8 (3)°. The cyclohexanone ring is nearly planar to the molecule plane making dihedral angle is 10.4 (2)°. In the crystal structure, there is only weak intramolecular interaction (Table 5). The packing diagram of the molecule is formed by stacking interactions along the *a* axis (Fig. 4).

Compound **3i** crystallizes in a triclinic system with space group *P* − 1 (Table 4). The dihedral angle between the phenylthio rings and bromophenyl rings are 25.2(4)° (left groups), 52.8(3)° (right groups). Phenylthio ring (C1/C6) is nearly perpendicular to molecule plane with 87.1(3)°. The dihedral angle between bromophenyl ring (C21/C26) and cyclohexanone ring is 87.3(3)° while the another ring (C8/C13) are inclined to cyclohexanone ring by 57.8 (4)°. In addition, phenylthio rings are nearly perpendicular cyclohexanone ring with 82.8(4)° and 77.2(4)°. In the crystal structure, molecules are linked by strong intermolecular C—H···S hydrogen bond to form an infinite chain along the *b* axis. This hydrogen bond also generates $R_2^2(10)$ ring motif with dimeric structure (Fig. 4) [64]. Moreover, there is a strong C—H···pi interaction between the C25 atom of the bromophenyl ring and another bromophenyl ring [Cg: C8/C9/C10/C11/C12/C13; C25–Cg 3.374(9) Å, H25···Cg 2.803(9) Å, C25–H25···Cg 120.7(8)°, symmetry code: 1 − x, 1 − y, 1 − z].

Compound **3j** crystallizes in a monoclinic system with lm space group (Table 4). The molecule has crystallographic mirror symmetry with x, 1 − y, z symmetry operator. The dihedral angle between the phenylthio ring and cyclohexanone ring is 57.6 (2)° while the phenylthio rings are inclined to bromophenyl ring by 33.3 (2)°. The cyclohexanone ring is nearly planar to the molecule plane making dihedral angle is 9.36 (14)°. The packing structure of the **3j** is stabilised by stacking interactions along the *b* axis (Fig. 4).

The expected absolute configuration for molecule **3h** and **3j** were confirmed by refinement of the Flack parameters (its value 0.01(11) A° for **3 h**, −0.027(9) A° for **3j**). Details of the molecular geometry having two stereogenic centers which is C5 atoms in molecule **3h** and **3j**, C7 and C20 atoms in molecule **3i** reveal that there are two enantiomeric forms (*R* and *S*) named with respect to the majority of chiral centers in the crystal structure of the compounds.

To examine the chemical details of enzymes function, the first step is to determine what functional groups are required for enzyme activity and to ascertain these functional groups [65]. In this study, X-ray crystallography provides an alternative and often-complementary means for elucidating functional groups at the enzyme active site. According to enzyme inhibition studies, comprising chlorophenyl and bromophenyl groups in **3h** and **3i** respectively are efficient both CA isoenzymes inhibitors. The X-ray crystallography reveals that the electron density of these groups in compounds less than similar groups in molecule **3j**. Therefore, the enzymes inhibition effects can be directly related to electron density of binding site of molecules [66].

3. Conclusion

The novel bis-thiomethylcyclohexanone compounds were investigated for AChE, BChE, hCA I, and hCA II enzymes inhibition effects. As we explained above, novel bis-sulfide compounds (**3a–j**) can be good candidate drugs, the same as AChE, BChE, and carbonic anhydrase inhibitor compounds, for therapy of some diseases like AD, epilepsy, glaucoma, gastric and duodenal ulcers, mountain sickness, osteoporosis or neurological disorders.

4. Experimentals

4.1. General

All chemicals were obtained from Sigma-Aldrich, Fluka, Merck or Alfa Aesar and were used without further purification. Melting points were determined on a Gallenkamp melting point apparatus. FTIR spectra were recorded on Bruker Tensor27 FTIR spectrometer using the KBr disc in the range of 4000–400 cm^{-1}. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian 200 MHz or Bruker Avance DPX 400 MHz spectrometer using $CDCl_3$ as a solvent. Splitting patterns are designated as follows; s, singlet; d, doublet; m, multiplet. Chemical shift (δ) values are given in ppm. Elemental analysis was done on a LECO CHNS-932 instrument.

4.2. Chemistry

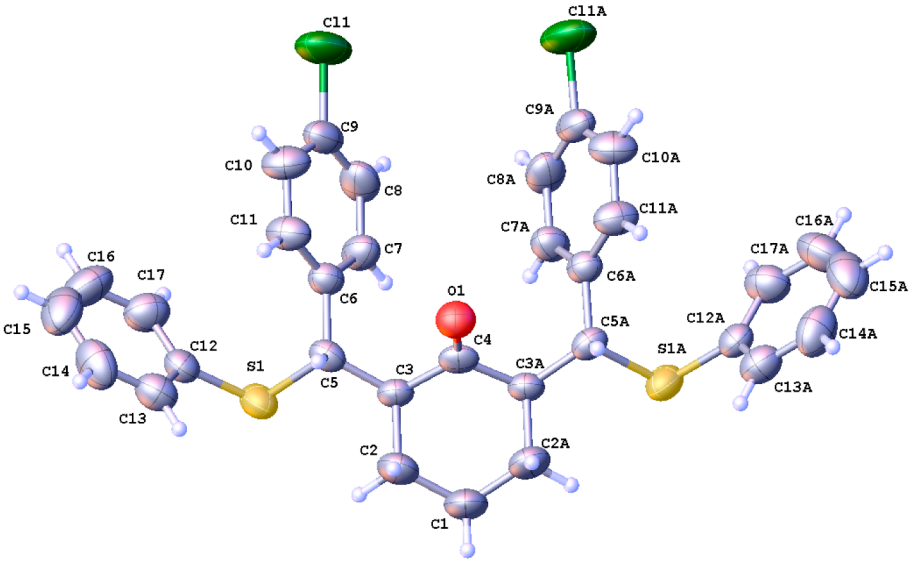
Bis-chalcones (**2a–j**) were synthesized following literature procedures by the condensation of cyclohexanone with aromatic aldehydes (**1a–j**) [42,43].

4.2.1. General procedure for synthesis of bis-thiomethylcyclohexanone derivatives (*3a–j*)

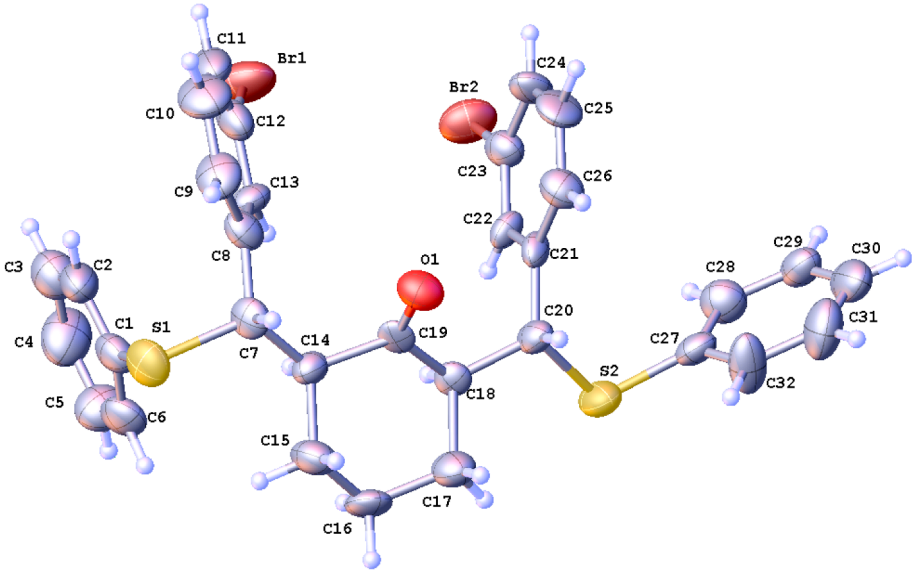
Bis-chalcone (1 eq.) was dissolved in 20 mL of DCM and Na (catalitic amount) added. After five minutes shaking in DCM, to reaction mixture was slowly added thiophenol (2.4 eq.) and the reaction was stirred at room temperature overnight. After completion of reaction, the mixture evaporated in vacuo. Pure bis-sulfide derivatives were obtained by recrystallization from methanol.

*2,6-Bis(phenyl(phenylthio)methyl)cyclohexanone (**3a**)*. Yield 62%; White solid; mp: 168–169 °C; FT-IR (KBr, cm^{-1}) ν: 3040–3025 (arom. C—H stretch.), 2958–2861 (alif. C—H stretch.), 1724 (C=O stretch.), 1622 (C=C stretch.), 780–700 (C—S stretch.); ^{1}H NMR(400 MHz, $CDCl_3$, δ, ppm): 7.19–7.06 (m, 20H), 4.67 (d, J = 7.6 Hz, 2H, CH—S), 2.89 (m, 2H, CH—C=O), 2.65 (m, 2H), 2.10 (m, 2H), 1.70–1.68 (m, 2H); ^{13}C NMR(100 MHz, $CDCl_3$, δ, ppm): 208.6, 142.1, 134.9, 132.6, 128.8, 128.2, 128.1, 127.2, 126.8, 57.5, 52.9, 33.1, 25.6; Elemental Analysis Calculated: C, 77.73; H, 6.07; S, 12.96. Found: C, 77.64; H, 6.12; S, 12.83.

*2,6-Bis(naphthalen-2-yl(phenylthio)methyl)cyclohexanone (**3b**)*. Yield 43%; White solid; mp 166–167 °C; FT-IR (KBr, cm^{-1}) ν: 3051(arom.

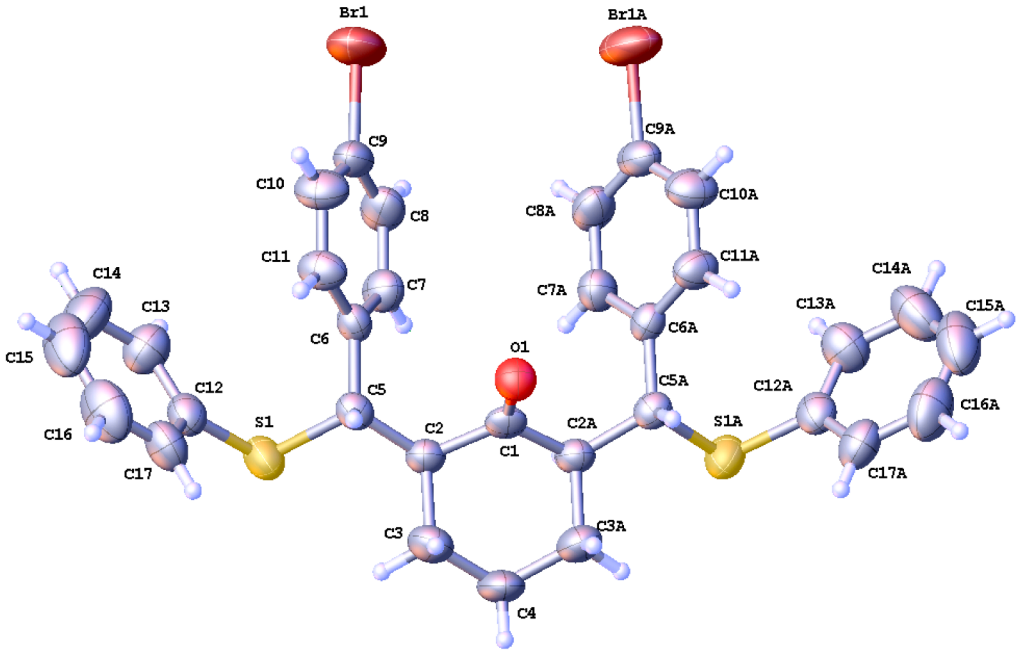


3h



3i

Fig. 3. Thermal ellipsoid view of the molecules with the atom numbering scheme and 75% probability.



3j

Fig. 3. (*continued*)

Table 3
Selected experimental parameters of the novel bis-sulfide compounds (**3h, 3i** and **3j**).

Bond lengths (Å)					
3h		**3i**		**3j**	
O1-C4	1.214(7)	O1-C19	1.206(8)	Br1-C9	1.899(5)
Cl1-C9	1.735(5)	S2-C20	1.861(8)	O1-C1	1.221(8)
S1-C5	1.852(4)	S2-C27	1.782(9)	S1-C5	1.857(5)
S1-C12	1.778(5)	Br2-C23	1.864(8)	S1-C12	1.771(5)
		Br1-C12	1.912(9)		
		S1-C1	1.770(9)		
		S1-C7	1.834(8)		
Bond angles (°)					
3 h		**3i**		**3j**	
C5-S1-C12	101.4(2)	C1-S1-C7	101.1(4)	C12-S1-C5	101.6(2)
O1-C4-C3	123.6(2)	C20-S2-C27	102.3(4)	S1-C5-*C2*	106.1(3)
Cl1-C9-C8	120.0(5)	O1-C19-C18	123.2(8)	O1-C1-*C2*	122.7(3)
		Br1-C12-C13	117.3(7)	Br1-C9-C8	120.4(4)
		Br2-C23-C24	119.8(6)		

C−H stretch.), 2923–2854 (alif. C−H stretch.), 1707 (C═O stretch.), 1630 (C═C stretch.), 742–692 (C−S stretch.); ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.63–7.00 (m, 24H), 4.79 (d, *J* = 7.6 Hz, 2H, CH−S), 3.00–2.97 (m, 2H, CH−C═O), 2.73–2.60 (m, 2H), 2.07–2.05 (m, 2H), 1.76–1.72 (m, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm): 208.3, 139.5, 134.9, 133.1, 132.5, 128.8, 128.0, 127.9, 127.6, 127.2, 126.8, 126.4, 126.0, 125.6, 57.6, 53.0, 33.2, 25.6; Elemental Analysis Calculated: C, 80.80; H, 5.72; S, 10.77. Found: C, 80.83; H, 5.80; S, 10.68.

2,6-Bis((3-nitrophenyl)(phenylthio)methyl)cyclohexanone ***(3c)***. Yield 36%; white solid; mp 138–139 °C; FT-IR (KBr, cm^{-1}) *ν*: 3081 (arom. C−H), 2943–2895 (alif. C−H), 1710 (C═O), 1534 (C═C), 1450 (NO_2 *asym.*), 1350 (NO_2 *sym.*), 732–687 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.78 (d, *J* = 8.0 Hz, 2H), 7.69 (s, 2H), 7.23 (d, *J* = 8.0 Hz, 2H), 7.20–7.00 (m, 12H,), 4.44 (d, *J* = 9.6 Hz, 2H, CH−S), 3.00–2.97 (m, 2H), 2.90–2.87 (m, 2H), 2.15–2.00 (m, 2H), 1.91–1.88 (m, 1H), 1.70–1.60 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm): 209.3, 147.8, 144.6, 134.3, 133.8, 132.6, 129.2, 128.7, 128.4, 122.5, 121.7, 57.3, 52.7, 35.1, 25.5; Elemental Analysis Calculated: C, 65.75; H, 4.79; S, 10.96. Found: C, 65.82; H, 4.90; S, 10.95.

2,6-Bis((4-nitrophenyl)(phenylthio)methyl)cyclohexanone ***(3d)***. Yield 44%; White solid; mp 162–164 °C; FT-IR (KBr, cm^{-1}) *ν*: 3083–3075 (arom. C−H), 2924–2857- (alif. C−H), 1711 (C═O), 1599 (C═C), 1519 (NO_2 *asym.*), 1347 (NO_2 *sym.*), 749–694 (C−S); ^{1}H NMR(400 MHz, $CDCl_3$, *δ*, ppm): 7.86 (d, *J* = 8.4 Hz, 4H), 7.31–7.05 (m, 14H), 4.47 (d, *J* = 8.4 Hz, 2H, CH−S), 2.98–2.92 (m, 2H, CH−C═O), 2.81–2.79 (m, 2H), 2.15–2.12 (m, 2H), 1.86–1.71 (m, 2H); ^{13}C NMR(100 MHz, $CDCl_3$, *δ*, ppm): 209.2, 149.8, 146.0, 133.7, 132.6, 129.2, 128.8, 128.4, 123.3, 57.0, 53.0, 34.1, 25.5; Elemental Analysis Calculated: C, 65.75; H, 4.79; S, 10.96. Found: C, 65.82; H, 4.92; S, 11.00.

2,6-Bis((3-fluorophenyl)(phenylthio)methyl)cyclohexanone ***(3e)***. Yield 40%; White solid; mp 168 °C; FT-IR (KBr, cm^{-1}) *ν*: 3056–3035 (arom. C−H), 2924–2855 (alif. C−H), 1697 (C═O), 1590 (C═C), 1146 (Ar-F), 763–690 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, *δ*, ppm): 7.16 (m, 10*H*), 6.99 (m, 2H), 6.77 (m, 2H), 6.75 (m, 2H), 6.73 (m, 2H), 4.50 (d, *J* = 8.0 Hz, 2H, CH−S), 2.93–2.86 (m, 2H, CH−C═O), 2.73–2.70 (m, 2H), 2.08–2.04 (m, 1H), 1.75–1.63 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, *δ*, ppm): 208.6, 163.9, 145.0, 144.7, 133.1, 129.4, 128.9, 127.7, 123.8, 114.9, 113.7, 57.4, 52.7, 33.9, 25.5; Elemental Analysis Calculated: C, 72,45; H, 5.28; S, 12.08. Found: C, 72.60; H, 5.43; S, 12.20.

Table 4
Crystal data and structure refinement parameters for the novel bis-sulfide compounds (**3h, 3i** and **3j**).

Crystal data	3h	3i	3j
Empirical formula	$C_{32}H_{28}Cl_2OS_2$	$C_{32}H_{28}Br_2OS_2$	$C_{32}H_{28}Br_2OS_2$
Formula weight	563.56	652.48	652.48
Temperature (K)	293(2)	292(2)	294(2)
Crystal system	monoclinic	triclinic	monoclinic
Space group	lm	*P*-1	lm
*Unit cell dimensionsa (*Å)	5.1312(5)	10.399(2)	5.0984(4)
*b (*Å)	25.692(2)	10.431(3)	25.8212(19)
*c (*Å)	10.9554(11)	14.373(4)	11.2384(6)
α (°)	90	108.01(2)	90
β (°)	97.941(9)	101.662(19)	98.313(6)
γ *(°)*	90	97.05(2)	90
Volume (Å^3)	1430.4(2)	1423.2(6)	1463.94(18)
Z	2	2	2
D_{calc} (g/cm^{-3})	1.308	1.523	1.480
Absorption coefficient (mm^{-1})	0.397	3.019	2.935
F (0 0 0)	588	660.0	660.0
Crystal size (mm)	0.659 × 0.395 × 0.053	0.335 × 0.242 × 0.166	0.241 × 0.211 × 0.092
Index ranges	$-6 \le h \le 3$, $-31 \le k \le 27$, $-13 \le l \le 13$	$-12 \le h \le 12$, $-12 \le k \le 12$, $-17 \le l \le 17$	$-6 \le h \le 6$, $-31 \le k \le 31$, $-8 \le l \le 13$
Reflections collected/unique	2733/1791	7467/7467	4554/2187
Data/restrains/parameters	1791/2/172	7467/12/335	2187/2/172
Goodness of fit on F^2	1.029	0.848	1.023
Final *R* indices [$I > 2\sigma(I)$]	$R_1 = 0.0465$, $wR_2 = 0.1151$	$R_1 = 0.0543$, $wR_2 = 0.1462$	$R_1 = 0.0340$, $wR_2 = 0.0603$
R indices (all data)	$R_1 = 0.0523 wR_2 = 0.1226$	$R_1 = 0.1367 wR_2 = 0.1704$	$R_1 = 0.0616 wR_2 = 0.0685$
Largest difference peak and hole (e Å^{-3})	0.23/−0.19	0.39/−0.39	0.24/−0.28

I:1-x, y, z.

Table 5
Interactions geometry (Å,°) for the novel bis-sulfide compounds (**3h**, **3i** and **3j**).

Compounds	D–H A	D–H	H···A	D···A	D–H···A
3h	*C*2-H2B…S1	0.97	2.74	3.1821(5)	108
3i	C15-H15B…S1	0.970(11)	2.719(8)	3.178(8)	109.5(7)
	C17-H17A…O1^{i}	0.969(12)	2.592(10)	3.544(10)	167.4(8)
	C17-H17B…S2	0.971(12)	2.683(8)	3.103(8)	106.6(7)
	C25-H25…Cg2	0.932(10)	2.803(9)	3.374(9)	120.7(8)
3j	C3-H3B…S1	0.970(6)	2.757(5)	3.192(4)	108.0(4)

2,6-Bis((4-fluorophenyl)(phenylthio)methyl)cyclohexanone ***(3f)***. Yield 38%; White solid; mp 144–145 °C; FT-IR (KBr, cm^{-1}) ν: 3068–3054 (arom. C−H), 2956–2857 (alif. C−H), 1706 (C═O), 1603 (C═C), 1226 (Ar-F), 750–690 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.19–7.09 (m, 10*H*), 6.97 (m, 4H), 6.72 (t, *J* = 8.4 Hz, 4H), 4.46 (d, *J* = 8.8 Hz, 2H, CH−S), 2.90–2.83 (m, 2H, CH−C═O), 2.73–2.70 (m, 2H), 2.07–2.03 (m, 1H), 1.75–1.60 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 208.4, 160.4, 137.9, 133.1, 129.6, 128.9, 127.6, 115.0, 114.8, 57.8, 52.3, 34.2, 25.6; Elemental Analysis Calculated: C, 72,45; H, 5.28; S, 12.08. Found: C, 72.53; H, 5.42; S, 11.95.

2,6-Bis((3-chlorophenyl)(phenylthio)methyl)cyclohexanone ***(3g)***. Yield 35%; White solid; mp 115–116 °C; FT-IR (KBr, cm^{-1}) ν: 3064–3058 (arom. C−H), 2948–2857 (alif. C−H), 1709 (C═O), 1570 (C═C), 1072 (Ar-Cl), 745–688 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.20–7.10 (m, 10*H*), 7.06 (m, 2H), 7.02 (m, 2H), 6.98 (m, 2H), 6.88 (m, 2H), 4.48 (d, *J* = 8.0 Hz, 2H, CH−S), 2.91–2.85 (m, 2H, CH−C═O), 2.71–2.68 (m, 2H), 2.08–2.04 (m, 1H), 1.77–1.62 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 208.5, 144.2, 134.0, 133.1, 129.3, 129.0, 128.1, 127.7, 127.1, 126.3, 57.3, 52.7, 33.7, 25.5; Elemental Analysis Calculated: C, 68.21; H, 4.97; S, 11.37. Found: C, 68.25; H, 5.12; S, 11.29.

2,6-Bis((4-chlorophenyl)(phenylthio)methyl)cyclohexanone ***(3h)***. Yield 52%; White solid; decomposition after 240 °C; FT-IR (KBr, cm^{-1}) ν: 3068–3054 (arom. C−H), 2926–2857 (alif. C−H), 1704 (C═O), 1600 (C═C), 1091 (Ar-Cl), 746–688 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.15 (m, 10*H*), 7.02 (d, *J* = 8.8 Hz, 4H), 6.93 (d, *J* = 8.4 Hz, 4H), 4.46 (d, *J* = 8.4 Hz, 2H, CH−S), 2.90–2.83 (m, 2H, CH−C═O), 2.74–2.70 (m, 2H), 2.08–2.03 (m, 1H), 1.74–1.61 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 209.1, 140.7, 134.0, 133.1, 132.5, 129.3, 129.0, 128.2, 127.7, 57.7, 52.4, 34.2, 25.6; Elemental Analysis Calculated: C, 68.21; H, 4.97; S, 11.37. Found: C, 68.12; H, 5.13; S, 11.22.

2,6-Bis((3-bromophenyl)(phenylthio)methyl)cyclohexanone ***(3i)***. Yield 36%; White solid; mp 120–121 °C; FT-IR (KBr, cm^{-1}) ν: 3062–3058 (arom. C−H), 2917–2856 (alif. C−H), 1709 (C═O), 1566 (C═C), 1069 (Ar-Br), 747–688 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.26–6.91 (m, 18H), 4.49 (d, *J* = 8.4 Hz, 2H, CH−S), 2.91–2.85 (m, 2H, CH−C═O), 2.71–2.68 (m, 2H), 2.07–2.00 (m, 1H), 1.74–1.62 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 208.5, 144.5, 134.0, 133.1, 131.0, 130.0, 129.7, 129.0, 127.8, 126.8, 122.3, 57.3, 52.7, 33.7, 25.5; Elemental Analysis Calculated: C, 58.90; H, 4.29; S, 9.82. Found: C, 58.84; H, 4.33; S, 9.96.

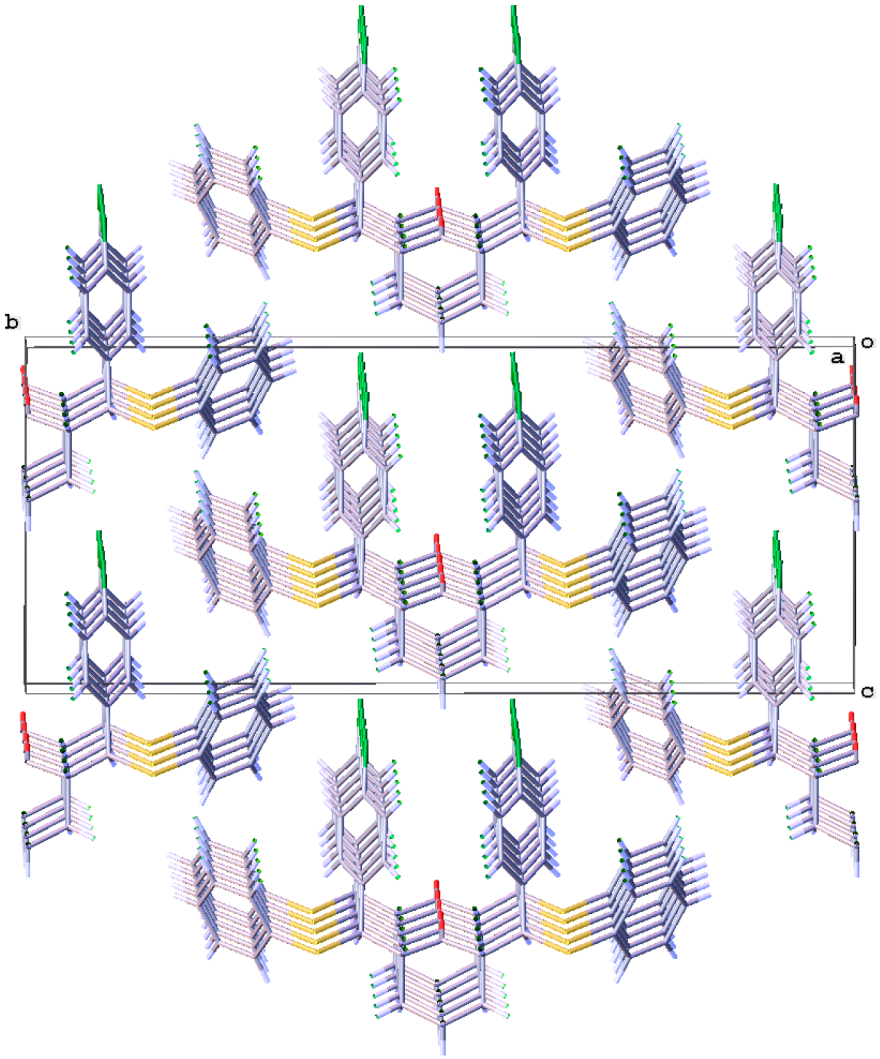
2,6-Bis((4-bromophenyl)(phenylthio)methyl)cyclohexanone ***(3j)***. Yield 48%; White solid; mp 166–167 °C; FT-IR (KBr, cm^{-1}) ν: 3066–3052 (arom. C−H), 2937–2853 (alif. C−H), 1706 (C═O), 1577 (C═C), 1068 (Ar-Br), 751–688 (C−S); ^{1}H NMR (400 MHz, $CDCl_3$, δ, ppm): 7.18–6.89 (m, 14H), 6.87 (d, *J* = 8.4 Hz, 4H), 4.44 (d, *J* = 8.8 Hz, 2H, CH−S), 2.89–2.82 (m, 2H, CH−C═O), 2.74–2.70 (m, 2H), 2.07–2.00 (m, 1H), 1.77–1.60 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ, ppm): 209.1, 141.2, 133.9, 133.1, 131.2, 129.7, 129.0, 127.7, 120.6, 57.6, 52.4, 34.2, 25.6; Elemental Analysis Calculated: C, 58.90; H, 4.29 S, 9.82. Found: C, 58.86; H, 4.42; S, 9.76.

4.3. X-ray crystallography

Single crystal X-ray diffraction data of **3h, 3i** and **3j** were collected at room temperature on an Rigaku-Oxford Xcalibur diffractometer with an Eos-CCD detector, operated at 50 kV and 40 mA using graphite monochromated Mo-Kα (λ = 0.71073 Å) radiation. Utilising CrysAlis^{Pro} software package, data collections and reductions along with absorption corrections were performed [67]. Structure solutions were performed using SHELXT [68] embedded in the Olex2 [69]. Refinement of coordinates and anisotropic thermal parameters of non-hydrogen atoms were carried out by the full-matrix least-squares method in SHELXL [70]. For the compounds, all hydrogen atoms were placed in geometrically idealized positions (C—H = 0.97 A° for methylene groups and C—H = 0.93 A° for aromatic groups). Detailed crystallographic data and refinement results are summarized in Table 4.

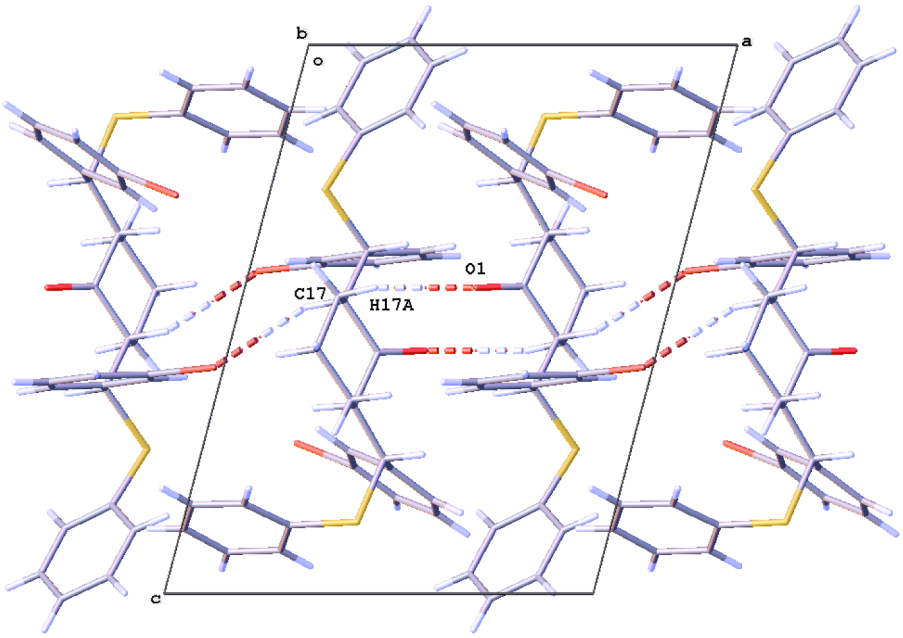
4.4. Biochemical studies

Both hCA isoenzymes inhibition effects of novel bis-sulfide compounds (**3a–j**) was measured according to Verpoorte et al. [71] conforming to previous studies [72–76] and recorded at 348 nm spectrophotometrically using p-nitrophenylacetate substrate (PNA) [77]. On the other hand, AChE and BChE inhibitory effects of novel bis-sulfide compounds (**3a–j**) were determined according to procedure of Ellman et al. [78] conforming to previous studies [79–84] and recorded at 412 nm spectrophotometrically using acetylthiocholine iodide and butyrylthiocholine iodide as substrates for the enzymatic reaction. 5,5′-Dithio-bis(2-nitro-benzoic) acid compound was used for the measurement of the AChE and BChE activities, respectively [85].

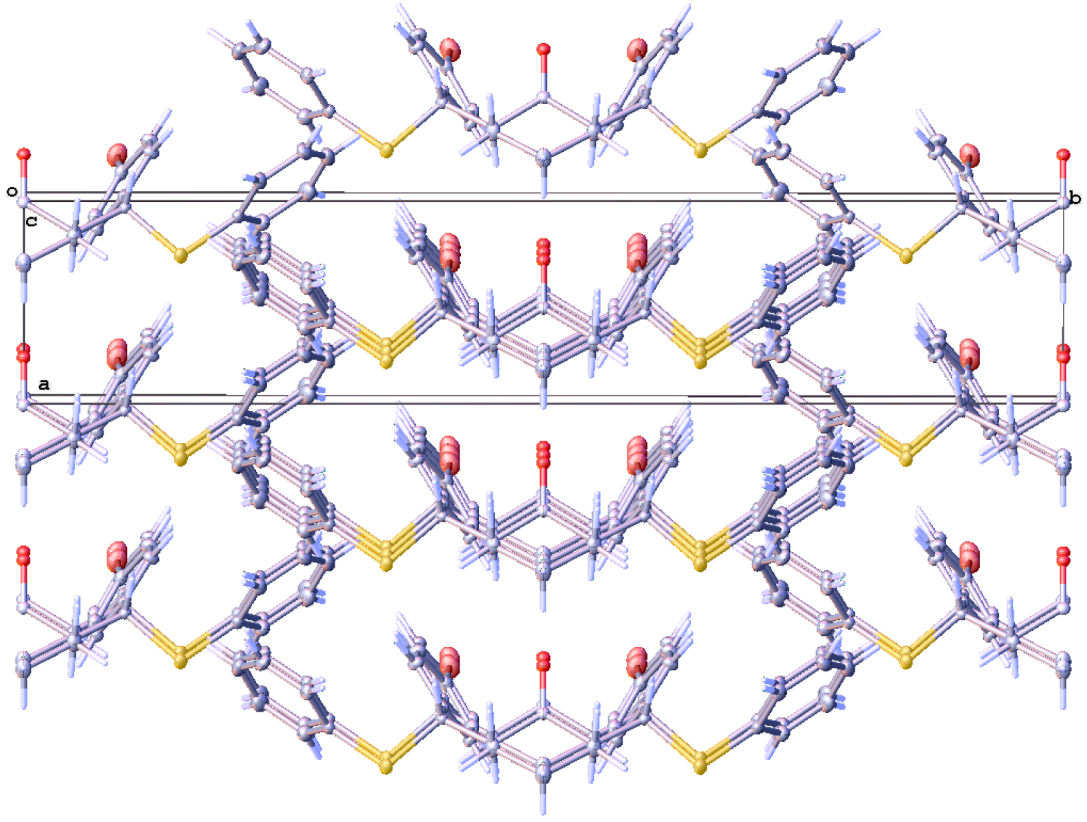


3h

Fig. 4. Packing structure of the novel bis-sulfide compound of **3h** via stacking interactions along the *a* axis, **(3i)** via strong C—H…O interactions along the *b* axis, **(3j)** via stacking interactions along the *b* axis.



3i



3j

Fig. 4. (*continued*)

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Appendix A. Supplementary material

Crystallographic data as .cif files for the structures reported in this paper have been deposited at the Cambridge Crystallographic Data Center with CCDC 1865288, 1865295 and 1865296 for the molecule **3h**, **3i** and **3j** respectively. Copies of the data can be obtained free of charge at www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Center, 12, Union Road, Cambridge CB2 1EZ, UK). Email: deposit@ccdc.cam.ac.uk. Supplementary data to this article can be found online at https://doi.org/10.1016/j.bioorg.2018.11.001.

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