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New supramolecules of bis(acylhydrazones)-linked bisphenol sulfide for Alzheimer's: targeting cholinesterases by *in vitro* and *in silico* approaches†

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In current research, two functional components, *i.e.*, hydrazone and bisphenol sulfide were combined to get useful supramolecules in medicinal chemistry. Herein **25** new 4,4′-thiodiphenol bis-acylhydrazones were synthesized in good to excellent yields. Initially ethyl-2-chloroacetate was reacted with 4,4′-thiodiphenol, which was further reacted with excess hydrazine hydrate to produce 2,2′-((thiobis(4,1-phenylene))bis(oxy))di(acetohydrazide), which was then combined with various aromatic and aliphatic aldehydes to get the desired products (hydrazones, **4a–4y**). The synthesized supramolecules were characterized by contemporary spectroscopic techniques such as ^{1}H NMR, ^{13}C NMR, and mass spectroscopy. The synthetic compound's cholinesterase blocking activity was tested against acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes where compounds **4n**, and **4h** showed excellent inhibitory potential for AChE, while **4b**, and **4h**, demonstrated most potent inhibition of BChE. The starting compound (**SM3**) and compounds **4h** and **SM3** depicted excellent dual inhibitory capabilities for both enzymes. The chemical basis of anticholinesterase activity was investigated using a structure-based molecular docking approach. The biological significance and the ease of synthesis of this class of compounds should be considered in therapeutic development for Alzheimer's disease treatments.

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1 Introduction

Alzheimer's disease is a degenerative neurological condition that affects cognition and behavior and makes everyday tasks difficult. The loss of cholinergic neurons is the primary reason for these alterations. In the end, it causes intellectual changes, memory loss, and social impairment.[1] The two main enzymes involved in the beginning of Alzheimer's disease are acetyl (AChE) and butyrylcholinesterase (BChE). Acetylcholinesterase (AChE) hydrolyzes acetylcholine, a neurotransmitter present in cholinergic synapses, while BChE co-regulates the activity of AChE. Cholinesterase inhibitors work against the actions of these enzyme to increase the amount of acetylcholine required for the neurotransmission process.[2] Cholinesterase inhibitors are among the most effective Alzheimer's disease treatments available right now. By suppressing AChE, certain medications treat Alzheimer's effectively. The current medications only produce symptomatic effects, therefore finding new cholinesterase inhibitors with more potency and less side effects is urgently needed to combat the effects of Alzheimer's.

The sulfide group and the Schiff's bases (hydrazide-hydrazones) are two important pharmacophores that have drawn medicinal chemists to create novel medications.[3] The hydrazones of aromatic carbonyl (aldehydes and ketones) compounds, have a strong conjugated system, excellent stability,[4] and are significant intermediates in the field of organic chemistry.[5] The unusual biological potential of Schiff's bases, including antibacterial[7] anti-inflammatory,[10] anti-tumor,[9] anticancer,[6] analgesic[11] and antifungal[8] properties, has received a lot of attention recently. Researchers are attracted to the synthesis of hydrazide-hydrazone derivatives, because of intriguing biological activities of this pharmacophore like anti-proliferative,[12] potential inhibitor to different enzymes,[13] anti-convulsant,[14] anti-microbial,[18] anti-malarial,[16] anti-leishmanial,[15] anti-mycobacterial,[17] anti-viral,[21] anti-HIV,[20] anti-pyretic[23] and anti-protozoal,[24] anti-trypanosomal,[22] anti-HIV,[25] anti-depressant,[26] anti-tubercular,[19] and anti-oxidant[27] activities

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which are crucial to organic and medicinal chemistry. Several commercially marketed hydrazide-hydrazone derivatives are available, for example nifurtimox (treats Chagas disease),[28] nifuroxazide (antibiotic to treat colitis and diarrhea), and nitrofurazone (antimicrobial agent), furazolidone (antibacterial agent and monoamine oxidase inhibitor), and nitrofurantoin (antibacterial medicine to treat urinary tract infections)[1] which shows the importance of hydrazide-hydrazone scaffold.[29]

In an effort to enhance the bio-chemical properties of compounds, the sulfide group and hydrazide-hydrazones were incorporated into a single molecule. This study sought to find new compounds with improved anticholinesterase activities[30–32] by synthesizing a series of bis(acylhydrazones) from bisphenol sulfide. Additionally, a well-known structure-based drug design approach *i.e.*, molecular docking was used to investigate the binding behavior of the highest, moderate, and least active molecules against both studied enzymes.

2 Results and discussion

2.1. Chemistry of compounds

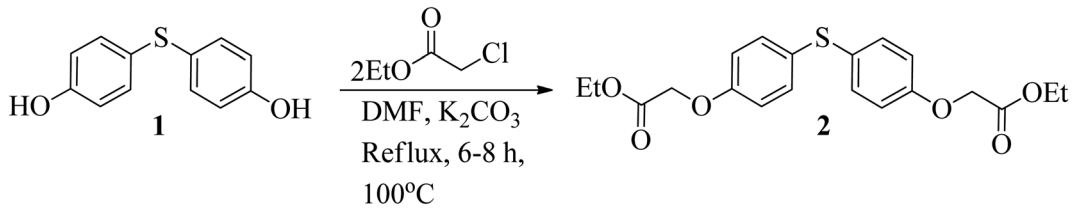
The esterification process was followed by hydrazination reaction to synthesize the aforementioned compounds (Schemes 1–3).[33] Diethyl 2,2′-((thiobis(4,1-phenylene))bis(oxy)) diacetate (**2**) was obtained by subjecting 4,4′-thiodiphenol (**1**) to reflux in dimethyl formamide (DMF) and reacting it with ethyl-2-chloroacetate (Scheme 1).[34]

In the process of being synthesized, 2,2′-((thiobis(4,1-phenylene))bis(oxy))di (acetohydrazide) (**3**) involved the reaction of 2,2′-((thiobis(4,2-phenylene))bis(oxy))diacetate (**2**) with an excess of hydrazine hydrate. This reaction was carried out in anhydrous methanol as the solvent (Scheme 2).[35]

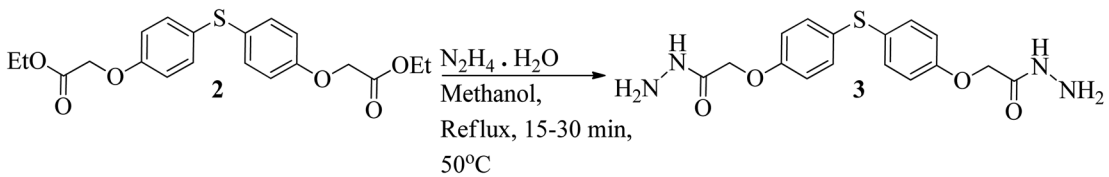
The hydrazide (**3**) was utilized in reactions with several aldehydes, both aromatic and aliphatic, in either DMF or ethyl alcohol which led to the synthesis of the matching bis(acylhydrazones) (**4a–4y**) with high yields ranging from 80% to 90% (Fig. 1). While considering the overall yields of the products obtained using different solvents, it can be inferred that DMF surpasses ethanol as a solvent for this reaction. This is likely due to the complete solubility of the hydrazide (**3**) in DMF. The enhanced solubility of the hydrazides in DMF makes it straightforward to separate the hydrazones by dissolving the reaction mixture in ice-cold water (Scheme 3). A collection of twenty-five (**25**) different bis(acylhydrazones) were prepared (Fig. 1) and their medicinal properties were evaluated *in vitro* by targeting acetyl and butyrylcholinesterase enzymes (Table 1).

2.2. Anticholinesterase activity

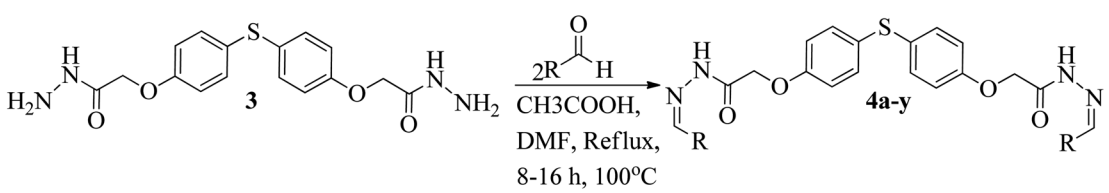
The cooperative function of AChE and BChE enzymes in preserving cholinomimetic activity has been shown by their inhibition studies. The rise in BChE activity in response to decrease in AChE activity suggests a different mechanism for the cholinergic breakdown. Additionally, clinical trials have shown that dual cholinesterase inhibitors outperform selective AChE inhibitors in terms of effectiveness. The inhibitory capability of the 25 synthetic supramolecules (**4a–4y**) were evaluated against both AChE and BChE to search dual cholinesterase inhibitors that may be considered as Alzheimer's disease therapy.



Scheme 1 Thiodiphenol (**1**) is esterified to produce diethyl 2,2′-((thiobis(4,1-phenylene))bis(oxy))diacetate (**2**).



Scheme 2 Synthesis of 2,2′-((thiobis(4,1-phenylene))bis(oxy))di(acetohydrazide) (**3**).



Scheme 3 Synthesis of bis(acylhydrazones) (**4a–4y**).

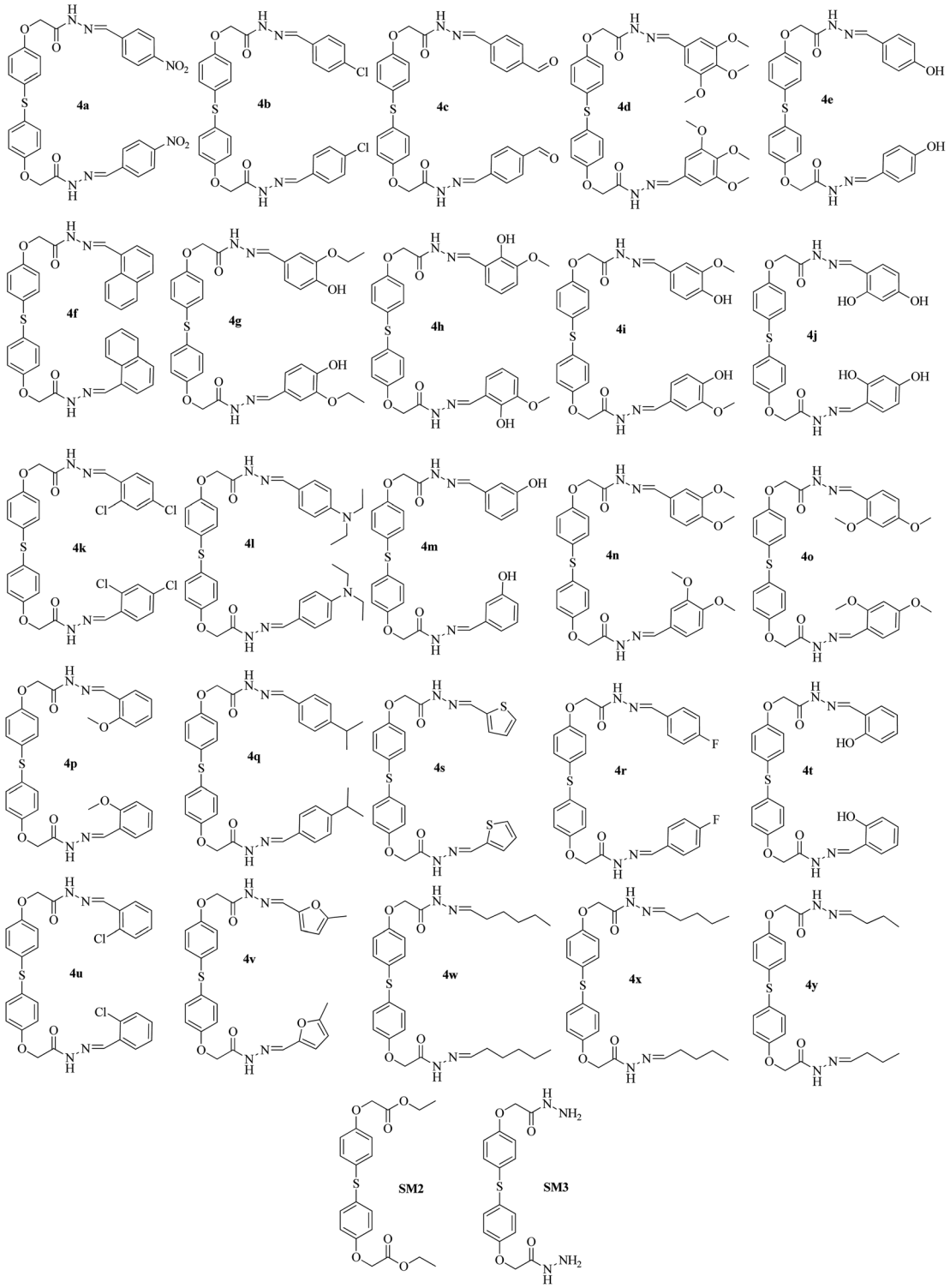


Fig. 1 The chemical structures of all the synthesized compounds.

Among all the compounds, **SM3**, **4n**, **4h**, **4l**, **4g**, **4t**, **4j** and **4o** showed good inhibitory activity for AChE with IC_{50} value in range of 23.1 to 40.2 μM, while **4b**, **4h**, **SM3**, **4s**, **4d**, and **4n** effectively inhibited the activity of BChE with IC_{50} ranging from 14.7 to 48.6 μM. We observed that compound **4h** and the starting molecule **SM3** have dual inhibitory properties for both AChE and BChE (Table 1). A well-known cholinesterase inhibitor, galantamine was used as a positive control which bears IC_{50} values of 29.5 μM and 27.8 μM for AChE and BChE, respectively. These findings suggests that bis(acylhydrazones), particularly **SM3** and **4h** holds promise as alternative therapeutic agents for the treatment of Alzheimer's disease.

Table 1 Acetyl and butyrylcholinesterase Inhibitory activities of compounds 4a–4y[a]

	AChE	BChE
Compounds	IC_{50} (μM) ± SD	IC_{50} (μM) ± SD
4a	NA	NA
4b	NA	14.7 ± 0.8297
4c	148.1 ± 0.7700	93.3 ± 0.8208
4d	67.0 ± 0.8623	44.7 ± 0.8461
4e	74.2 ± 0.7970	142.5 ± 0.7698
4f	131.4 ± 0.8634	117.1 ± 0.8046
4g	36.3 ± 0.7874	239.2 ± 0.8209
4h	27.8 ± 0.7232	19.0 ± 0.8625
4i	45.4 ± 0.6736	65.7 ± 0.7662
4j	39.1 ± 0.7811	84.6 ± 0.7276
4k	47.3 ± 0.7912	75.1 ± 0.8628
4l	33.5 ± 0.9318	109.4 ± 0.8708
4m	51.1 ± 0.7283	60.3 ± 0.6995
4n	25.7 ± 0.8137	48.6 ± 0.6727
4o	40.2 ± 0.8223	55.4 ± 0.7870
4p	49.1 ± 0.6934	110.2 ± 0.8135
4q	192.8 ± 0.6493	119.6 ± 0.6420
4r	NA	NA
4s	91.5 ± 0.6153	36.4 ± 0.7022
4t	37.7 ± 0.6853	78.9 ± 0.6850
4u	235.1 ± 0.7335	113.5 ± 0.6950
4v	NA	NA
4w	NA	NA
4x	186.3 ± 0.8761	103.1 ± 0.8765
4y	122.6 ± 0.9429	101.1 ± 0.9378
SM2	NA	NA
SM3	23.1 ± 0.6540	21.8 ± 0.8761
Galantamine	29.5 ± 0.9036	27.8 ± 0.8740

[a] NA = not active, SD = standard deviation.

2.3. Molecular docking analysis

The binding modes of most active, moderate, and least active inhibitors of both AChE and BChE were deduced by *in silico* structure-based drug design approach, *i.e.*, molecular docking. Compounds **SM3** and **4h** are among the most potent inhibitors of both the enzymes, similarly, **4i** and **4k** serves as moderate active inhibitors and **4q** is among the least active inhibitors for both AChE and BChE enzymes, therefore, only these molecules were selected for docking studies.

Before docking our synthesized compounds, the standard inhibitor, galantamine was manually docked into the active site of Electric eel AChE (**https://alphafold.com/entry/O42275**) and Equine BChE (**https://alphafold.com/entry/P81908**) to deduce its binding mode in these enzymes. The electric eel AChE and equine BChE structures was selected for docking studies because these sources were used in the *in vitro* experiments. Several residues including Asp96, Trp108, Gly142-Gly144, Tyr146, Ser147, Tyr155, Glu224, Ser225, Trp258, Phe313, Phe315, Tyr355, Phe356, Tyr359, Glu352, and His494, Gly495 constitutes the active site of electric eel AChE, where galantamine binds with the side chain of Glu224 through hydrogen bond, while Trp108 provides hydrophobic interactions to galantamine. The active site of equine BChE is constituted by Asp70, Ser79, Trp82, Asn83, Tyr114, Gly115-Gly117, Phe118, Gly121, Tyr128, Glu197, Ser198, Ala199, Trp231, Phe329, Tyr332, His438, Gly439, and Ile442. In the active site of equine BChE, Glu197, and Gly116 mediates hydrogen bonds with the –OH group of galantamine. When our selected inhibitors were docked into the active site of AChE, these molecules showed excellent binding interactions and the bisphenol sulfide moiety of these molecules resides in the middle of AChE active site, while one of the acylhydrazone moieties is located at the gorge of the active site, and the other acylhydrazone moiety is located near the entrance of AChE active site. The acylhydrazone moieties of most active inhibitor of AChE, **SM3** mediates strong hydrogen bonds with multiple residues including Gly311, Trp108, and Arg314, these multiple hydrogen bonding within the active site is responsible for the higher AChE inhibitory activity of **SM3**. Similarly, one of the acyl hydrazone moiety of **4h** and **4i** formed H-bond with side chain of Ser147 and Tyr155, respectively, while their substituted methoxy-phenol ring forms hydrophobic interaction with the side chain of Trp108 in the active site of AChE. The less hydrogen bond interactions of **4h** and **4i** with the active site residues of AChE as compared to **SM3** leads to the moderate activity of **4h** and **4i**. Likewise, the compound **4k** mediates H-bond and hydrophobic interaction with Ser147 and Trp108 through its acylhydrazone and its linked dicholorphenyl ring, respectively. In addition, the other acylhydrazone also forms H-bond with backbone of Phe313 at the entrance of the active site. While the least active molecule, **4q** mediates only bidentate interaction with Ser149 and Tyr155. The compounds possess docking scores in range of −7.73 to −3.04 kcal mol^{-1}, where both highly active compounds, **SM3** (−7.73kcal mol^{-1}) and **4h** (−7.18 kcal mol^{-1}) exhibited higher docking score than galantamine (−5.69 kcal mol^{-1}) which is in good agreement with the IC_{50} values of these compounds. The binding interactions of the selected compounds are shown in Fig. 2A.

The compound **4h** shows excellent inhibitory potency for BChE, when docked into the active site of BChE, this compound reflects multiple interactions with Asp283, Tyr332, and Trp82. We observed that one of the substituted methoxyphenol group formed H-bond with the side chain of Asp283 instead of the acylhydrazone moiety of the compound. While the diphenylsulfane ring forms hydrophobic interaction (π–π) with Trp82, similarly the other substituted acylhydrazone moiety formed hydrophobic interaction (π–H) with Tyr332. In compound **SM3**, both the acetohydrazide groups formed strong H-bonds with the side chains of Ser287 and Gly115. Interestingly, the diphenylsulfane of compounds **4i**, **4k** and **4q** accepts H-bonds with the side chain of Ser198, while the diphenylsulfane of **4i** and **4k** and the substituted cumene of **4q** formed hydrophobic interactions with trp82, Phe329, and Asn68, respectively. It is clear that due to the higher number of binding interactions with the residues of active site, these molecules exhibits high inhibitory activity, while the activity of compounds are reduced when their binding interactions are reduced. The compounds' docking scores vary from −9.52 to −3.36 kcal mol^{-1} (Table 2). Again, we obtained higher docking scores of **4h** (−9.52 kcal mol^{-1}), **SM3** (−7.15 kcal mol^{-1}) and **4i**

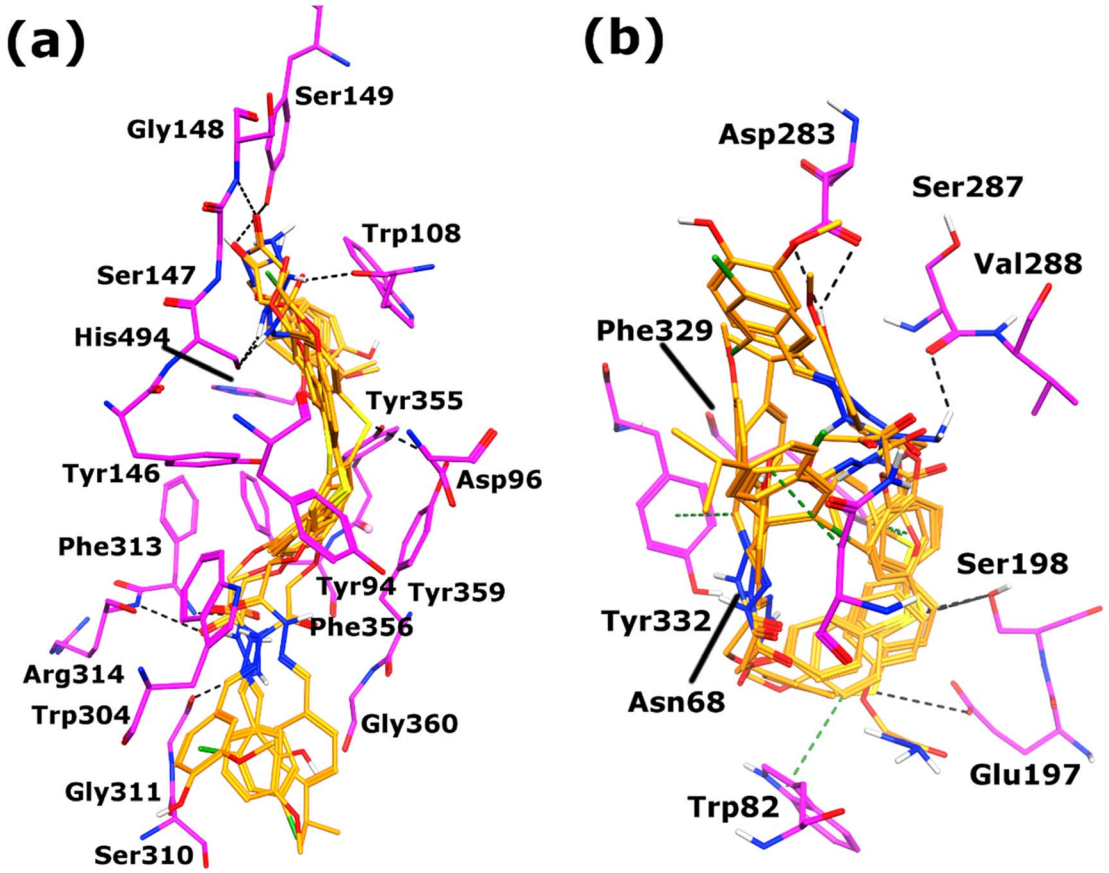


Fig. 2 The binding interactions of compounds are shown in the active site of (A) AChE and (B) BChE enzymes. The active site residues are shown in magenta stick model, H-bonds and hydrophobic interactions are displayed in black and green dotted lines, respectively. The ligands are given in orange stick model.

(−6.98 kcal mol^{-1}) as compared to galantamine (−6.66 kcal mol^{-1}) while **4k** and **4q** bears lesser docking scores than the standard inhibitor. The molecular interactions of compounds **4h**, **SM3**, **4i**, **4k**, and **4q** are summarized in Table 2 and presented in Fig. 2B.

3 Methodology

3.1. General procedure

In addition to freshly distilled solvents, dry solvents were sometimes utilized as well. The progress of the reactions was monitored through Thin Layered Chromatography performed on pre-coated Kieselgel-60 HF 254 cards. Standard protocols were followed, and all compounds were synthesized using clean glassware. Chemical reactions were carried out using reagents purchased from Sigma Aldrich. A number of contemporary spectroscopic methods were used to characterize the newly synthesized molecules, including ^{13}C NMR, ^{1}H NMR, and HRMS-EIMS.

3.2. Synthesis of diethyl 2,2′-((thiobis(4,1-phenylene)) bis(oxy))diacetate (2)

8 mmol of 4,4′-thiodiphenol and 16 mmol of K_2CO_3 were dissolved in DMF, and the mixture was then refluxed for 30 minutes. The reaction mixture was then given 12 mmol of ethyl-2-chloroacetate for esterification. An additional 6 to 8 hours were spent refluxing the reaction mixture. TLC was used to continually track the reaction's progress using the *n*-hexane/ethyl acetate solvent combination (6 : 4). White precipitates were created when the hot reaction mixture was poured into ice-cold water at the end of the reaction. The precipitates were filtered and then let to air dry.

Yield: 8 g (97%); white solid; mp 107–109 °C.

HR-MS (EIMS): *m*/*z* $[M + H]^+$ calcd for $C_{20}H_{22}O_6S$: 390.4500, found: 390.1031.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 1.230 (t, J = 7.0 Hz, 6H, 2 –CH_3), 4.902 (q, J = 7.5 Hz, 4H, 2 –CH_2–), 4.6 s (4H, 2 –CH_2–), 7.16 (d, J = 8.2 Hz, 4H, Ar–H), 7.38 (d, J = 8.2 Hz, 4H, Ar–H).

^{13}C NMR (600.153 MHz, DMSO-d_6): (δ ppm): 168.49, 161.16, 134.04, 129.30, 115.45, 64.68, 60.85, 51.96, 13.99.

3.3. 2,2′-((Thiobis(4,1-phenylene)bis(oxy)) di(acetohydrazide)) syntheses (3)

8000 mg (8 g, 8 mmol) of diethyl 2,2′-((thiobis(4,1-phenylene)) bi(oxy))diacetate **(2)** and an excess quantity of hydrazine hydrate were combined in 50 mL of methanol to create the hydrazide of the ester. A white precipitate of compound (3) was

Table 2 The docking results of **SM3**, **4h**, **4i**, **4k**, **4q** in the active site of AChE and BChE[a]

		AChE-ligands interactions			
Compounds	Docking score (kcal mol^{-1})	LA	RA	Interaction	Distance (Å)
SM3	−7.73	N31	O-GLY311	HBD	2.09
		N38	O-TRP108	HBD	2.54
		O30	N-ARG314	HBA	2.19
4h	−7.18	N28	OG-SER147	HBD	2.52
		6-Ring	6-Ring-TRP108	π–π	2.81
Galantamine	−5.69	O18	OE2-GLU224	HBD	2.70
4k	−5.41	N36	OG-SER147	HBD	2.87
		O30	N-PHE313	HBA	2.18
		6-Ring	6-Ring-TRP108	π–π	2.61
4i	−5.39	O38	OH-TYR155	HBA	2.44
		6-Ring	5-Ring-TRP108	π–π	2.90
		6-Ring	6-Ring-TRP108	π–π	2.65
4q	−3.04	O38	N-SER149	HBA	2.13
		O38	OH-TYR155	HBA	2.10

Compounds	BChE IC_{50} (μM)	BChE-ligands interactions			
4h	−9.52	O72	OD1-ASP283	HBD	2.38
		O72	OD2-ASP283	HBD	2.30
		C42	6-Ring-TYR332	H–π	2.70
		6-Ring	5-Ring-TRP82	π–π	2.61
SM3	−7.15	N41	O-SER287	HBD	2.14
		O30	N-GLY115	HBA	2.39
4i	−6.98	S11	OG-SER198	HBA	2.12
		C8	5-Ring-TRP82	H–π	2.81
Galantamine	−6.66	O18	OE2-GLU197	HBD	2.76
		O18	N-GLY116	HBA	2.04
4k	−4.12	S11	OG-SER198	HBA	2.51
		6-Ring	CE1-PHE329	H–π	2.94
4q	−3.36	S11	OG-SER198	HBA	2.52
		6-Ring	CB-ASN68	H–π	2.56

[a] LA = ligand atom, RA = receptor Atom, HBA = hydrogen bond acceptor, HBD = hydrogen bond donor.

created after a 30 minute reflux time. The precipitate was separated, filtered, and then dried.

Yield: 90% (5581 mg); white solid; mp 227–229 °C. HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{16}H_{18}N_4O_4S$: 362.4020, found: 362.1020.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 9.08 (s, 4H, 2-NH_2), 4.22 (s, 2H, 2-NH–), 4.590 (s, 4H, 2-CH_2–), 7.160 (d, J = 8.2 Hz, 4H, Ar–H), 7.381 (d, J = 8.2 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 169.11, 163.66, 134.54, 129.80, 115.95, 66.36.

3.4. Basic steps for producing 2,2′-((thiobis(4,1-phenylene)) bis(oxy))di(acetohydrazide) bis(acylhydrazones) (3)

Using compound (**3**) as the starting material, bis(acylhydrazones) were synthesized by reacting it with various aromatic and aliphatic aldehydes in a 2 : 1 ratio. The mixture was refluxed at 120 °C for 8–16 hours while the reactions took place using DMF as the solvent. The resulting mixture was quickly cooled by submerging it in ice-cold water after refluxing. The resultant bi(acylhydrazone) precipitates were filtered, separated, and dried for further use.

3.4.1. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-nitrobenzylidene)acetohydrazide) 4a. Yield: 90% (160 mg); yellow solid; mp 252–254 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{24}N_6O_8S$: 628.1400, found: 628.2525.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.686 (s, 2H, 2-NH–), 4.733 (s, 4H, 2-CH_2–), 8.428, 8.408 (s, 2H, 2 =CH–), 6.999, 7.053 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.510, 7.558 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.929, 7.981 (d, d, J = 8.4 Hz, 4H, Ar–H), 8.252, 8.283 (d, d, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 169.52, 164.85, 162.33, 157.37, 147.98, 147.82, 145.52, 141.54, 140.47, 140.32, 132.71, 128.11, 127.95, 127.36, 127.26, 124.08, 124.02, 115.16, 114.99, 66.60, 64.85, 35.81, 30.80.

3.4.2. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-chlorobenzylidene)acetohydrazide) 4b. Yield: 88% (150 mg); white solid; mp 240–242 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{24}Cl_2N_4O_4S$: 606.0900, found: 606.1993.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.687 (s, 2H, 2-NH–), 4.689 (s, 4H, 2-CH_2–), 8.311, 7.997 (s, 2H, 2 =CH–), 6.980,

7.046 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.484, 7.506 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.542, 7.566 (d, d, J = 8.4 Hz, 4H, Ar–H), 7.705, 7.740 (d, d, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 169.15, 164.42, 162.33, 157.47, 157.30, 156.93, 146.67, 142.59, 134.65, 134.41, 133.11, 132.98, 132.67, 128.96, 128.90, 128.80, 127.34, 127.28, 127.24, 115.15, 114.96, 66.62, 64.80, 35.80, 30.80.

3.4.3. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-formylbenzylidene)acetohydrazide) 4c. Yield: 85% (152 mg); yellowish solid; mp 278–280 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{32}H_{26}N_4O_6S$: 594.1600, found: 594.2686.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.686 (s, 2H, 2-NH–), 10.015 (s, 2H, 2CHO), 4.723 (s, 4H, 2-CH_2–), 8.403, 8.085 (s, 2H, 2 =CH–), 6.997, 7.054 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.523, 7.558 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.896, 7.910 (d, d, J = 8.4 Hz, 4H, Ar–H), 7.918, 7.945 (d, d, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 192.72, 169.39, 164.67, 162.33, 146.57, 142.55, 139.66, 139.51, 136.74, 129.96, 129.93, 127.68, 127.52, 127.36, 127.30, 127.25, 115.17, 114.99, 66.64, 64.85, 35.80, 30.81.

3.4.4. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-3,4,5-trimethoxybenzylidene)acetohydrazide) 4d. Yield: 90% (180 mg); white solid; mp 248–250 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{36}H_{38}N_4O_{10}S$: 718.2300, found: 718.3526.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.599 (s, 2H, 2-NH–), 4.691 (s, 4H, 2-CH_2–), 3.804 (s, 6H, 2-CH_3), 3.688 (s, 12H, 4-CH_3), 8.256, 7.925 (s, 2H, 2 =CH–), 6.977, 7.051 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.521, 7.559 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.012 (s, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 169.14, 164.22, 153.19, 148.02, 143.66, 139.38, 139.17, 129.62, 129.54, 127.38, 127.31, 127.27, 127.20, 115.17, 114.91, 104.43, 104.29, 66.64, 64.89, 60.15, 55.99.

3.4.5. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-hydroxy benzylidene)acetohydrazide) 4e. Yield: 80% (134 mg); white solid; mp 164–166 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{30}H_{26}N_4O_6S$: 570.1600, found: 570.2664.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.368 (s, 2H, 2-NH–), 9.934 (s, 2H, 2-OH), 4.723 (s, 4H, 2-CH_2–), 8.214, 7.903 (s, 2H, 2 =CH–), 6.972, 7.046 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.505, 7.518 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.798, 6.812 (d, d, J = 8.4 Hz, 4H, Ar–H), 7.541, 7.556 (d, d, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 168.68, 163.88, 162.33, 159.53, 159.32, 157.51, 157.43, 157.04, 156.97, 148.30, 144.18, 133.14, 132.98, 132.63, 132.45, 128.93, 128.69, 127.37, 127.32, 127.28, 127.22, 125.09, 125.03, 115.72, 115.70, 115.16, 114.92, 66.67, 64.78, 35.81, 30.81.

3.4.6. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-naphthalen-1-ylmethylene)acetohydrazide) 4f. Yield: 82% (151 mg); white solid; mp 216–218 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{38}H_{30}N_4O_4S$: 638.2000, found.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.666, 11.648 (s, 2H, 2-NH–), 4.755 (s, 4H, 2-CH_2–), 9.010, 8.687 (s, 2H, 2 =CH–), 7.023, 7.110 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.535, 7.550 (d, d, J = 8.2 Hz, 4H, Ar–H), 8.799, 8.818 (dd, J = 8.4 Hz, 2H, Ar–H), 8.012, 8.022 (ddd, J = 8.4 Hz, 2H, Ar–H), 8.614, 8.628 (dd, J = 8.4 Hz, 2H, Ar–H), 7.910, 7.999 (dd, J = 8.4 Hz, 2H, Ar–H), 7.565, 7.591 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.601, 7.659 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.635, 7.659 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 169.68, 163.88, 162.33, 159.53, 159.32, 157.51, 157.43, 157.04, 156.97, 148.30, 144.18, 133.14, 132.98, 132.63, 132.45, 128.93, 128.69, 127.37, 127.32, 127.28, 127.22, 125.09, 125.03, 115.74, 115.70, 115.16, 114.92, 66.67, 64.78, 35.81, 30.81.

3.4.7. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-3-ethoxy-4-hydroxybenzylidene)acetohydrazide) 4g. Yield: 84% (160 mg); white solid; mp 196–198 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{34}H_{34}N_4O_8S$: 658.2100, found: 658.3287.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.418, 11.378 (s, 2H, 2-NH–), 9.448 (s, 2H, 2-OH), 4.653 (s, 4H, 2-CH_2–), 1.307, 1.350 (t, J = 7.0 Hz, 6H, 2-CH_3), 4.029, 4.040 (q, J = 7.5 Hz, 4H, 2-CH_2–) 8.188, 7.783 (s, 2H, 2 =CH–), 7.046, 7.079 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.532, 7.569 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.506, 7.519 (d, J = 8.4 Hz, 2H, Ar–H), 7.240, 7.250 (dd, J = 8.4 Hz, 2H, Ar–H), 6.953, 6.957 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 168.77, 163.89, 162.33, 157.54, 149.36, 149.08, 148.55, 147.19, 147.12, 144.27, 127.37, 127.30, 127.26, 127.19, 125.46, 122.10, 121.33, 115.62, 115.57, 115.16, 114.90, 110.96, 110.51, 66.68, 6486, 63.91, 35.80, 30.80, 14.74, 14.71.

3.4.8. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2-hydroxy-3-methoxybenzylidene)acetohydrazide) 4h. Yield: 87% (160 mg); white solid; mp 235–237 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{32}H_{30}N_4O_8S$: 630.1800, found: 630.2939.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.525 (s, 2H, 2-NH–), 10.730 (s, 2H, 2-OH), 4.795 (s, 4H, 2-CH_2–), 3.811 (s, 6H, 2-OCH_3) 8.569, 8.334 (s, 2H, 2 =CH–), 7.124, 7.309 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.509, 7.851 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.965, 6.992 (d, J = 8.4 Hz, 2H, Ar–H), 6.795, 6.853 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.024, 7.066 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 168.72, 164.21, 157.48, 156.97, 156.89, 148.22, 148.02, 147.98, 147.13, 146.01, 141.36, 133.23, 133.05, 127.41, 127.35, 127.30, 127.24, 120.63, 120.46, 119.21, 119.09, 118.93, 117.91, 115.19, 114.94, 113.96, 66.57, 64.86, 55.89.

3.4.9. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-hydroxy-3-methoxybenzylidene)acetohydrazide) 4i. Yield: 80% (146 mg); brown solid; mp 243–245 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{32}H_{30}N_4O_8S$: 630.1800, found: 630.2958.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.429, 11.392 (s, 2H, 2-NH–), 9.570 (s, 2H, 2-OH), 4.658 (s, 4H, 2-CH_2–), 3.800 (s, 6H, 2-OCH_3) 8.203, 7.940 (s, 2H, 2 =CH–), 6.957, 6.970 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.519, 7.570 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.258, 7.269 (d, J = 8.4 Hz, 2H, Ar–H), 7.048, 7.088 (dd, J = 8.4 Hz, 2H, Ar–H), 6.808, 6.820 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 168.78, 163.89, 162.34, 159.46, 157.55, 157.46, 157.05, 156.96, 149.12, 148.84,

148.54, 148.06, 147.98, 144.27, 132.98, 132.43, 127.37, 127.31, 127.27, 127.20, 125.50, 125.46, 122.20, 121.36, 115.55, 115.47, 115.17, 114.91, 109.69, 109.12, 66.68, 64.85, 55.63, 55.60, 35.81, 20.21.

3.4.10. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2,4-dihydroxybenzylidene)acetohydrazide) 4j. Yield: 85% (150 mg); brown solid; mp 276–278 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{26}N_4O_8S$: 602.1500, found: 602.2595.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.257 (s, 2H, 2-NH–), 10.038 (s, 2H, 2-OH), 4.684 (s, 4H, 2-CH$_2$–), 8.413, 8.172 (s, 2H, 2 =CH–), 6.967, 7.058 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.532, 7.575 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.474, 7.488 (d, J = 8.4 Hz, 2H, Ar–H), 6.303, 6.337 (dd, J = 8.4 Hz, 2H, Ar–H), 7.267, 7.281 (d, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm: 172.69, 168.26, 163.83, 162.29, 160.67, 159.67, 158.14, 157.14, 149.55, 142.67, 133.03, 131.21, 127.39, 127.33, 127.28, 115.19, 114.92, 112.07, 107.86, 102.68, 102.44, 66.85, 65.42, 35.81, 30.72.

3.4.11. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2,4-dichlorobenzylidene)acetohydrazide) 4k. Yield: 90% (156 mg); white solid; mp 270–272 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{22}C_{l4}N_4O_4S$: 674.0100, found: 674.1286.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.866, (s, 2H, 2-NH–), 4.715 (s, 4H, 2-CH$_2$–), 8.703, 8.342 (s, 2H, 2 =CH–), 6.992, 7.061 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.500, 7.564 (d, d, J = 8.2 Hz, 4H, Ar–H), 8.028, 8.043 (dd, J = 8.4 Hz, 2H, Ar–H), 7.952, 7.967 (dd, J = 8.4 Hz, 2H, Ar–H), 7.12 (d, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 169.30, 164.66, 157.37, 142.86, 138.86, 135.24, 135.00, 133.97, 133.68, 132.70, 130.58, 130,41, 129.41, 129.38, 128.33, 128.17, 128.07, 127.97, 127.40, 127.35, 127.28, 127.24, 115.19, 114.99, 66.69, 64.84, 35.86, 14.30.

3.4.12. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-(diethylamino)benzylidene)acetohydrazide) 4l. Yield: 91% (180 mg); yellowish solid; mp 175–177 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{38}H_{44}N_6O_4S$: 680.3100, found: 680.4315.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.272, 11.216 (s, 2H, 2-NH–), 4.646 (s, 4H, 2-CH$_2$–), 8.143, 7.843 (s, 2H, 2 =CH–), 1.081, 1.100 (t, J = 7.0 Hz, 12H, 4-CH$_3$), 3.297, 3.373 (q, J = 7.5 Hz, 8H, 4-CH$_2$–), 6.966, 7.048 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.405, 7.519 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.660, 6.688 (dd, J = 8.4 Hz, 4H, Ar–H), 7.544, 7.570 (dd, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 189.48, 168.36, 163.50, 148.97, 148.86, 148.78, 144.74, 128.85, 128.56, 127.36, 127.30, 127.22, 120.44, 115.15, 114.90, 111.11, 110.63, 66.71, 64.80, 43.75, 12.45.

3.4.13. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-3-hydroxybenzylidene)acetohydrazide) 4m. Yield: 87% (145 mg); brown solid; mp 160–162 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{26}N_4O_6S$: 570.1600, found: 570.2672.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.542, 11.523 (s, 2H, 2-NH–), 4.679 (s, 4H, 2-CH$_2$–), 8.239, 8.229 (s, 2H, 2 =CH–), 9.611 (s, 2H, 2-OH), 7.094, 7.148 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.920, 7.940 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.966, 6.980 (d, J = 8.4 Hz, 2H, Ar–H), 6.806, 6.819 (dd, J = 8.4 Hz, 2H, Ar–H), 7.212, 7.238 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.512, 7.576 (ddd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 168.95, 164.26, 162.33, 157.70, 157.66, 157.47, 157.40, 156.95, 148.06, 144.08, 135.40, 135.24, 133.17, 133.00, 132.68, 132.52, 129.89, 127.39, 127.34, 127.31, 127.26, 118.86, 118.45, 117.56, 117.31, 115.16, 114.92, 112.77, 112.71, 66.65, 64.74, 35.80, 30.80.

3.4.14. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-3,4-dimethoxybenzylidene)acetohydrazide) 4n. Yield: 81% (155 mg); reddish solid; mp 246–248 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{34}N_4O_8S$: 658.2100, found: 658.5057.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.466 (s, 2H, 2-NH–), 4.672 (s, 4H, 2-CH$_2$–), 8.249, 7.940 (s, 2H, 2 =CH–), 3.783 (s, 6H, 2-OCH$_3$), 3.789 (s, 6, 2-OCH$_3$), 7.050, 7.193 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.570, 7.940 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.974, 7.000 (dd, J = 8.4 Hz, 2H, Ar–H), 7.289, 7.313 (dd, J = 8.4 Hz, 2H, Ar–H), 7.520, 7.545 (d, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 168.90, 164.02, 162.33, 157.55, 157.47, 157.04, 156.96, 150.88, 150.64, 149.10, 149.07, 148.21, 143.92, 133.16, 132.99, 132.44, 127.38, 127.31, 127.26, 127.21, 126.83, 126.79, 121.95, 121.27, 115.17, 114.92, 111.55, 108.77, 108.40, 66.68, 64.88, 5561, 55.51, 38.30, 30.80.

3.4.15. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2,4-dimethoxybenzylidene)acetohydrazide) 4o. Yield: 85% (158 mg); yellowish solid; mp 275–277 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{34}H_{34}N_4O_8S$: 658.2100, found: 658.3267.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.458, 11.409 (s, 2H, 2-NH–), 4.636 (s, 4H, 2-CH$_2$–), 8.574, 8.249 (s, 2H, 2 =CH–), 3.783 (s, 6H, 2-OCH$_3$), 3.789 (s, 6H, 2-OCH$_3$), 7.032, 7.046 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.509, 7.569 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.581, 6.616 (d, J = 8.4 Hz, 2H, Ar–H), 6.954, 6.968 (dd, J = 8.4 Hz, 2H, Ar–H), 7.744, 7.776 (d, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 168.66, 163.83, 162.56, 162.35, 159.24, 159.06, 157.52, 157.44, 143.55, 139.65, 132.62, 127.35, 127.30, 127.21, 126.76, 115.14, 114.93, 106.52, 106.44, 98.34, 98.17, 66.63, 64.83, 55.82, 55.48, 35.81, 30.80.

3.4.16. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2-methoxybenzylidene)acetohydrazide) 4p. Yield: 92% (161 mg); white solid; mp 256–258 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{32}H_{30}N_4O_6S$: 598.1900, found: 598.3018.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.615, 11.567 (s, 2H, 2-NH–), 4.680 (s, 4H, 2-CH$_2$–), 8.698, 8.364 (s, 2H, 2 =CH–), 3.861 (s, 6H, 2-OCH$_3$), 7.058, 7.117 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.826, 7.813 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.983, 7.025 (dd, J = 8.4 Hz, 2H, Ar–H), 7.023, 7.428 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.523, 7.579 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.875, 7.863 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 169.13, 164.34, 159.57, 147.92, 143.70, 135.57, 135.43, 132.65, 129.98, 129.94, 127.39, 127.33, 127.28, 127.23, 120.09, 119.63, 116.37, 115.92, 115.17, 114.94, 111,64, 111.34, 66.65, 64.83, 55.21.

3.4.17. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-isopropylbenzylidene)acetohydrazide) 4q. Yield: 83% (151 mg); white solid; mp 207–209 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{36}H_{38}N_4O_4S$: 622.2600, found: 622.3745.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.544 (s, 2H, 2-NH–), 4.679 (s, 4H, 2-CH$_2$–), 8.303, 7.982 (s, 2H, 2 =CH–), 1.194–1.205 (d, J = 7.4 Hz, 12H, 4-CH$_3$), 2.980 (heptet, J = 7.5 Hz, 2H, 2-CH–), 6.966, 7.055 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.512, 7.572 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.602, 7.623 (d, d, J = 8.4 Hz, 4H, Ar–H), 7.295, 7.320 (d, d, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 168.95, 164.18, 157.50, 157.43, 156.96, 150.83, 150.60, 148.03, 142.93, 133.16, 132.66, 132.50, 131.84, 131.72, 127.38, 127.33, 127.26, 127.05, 126.83, 126.78, 115.16, 114.93, 66.66, 64.81, 33.40, 33.38, 23.68.

3.4.18. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-thiophen-2-ylmethylene)acetohydrazide) 4r. Yield: 84% (136 mg); yellowish solid; mp 249–251 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{26}H_{22}N_4O_4S_3$: 550.0800, found: 550.1864.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.550 (s, 2H, 2-NH–), 4.668 (s, 4H, 2-CH$_2$–), 8.549, 8.190 (s, 2H, 2 =CH–), 6.943, 7.047 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.439, 7.521 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.116, 7.130 (dd, J = 8.4 Hz, 2H, Ar–H), 7.631, 7.663 (dd, J = 8.4 Hz, 2H, Ar–H), 7.535, 7.544 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 168.65, 164.15, 157.44, 156.99, 156.92, 143.17, 139.03, 138.86, 138.67, 133.02, 132.15, 130.56, 139.12, 128.62, 127.94, 127.89, 127.39, 127.32, 127.27, 115.18, 114.98, 66.68, 64.57, 38.90, 35.80.

3.4.19. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-4-fluorobenzylidene)acetohydrazide) 4s. Yield: 90% (150 mg); white solid; mp 273–274 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{24}F_2N_4O_4S$: 574.1500, found: 574.2565.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.600 (s, 2H, 2-NH–), 4.687 (s, 4H, 2-CH$_2$–), 8.335, 8.007 (s, 2H, 2 =CH–), 6.993, 7.052 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.510, 7.560 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.258, 7.279 (d, d, J = 8.4 Hz, 4H, Ar–H), 7.750, 7.787 (d, d, J = 8.4 Hz, 4H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 169.08, 164.33, 164.03, 162.38, 162.24, 157.49, 157.41, 156.94, 146.88, 142.73, 133.00, 132.67, 132.49, 130.77, 130.66, 129.38, 129.32, 129.20, 129.14, 127.34, 127.28, 127.24, 127.01, 116.01, 115.94, 115.86, 115.80, 115.16, 114.95, 66.65, 64.81, 38.30, 35.80, 30.40.

3.4.20. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2-hydroxybenzylidene)acetohydrazide) 4t. Yield: 80% (140 mg); pink solid; mp 224–226 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{26}N_4O_6S$: 570.6200, found: 570.2663.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.819, 11.535 (s, 2H, 2-NH–), 4.720 (s, 4H, 2-CH$_2$–), 8.560, 8.306 (s, 2H, 2 =CH–), 11.044 and 10.049 (s, 2H, 2-OH), 6.969, 7.055 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.511, 7.583 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.839, 6.851 (dd, J = 8.4 Hz, 2H, Ar–H), 6.864, 6.914 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.223, 7.297 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.699, 7.712 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 168.72, 164.25, 157.41, 156.98, 156.90, 156.44, 148.37, 141.52, 133.24, 133.06, 131.54, 131.23, 129.34, 127.41, 127.35, 127.30, 127.24, 126.58, 120.05, 119.44, 119.40, 118.65, 116.42, 116.17, 112.20, 114.95, 66.56,64.89, 38.40, 35.90, 30.80.

3.4.21. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-2-chlorobenzylidene)acetohydrazide) 4u. Yield: 88% (160 mg); white solid; mp 230–232 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{30}H_{24}Cl_2N_4O_4S$: 606.5100, found: 606.2000.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.851, 11.778 (s, 2H, 2-NH–), 4.710 (s, 4H, 2-CH$_2$–), 8.749, 8.397 (s, 2H, 2 =CH–), 6.982, 7.067 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.512, 7.556 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.570, 7.585 (dd, J = 8.4 Hz, 4H, Ar–H), 7.409, 7.448 (ddd, J = 8.4 Hz, 2H, Ar–H), 7.956, 8.030 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 169.25, 164.59, 157.39, 143.91, 139.90, 133.28, 132.98, 132.69, 131.65, 131.41, 131.29, 129.96, 129.91, 127.67, 127.63, 127.41, 127.35, 127.30, 127.24, 127.11, 126.98, 115.20, 114.98, 66.69, 64.85, 35.80, 30.70.

3.4.22. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-(5-methylfuran-2-yl)methylene)acetohydrazide) 4v. Yield: 82% (130 mg); brown solid; mp 148–150 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{28}H_{26}N_4O_6S$: 546.6000, found: 546.0000.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.453, 11.436 (s, 2H, 2-NH–), 4.657 (s, 4H, 2-CH$_2$–), 8.123, 7.810 (s, 2H, 2 =CH–), 2.324 (s, 6H, 2-CH$_3$), 6.936, 7.028 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.499, 7.571 (d, d, J = 8.2 Hz, 4H, Ar–H), 6.244 (dd, J = 8.4 Hz, 2H, Ar–H), 6.783, 6.795 (dd, J = 8.4 Hz, 2H, Ar–H).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 169.67, 164.08, 157.46, 154.71, 154.55, 147.71, 147.53, 137.67, 134.17, 127.38, 127.32, 127.28, 127.23, 115.64, 115.39, 115.18, 114.92, 108.59, 66.72, 64.62, 13.56, 13.49.

3.4.23. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-hexylidene)acetohydrazide) 4w. Yield: 86% (125 mg); brown solid; mp 128–130 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{28}H_{38}N_4O_4S$: 526.7000, found: 526.0000.

^{1}H NMR (DMSO-d$_6$, 600.150 MHz, δ ppm): 11.605 (s, 2H, 2-NH–), 4.811 (s, 4H, 2-CH$_2$–), 7.940, 7.810 (s, 2H, 2 =CH–), 2.324 (s, 6H, 2-CH$_3$), 6.921, 6.935 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.489, 7.500 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.381 (t, J = 7.5 Hz, 1H, –N=CH–), 7.974 (t, J = 7.5 Hz, 1H, –N=CH–), 2.180 (q, J = 7.2 Hz, 4H, 2-CH$_2$–), 1.460–1.500 (quintet, J = 7.3 Hz, 4H, 2-CH$_2$–), 1.236–1.276 (sextet, J = 7.3 Hz, 4H. 2-CH$_2$–), 0.839–0.903 (t, J = 7.5 Hz, 6H. 2-CH$_3$).

^{13}C NMR (600.150 MHz, DMSO-d$_6$): (δ ppm): 192.72, 169.39, 164.67, 162.33, 146.57, 142.55, 139.66, 139.51, 136.74, 129.96, 129.93, 127.68, 127.52, 127.36, 127.30, 127.25, 115.17, 114.99, 66.64, 64.85, 35.80, 30.81,24.80, 22.30, 18.40, 14.30.

3.4.24. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-pentylidene)acetohydrazide) 4x. Yield: 90% (145 mg); white solid; mp 117–119 °C.

HR-MS (EIMS): *m*/*z* [M + H]$^+$ calcd for $C_{26}H_{34}N_4O_4S$: 498.6400, found: 498.0000.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.605 (s, 2H, 2-NH–), 4.811 (s, 4H, 2-CH_2–), 7.940, 7.810 (s, 2H, 2 =CH–), 2.324 (s, 6H, 2-CH_3), 6.921, 6.935 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.489, 7.500 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.381 (t, J = 7.5 Hz, 1H, –N=CH–), 7.974 (t, J = 7.5 Hz, 1H, –N=CH–), 2.180 (q, J = 7.2 Hz, 4H, 2-CH_2–), 1.460–1.500 (quintet, J = 7.3 Hz, 4H, 2-CH_2–), 1.236–1.276 (sextet, J = 7.3 Hz, 4H. 2-CH_2–), 0.839–0.903 (t, J = 7.5 Hz, 6H. 2-CH_3).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 169.52, 164.85, 162.33, 157.37, 147.98, 147.82, 145.52, 141.54, 140.47, 140.32, 132.71, 128.11, 127.95, 127.36, 127.26, 124.08, 124.02, 115.16, 114.99, 66.60.64.85, 35.81, 30.80, 28.70, 26.80, 14.30.

3.4.25. 2,2′-((Thiobis(4,1-phenylene))bis(oxy))bis(*N*′-((*E*)-butylidene)acetohydrazide) 4y. Yield: 82% (120 mg); brown solid; mp 120–122 °C.

HR-MS (EIMS): m/z [M + H]$^+$ calcd for $C_{24}H_{30}N_4O_4S$: 470.5900, found: 470.0000.

^{1}H NMR (DMSO-d_6, 600.150 MHz, δ ppm): 11.632 (s, 2H, 2-NH–), 4.812 (s, 4H, 2-CH_2–), 7.940, 7.810 (s, 2H, 2 =CH–), 2.324 (s, 6H, 2-CH_3), 6.896, 6.990 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.386, 7.459 (d, d, J = 8.2 Hz, 4H, Ar–H), 7.648 (t, J = 7.5 Hz, 1H, –N=CH–), 6.928 (t, J = 7.5 Hz, 1H, –N=CH–), 2.232–2.243 (q, J = 7.2 Hz, 4H, 2-CH_2–), 1.223–1.531 (heptet, J = 7.3 Hz, 4H. 2-CH_2–), 0.863–0.915 (t, J = 7.5 Hz, 6H. 2-CH_3).

^{13}C NMR (600.150 MHz, DMSO-d_6): (δ ppm): 168.95, 164.18, 157.50, 157.43, 156.96, 150.83, 150.60, 148.03, 143.94, 133.16, 132.66, 132.50, 131.84, 131.72, 127.38, 127.33, 127.29, 127.26, 127.05, 126.83, 126.78, 115.16, 114.93, 66.66, 64.81, 33.40, 33.38, 23.68, 20.20, 18.80.

3.5. Anticholinesterase activity

DTNB, butyrylcholine iodide, potassium phosphate buffer (pH 8.0), acetylcholine iodide, galantamine, and two enzymes—equine butyrylcholinesterase and electric eel acetylcholinesterase (type-IV-S) were utilized in this study. The spectrophotometric approach developed by Elman was used to perform the inhibition investigations on AChE and BChE. The substrates used in this approach were butyrylcholine iodide and acetylcholine iodide.

Finally, two mixes were made with 5 mL each of AChE (0.03 g mL^{-1}) and BChE (0.01 g mL^{-1}). The sample chemical was added to each combination in amounts ranging from 125 to 1000 g mL^{-1} along with DNTB (5 μL), and each mixture was then incubated for 15 minutes at 30 °C. 5 μL of substrates were then added to each mixture after the incubation period, and the reaction was then observed using a UV-Visible spectrophotometer for 4 minutes at 412 nm. The mixture took on a yellow hue, which was an indication that the 5-thio-2-nitrbenzoate anion was formed as a consequence of the interaction between the thiocholines and the DNTB. A control reaction mixture without enzymes and samples was also looked at to investigate the non-enzymatic hydrolysis of the substrate. Using the supplied formulae, enzyme activity and percent inhibition were computed.

$$V = \frac{V_{\text{abs}}}{Dt}$$

$$\%\ \text{Age enzyme activity} = \frac{V}{V_{\max}} \times 100$$

$$\%\ \text{Age enzyme activity} = 100 - \%\ \text{enzyme activity}$$

where, V = the rate of reaction in the presence of inhibitor. $V_{\max}$ = the rate of reaction without inhibitor.

3.6. Molecular docking simulations

Molecular docking was performed on Molecular Operating Environment (MOE version 2020.0901)[36,37] by using the three-dimensional structures of electric eel AChE (**https://alphafold.com/entry/O42275**) and equine BChE (**https://alphafold.com/entry/P81908**) from AlphaFold protein structure database (**https://alphafold.com/**). Both the structures were treated by MOE's QuickPrep module to add hydrogen atoms and to calculate partial charges (according to AMBER10:EHT force field). *Tetronarce californica* acetylcholinesterase (PDB code: 1DX6) in complex with galantamine was taken from Protein data bank and superimposed with both the models to deduce the binding configuration of galantamine in electric eel AChE and equine BChE. Active site was defined as 3.5 Å vicinity of galantamine binding region to dock the compounds. The ligands were drawn on MOE and partial charges (with MMFF-94x forcefield) were added on ligands, and each structure was minimized with gradient of 0.1 RMS kcal mol^{-1} Å^{-1}. After preparing ligands and protein files, the selected compounds were docked at the active site (around 3 Å of galantamine binding region) by the Triangle Matcher docking algorithm and London dG scoring function, and 30 conformations of each ligand were saved. After docking, the protein ligand interactions were analyzed on MOE's interface and diagrams are illustrated by MOE. The 2D-interactions of docked molecules within the active site of acetyl and butyryl cholinesterase enzymes are shown in ESI (Fig. S82 and S83†).

4 Conclusion

Using straightforward, simple, varied, and sequential synthetic approach, **25** (**4a–y**) novel supramolecules comprising of bisphenol sulfide and hydrazone functionalities were synthesized. Herein, DMF, methanol, and acetic acid are found as the most efficient solvents for these alterations. Phenolic hydroxy was often used for synthetic modifications (CH_3COOH). The synthesized functionalities were tested *in vitro* for their potential cholinergic activities by blocking acetylcholinesterase and butyrylcholinesterase enzymes. Among the synthesized hits, **4n**/**4h** and **4b**/**4h** exhibited excellent inhibitory potential for AChE and BChE, respectively. While the starting compound, **SM3** and compound **4h** have dual inhibitory capabilities for AChE and BChE. In addition, the molecular docking results of most active (**4h** and **SM3**), intermediate (**4i** and **4k**), and least active (**4q**) inhibitors of both AChE and BChE shows strong correlation

with our *in vitro* findings. The results indicate a strong potential for bis(acylhydrazones) as promising drug candidates for the treatment of Alzheimer's disease.

Author contributions

All authors declare that they have all participated in the design, execution, and analysis of the paper and approved the final version.

Conflicts of interest

There is no conflict of interest.

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