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Synthesis, molecular docking studies, and biological evaluation of novel alkyl bis(4-amino-5-cyanopyrimidine) derivatives

Imen Boualia[1] | Chamseddine Derabli[1] | Raouf Boulcina[1,2] | Chawki Bensouici[3] | Muhammet Yildirim[4] | Arzu Birinci Yildirim[5] | El Hassen Mokrani[6] | Abdelmadjid Debache[1]

[1]Laboratory of Synthesis of Molecules with Biological Interest, Mentouri–Constantine University, Constantine, Algeria

[2]Faculty of Technology, Batna 2 University, Batna, Algeria

[3]Biotechnology Research Center, Constantine, Algeria

[4]Department of Chemistry, Faculty of Sciences and Arts, Abant Izzet Baysal University, Bolu, Turkey

[5]Department of Field Crops, Faculty of Agriculture and Natural Sciences, Abant İzzet Baysal University, Bolu, Turkey

[6]Department of Biochemistry and Cellular and Molecular Biology, Laboratory of Applied Biochemistry, Faculty of Natural and Life Sciences, Mentouri–Constantine University, Constantine, Algeria

Correspondence
Dr. Raouf Boulcina, Laboratory of Synthesis of Molecules with Biological Interest, Mentouri–Constantine University, 25000 Constantine, Algeria.
Email: r.boulcina@univ-batna2.dz

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Abstract

A series of bis(4-amino-5-cyano-pyrimidines) was synthesized and evaluated as dual inhibitors of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). To further explore the multifunctional properties of the new derivatives, their antioxidant and antibacterial activities were also tested. The results showed that most of these compounds could effectively inhibit AChE and BChE. Particularly, compound **7c** exhibited the best AChE inhibitory activity ($IC_{50} = 5.72 \pm 1.53\ \mu M$), whereas compound **7h** was identified as the most potent BChE inhibitor ($IC_{50} = 12.19 \pm 0.57\ \mu M$). Molecular modeling study revealed that compounds **7c**, **7f**, and **7b** showed a higher inhibitory activity than that of galantamine against both AChE and BChE. Anticholinesterase activity of compounds **7h**, **7b**, and **7c** was significant in vitro and in silico for both enzymes, since these compounds have hydrophobic rings (Br-phenyl, dimethyl, and methoxyphenyl), which bind very well in both sites. In addition to cholinesterase inhibitory activities, these compounds showed different levels of antioxidant activities. Indeed, in the superoxide–dimethyl sulfoxide alkaline assay, compound **7j** showed very high inhibition ($IC_{50} = 0.37 \pm 0.28\ \mu M$). Also, compound **7l** exhibited strong and good antibacterial activity against *Staphylococcus epidermidis* and *Staphylococcus aureus*, respectively. Taking into account the results of biological evaluation, further modifications will be designed to increase potency on different targets. In this study, the obtained results can be a new starting point for further development of multifunctional agents for the treatment of Alzheimer's disease.

KEYWORDS
alkyl bis(4-amino-5-cyano-pyrimidines), antibacterial activity, anticholinesterase activity, antioxidant effect, molecular docking studies

1 | INTRODUCTION

Alzheimer's disease (AD) as a neurodegenerative disorder is manifested by progressive deterioration of intellectual and cognitive functions, memory loss, and personality changes.[1,2] One of the major therapeutic strategies adopted for primarily symptomatic AD is based on the cholinergic hypothesis targeting cholinesterase enzymes (acetylcholinesterase and butyrylcholinesterase).[3] Inhibition of the hydrolysis of acetylcholine by blocking its metabolic enzyme acetylcholinesterase (AChE) increases the ACh concentration and provides a possible symptomatic treatment option for AD. On the contrary, butyrylcholinesterase (BuChE) has recently been considered as a potential target because it also plays an important role in regulating ACh level.[4] However, due to the complex pathologies of AD, the current AChE inhibitors such as donepezil and galantamine (Figure 1) that modulate a single target can only improve clinical symptoms but

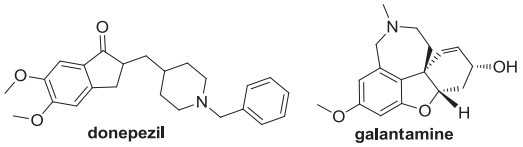


FIGURE 1 Acetylcholinesterase inhibitors in use

cannot mitigate the progression of the disease process underlying the AD.[5] As reported in some earlier studies, inhibition of butyrylcholinesterase (BuChE) represents an important therapeutic target for AD. It has also been recently reported that dual inhibition of AChE and BuChE might alleviate AD symptoms owing to the key role of BuChE in hydrolysis of ACh. It is essential to ensure that the drug inhibits both AChE and BuChE.[6] Moreover, oxidative injury caused by free radical formation and iron accumulation has been revealed to be another factor in AD pathogenesis.[7]

On the contrary, the pyrimidine core is found in many natural products and pharmaceuticals, which display a range of significant biological properties, such as phosphatidyl inositol 3-kinase inhibitors,[8] A2A receptor antagonists,[9] anti-HIV,[10] and antibacterial.[11,12]

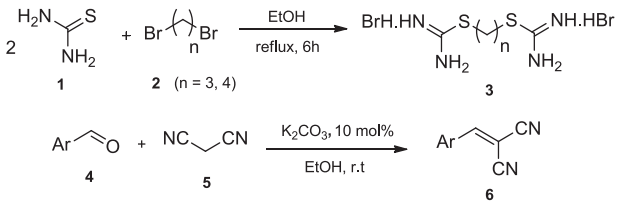
Therefore, many researchers have contributed to the development of efficient strategies for their construction. Pyrimidines have been synthesized via one-pot three-component coupling of terminal alkynes, elemental sulfur, and carbodiimides.[13] In addition, pyrimidine derivatives have been obtained from a single-step reaction[14] or by a titanium-catalyzed one-pot multicomponent coupling reaction.[15] Also, a new series of pyrimidine derivatives has been synthesized via tandem aza-Wittig and annulation reactions.[16]

As per our continuous interest in the synthesis of the biologically important heterocycles, herein, we further report a simple, and cost-effective, and high-yielding method for synthesis of a novel series of highly functionalized 2-amino-3-cyano-4-aryl-6-sulfapyrimidines as cholinesterase inhibitors. In addition, molecular docking analysis was also performed to disclose the binding interaction template of the most active inhibitors to the amino acid residues composing the active sites of the AChE and butyrylcholinesterase (BChE) enzymes and the findings are presented below. Antioxidant activity and antibacterial effects of these compounds are also discussed.

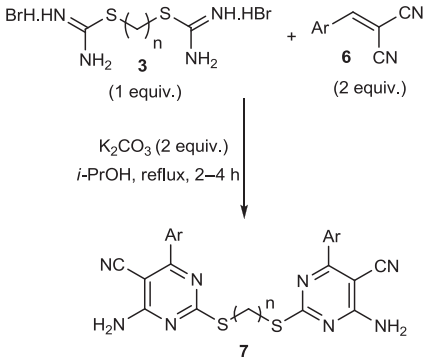
2 | RESULTS AND DISCUSSION

2.1 | Synthesis

The synthesis of the intermediates and target compounds was accomplished according to the steps depicted in Schemes 1 and 2. The present synthetic strategy begins with the synthesis of 2-alkylisothiouronium bromides **3** that were not commercially available, and were thus obtained from the condensation of thiourea **1** with the corresponding alkyl bromides **2** by prolonged heating of the mixture in ethanol at reflux. On the contrary, the condensation between various substituted benzaldehydes **4** and malononitrile **5** gave rise to the formation of



SCHEME 1 Preparation of 2-alkylisothiouronium bromides **3** and arylidenemalononitriles **6**



SCHEME 2 Synthesis of bis(4-amino-5-cyano-pyrimidines) **7**

2-arylidenemalononitriles **6** in the presence of a catalytic amount of potassium carbonate in ethanol at room temperature (Scheme 1).

For the synthesis of compound **7**, the condensation of the precursors **3** and **6** was carried out using various catalysts under different conditions. At the outset of our synthetic work, we attempted to find a feasible and efficient pathway to pyrimidine formation under mild conditions. In the first trial experiment, a reaction between 2-benzylidenemalononitriles **6a** and 2-propylisothiouronium bromide **3a** in ethanol was left at room temperature for 24 hr. After simple work-up, the corresponding product was isolated in very poor amounts. The use of K_2CO_3 as a catalyst in ethanol at reflux facilitated the reaction to some extent, but none exceeded the yield of 50%. However, the reaction was most efficient when two equivalents of K_2CO_3 was used in isopropanol under reflux.

Using the most efficient condition established above, we next attempted to extend the process to a series of 2-alkylisothiouronium bromides **3** and 2-arylidenemalononitriles **6** having electron-withdrawing and electron-donating groups. The results showed that most of the reactions afforded the desired products (**7a–p**) in good yields, indicating that the designed synthetic method using K_2CO_3 as catalyst was very appropriate (Table 1).

^{1}H-NMR spectra of compounds **7** revealed the NH_2 protons to be a singlet at δ 5.71–6.09 ppm, CH_2 groups protons were at δ 1.78–3.79 ppm, and aromatic protons were in the range of δ 7.01–8.63 ppm. The structures of all the newly synthesized compounds were confirmed by the infrared, ^{1}H NMR, ^{13}C NMR, and HRMS spectral data, which were in full agreement with their structures.

TABLE 1 Synthesis of bis(4-amino-5-cyano-pyrimidines) **7a–p**

Entry	Ar	*n*	Product	Time (hr)	Yield[a] (%)
1	C_6H_5	3	**7a**	1	65
2	4-(Me)-C_6H_4	3	**7b**	3	58
3	3-(MeO)-C_6H_4	3	**7c**	4	67
4	4-(MeO)-C_6H_4	3	**7d**	3	64
5	4-NO_2-C_6H_4	3	**7e**	3	64
6	3-NO_2-C_6H_4	3	**7f**	4	79
7	4-Cl-C_6H_4	3	**7g**	3	70
8	4-Br-C_6H_4	3	**7h**	4	69
9	C_6H_5	4	**7i**	4	63
10	4-Me-C_6H_4	4	**7j**	4	62
11	3-(MeO)-C_6H_4	4	**7k**	3	73
12	4-(MeO)-C_6H_4	4	**7l**	4	68
13	4-NO_2-C_6H_4	4	**7m**	2.5	68
14	3-NO_2-C_6H_4	4	**7n**	3	69
15	4-Cl-C_6H_4	4	**7o**	4	60
16	4-Br -C_6H_4	4	**7p**	3	72

[a]Yields of pure isolated products.

2.2 | In vitro cholinesterases inhibition studies

In a very recent study, Xu et al.[17] studied the BACE1 activity of 4-amino-5-cyano-pyrimidine compounds, and they found at least three derivatives, which were very similar to our scaffolds, had very strong BACE1 inhibition effects with very low IC_{50} levels. For this purpose, we study herein the anti-ChE activity of bis(4-amino-5-cyanopyrimidine) derivatives.

We selected arbitrarily ten bis(4-amino-5-cyano-pyrimidines) **7**, which were tested for their in vitro inhibitory activities against AChE from *Electrophorus electricus* (eel-AChE) and BChE from horse serum (eqBChE) according to the Ellman method with some modifications.[18] The efficacy is expressed as IC_{50} values, representing the concentration of an inhibitor required to decrease the enzymatic activity by 50%. The results were compared with those obtained for galantamine, a clinically used ChE inhibitor which acts as a competitive inhibitor.

As shown in Table 2, derivatives of **7** exhibited AChE inhibitory activities with IC_{50} values ranging from 5.72 ± 1.53 to 35.08 ± 0.55 μM. The strongest inhibitory activity towards AChE and BChE was displayed by bis-pyrimidine hybrids with a three-carbon atom linker. The elongation of the tether led to a decreased activity, except for compound **7k,** which gave good activity.

On the contrary, the chemical nature of the substituents as well as their positions on the benzene ring greatly influenced the activity. In general, the inhibitory potency of the bis-pyrimidine derivatives against AChE was as follows: *meta*-substituted > *para*-substituted. However, the inhibitory potency against BChE was reversed: *para*-substituted > *meta*-substituted. In addition, the inhibitory activity against AChE was highly dependent on the nature of the ring substituent. Compounds containing a methoxyl or nitro group possessed much higher activity than did those compounds containing a bromo group. Among these compounds, **7c**, bearing a *m*-methoxyphenyl group at position 4 of the pyrimidine ring, showed the highest AChE inhibitory activity, followed by **7f**, with a *m*-nitrophenyl group at position 4 of the pyrimidine ring, with IC_{50} values of 5.72 ± 1.53 and 9.09 ± 1.66 μM, respectively.

Similarly, these compounds showed BChE inhibitory activity, with IC_{50} values ranging from 12.19 ± 0.57 to 123.09 ± 1.14 μM. Compound **7h** bearing a *p*-bromophenyl group at position 4 of the pyrimidine ring, with an IC_{50} value of 12.19 ± 0.57, had a higher inhibitory activity than that of galantamine (IC_{50} = 31.77 ± 2.22 μM), whereas other compounds such as **7f** and **7c** had similar or lower inhibitory effects with IC_{50} values of 32.32 ± 1.42 and 44.41 ± 2.32 μM, respectively.

The inhibition selectivity of the newly synthesised compounds was determined for AChE and BChE (Table 2). Overall, all of the compounds had a good preference for AChE, among which three compounds displayed a 5–10 times higher inhibition selectivity toward AChE over BChE. The most selective AChE inhibitor was compound **7k**, followed by **7c** and **7b**, whose affinities were 10.11, 7.76, and 5.72 times higher than that for BChE, respectively. The lowest inhibition selectivity, only by 0.77 and 1.6 times, was obtained for compounds **7h** and **7n**, respectively.

2.3 | Molecular docking studies

Molecular docking calculations of the designed compounds into the active sites of AChE and BChE were performed using FlexX, which is based on an incremental construction of ligands.

First, the binding energy was predicted between the synthesized compounds and both AChE and BChE active sites using molecular docking. From Table 2 it appears that the experiment data of in vitro ChE inhibitory activity showed a satisfactory agreement with the molecular docking results. According to their binding energies (ΔG) and their experimental IC_{50} values, compounds **7c**, **7f**, and **7b** were found to exhibit a higher inhibitory activity than that of galantamine against both AChE and BChE (see Table 2).

The most promising inhibitors **7c** and **7h** from in vitro assays were selected for further investigations of their binding mode with AChE and BChE active sites respectively. As shown in Figure 2, **7c** fit well the entrance until the bottom of AChE-active gorge making five hydrogen bonds.

The first pyrimidine ring was involved in three hydrogen bonds, two of them by its carbonitrile group with Ser293 and one by its amine group with Trp289. The amine group of the second ring forms the fourth hydrogen bond with Tyr341 while the last hydrogen bond was observed between the carbonitrile group of the same ring and Phe295. It should be noted that the two methoxyphenyl rings performed an important role in stabilization of **7c** inside the pocket by making several hydrophobic interactions with Tyr124, Tyr337, Tyr341, Phe338, Phe297, Ser293, Val294, Gly342, and Glu292.

On the contrary, **7h** covers the entire BChE active site pocket making four hydrogen bonds (Figure 3). Two of them were observed

TABLE 2 The inhibitory concentration (IC_{50}), calculated binding energy and selectivity of selected bis(4-amino-5-cyano-pyrimidines) **7** investigated against AChE and BChE

	AChE			BChE		
Compound	IC_{50} (μM)[a]	Selectivity[b]	Binding energy (kJ/mol)	IC_{50} (μM)[a]	Selectivity[c]	Binding energy (kJ/mol)
7b	11.37 ± 2.12	5.72	−21.88	65.05 ± 1.14	0.17	−25.68
7c	5.72 ± 1.53	7.76	−32.11	44.41 ± 2.32	0.13	−33.39
7e	32.43 ± 0.61	3.80	−19.14	123.09 ± 1.14	0.26	−18.94
7f	9.09 ± 1.66	3.56	−30.66	32.32 ± 1.42	0.28	−34.52
7h	15.92 ± 0.32	0.77	−18.10	12.19 ± 0.57	1.31	−33.78
7j	17,58 ± 0.07	4.57	−19.34	80.33 ± 0.17	0.22	−20.58
7k	9.40 ± 0.16	10.11	−29.56	95.08 ± 0.56	0.099	−20.88
7m	17.26 ± 0.60	4.28	−20.15	73.95 ± 0.75	0.24	−19.85
7n	35.08 ± 0.55	1.60	−18.30	55.96 ± 0.07	0.63	−32.48
7p	30.42 ± 0.11	2.03	−20.96	61.78 ± 0.44	0.49	−18.84
Galantamine	21.82 ± 4.00	5.54	−18.27	31.77 ± 2.22	0.18	−20.97

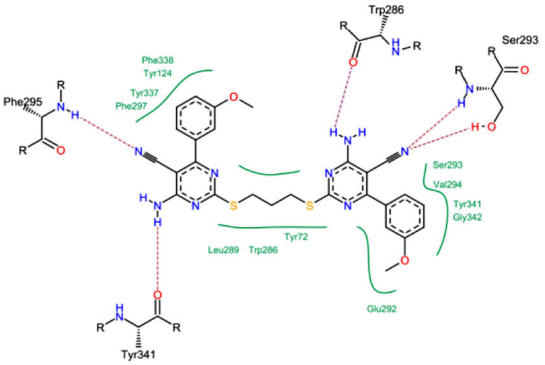
Abbreviations: AChE, acetylcholinesterase; BChE, butyrylcholinesterase; SD, standard deviation.
[a]IC_{50} values expressed are means ± SD of three parallel measurements ($p < 0.05$).
[b]Selectivity for AChE defined as IC_{50}(BChE)/IC_{50}(AChE).
[c]Selectivity for BChE defined as IC_{50}(AChE)/IC_{50}(BChE).

between the amine group of the first ring and the two residues Ser287 and Thr284. The two other hydrogen bonds were formed between the carbonitrile group of the second ring and the two residues Gly116 and His448 of BChE. This last residue is considered as a critical member of the BChE catalytic triad.[19]

7h was well stabilized in the BChE active pocket because of its two Br-phenyl rings making hydrophobic interactions with Tyr332, Asp70, Gly117, Gly116, Phe329, Phe398, Ser198, Pro285, and His448.

2.4 | Antioxidant activity studies

Oxidative stress is critical to the etiology of many chronic and degenerative diseases such as cancer, cardiovascular diseases, diabetes, obesity, epilepsy, and AD.[20] Therefore, the synthesis of compounds with antioxidant potential has received great attention from researchers.[21] Thus, the antioxidant capabilities of the synthesized compounds were also evaluated using cupric reducing antioxidant capacity (CUPRAC) assay, reducing power as well as β-carotene, and superoxide–DMSO alkaline methods. For CUPRAC, compound **7p** showed the most promising antioxidant effect with an IC_{50} value of 25.57 ± 0.60 μM (Table 3), that is approximately equal in efficacy to butylated hydroxytoluene (BHT; IC_{50} = 24.28 ± 3.22 μM) and two-fold more effective than butylated hydroxyanisole (BHA; IC_{50} = 36.62 ± 0.27 μM), followed by **7j** with IC_{50} = 26.50 ± 1.51 μM. α-Tocopherol and ascorbic acid were used as standards for inhibition in reducing power assay and the results are given as the $A_{0.50}$ value. According to this method, all tested compounds did not show any significant activity. On the contrary, **7f** (IC_{50} = 4.91 ± 0.32 μM) and **7b** (IC_{50} = 9.5 ± 1.30 μM) for inhibition in β-carotene assay, had the



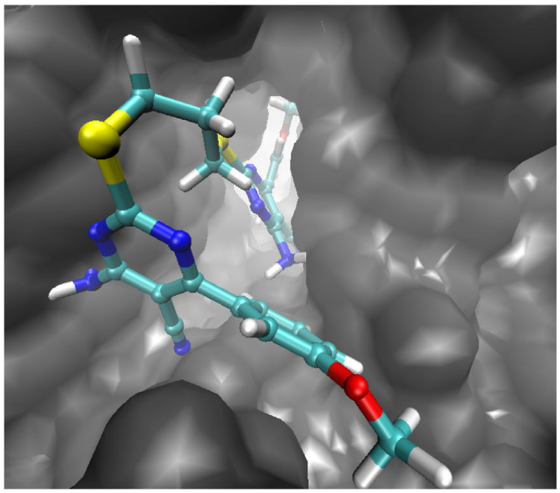


FIGURE 2 Binding mode prediction of **7c** into the acetylcholinesterase active site

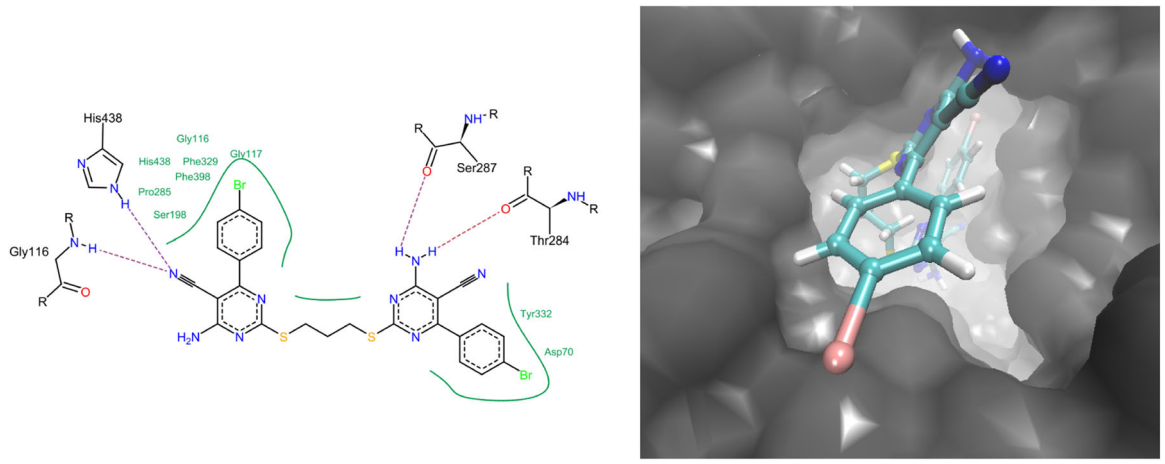


FIGURE 3 Binding mode prediction of **7h** into the butyrylcholinesterase active site

highest IC_{50} values among the selected products. Also, the compound **7j** (IC_{50} = 0.37 ± 0.28 μM), followed by **7p** (IC_{50} = 18.57 ± 1.22 μM) and **7m** (IC_{50} = 23.23 ± 1.33 μM) showed the best inhibition for superoxide–DMSO alkaline assay (Table 3).

2.5 | Antibacterial activity studies

Recently, the emergence of bacterial resistance to famous antibiotics (β-lactams, cephalosphorins, and quinolones) due to their prolonged use has generated some risks for patients like high mortality, longer hospital stays and costs, and risk of secondary infections.[22,23] Therefore, development of new antimicrobial agent candidates has been the prime purpose of researchers working in the area of medicinal chemistry and drug industry for the last two decades. With this design, the Kirby–Bauer (disk diffusion) method[24] was used for the evaluation of the susceptibility of the synthesized bis-pyrimidines (**7**) to different bacteria. The antibacterial properties of compounds **7a**, **7d**, **7g**, **7i**, **7l**, and **7o** were tested against 10 bacterial strains (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Serratia marcescens*, *Proteus vulgaris*, *Enterobacter cloacae*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Staphylococcus epidermidis* , and *Streptococcus pyogenes*; Table 4).

According to the test results, none of the compounds (**7**) exhibited antibacterial effects against any Gram-negative bacteria.

TABLE 3 Antioxidant activity of selected bis(4-amino-5-cyano-pyrimidines) **7** using CUPRAC, reducing power, β-carotene, and superoxide–DMSO alkaline assays

	$A_{0.50}$ (μM)[a]		IC_{50} (μM)[a]	
Compound	CUPRAC assay	Reducing power assay	β-Carotene assay	Superoxide anion-scavenging assay
7b	238.42 ± 0.46	270.38 ± 0.10′	9.5 ± 1.30	238.96 ± 0.46
7c	231.90 ± 0.22	>200	15.48 ± 0.32	236.72 ± 0.74
7e	201.64 ± 0.63	298.16 ± 0.22	10.44 ± 0.82	57.19 ± 0.61
7f	203.24 ± 1.66	222.81 ± 0.82	4.91 ± 0.32	81.11 ± 1.89
7h	338.36 ± 0.72	>200	32.01 ± 0.55	210.35 ± 0.67
7j	26.50 ± 1.51	>200	20.07 ± 1.06	0.37 ± 0.28
7k	179.53 ± 0.95	259.49 ± 1.12	160.95 ± 1.61	410.25 ± 1.44
7m	177.05 ± 0.15	>200	25.58 ± 0.25	23.23 ± 1.33
7n	400.71 ± 1.63	>200	80.75 ± 1.83	331.02 ± 0.42
7p	25.57 ± 0.60	213.30 ± 0.20	17.19 ± 0.38	18.57 ± 1.22
BHT[b]	24.28 ± 3.22	>200	4.77 ± 0.05	>200
BHA[b]	36.62 ± 0.27	44.32 ± 4.82	4.99 ± 0.11	>200
α-Tocopherol	46.24 ± 3.38	81.10 ± 5.53	4.15 ± 0.06	52.17 ± 5.87
Ascorbic acid	70.57 ± 0.51	38.44 ± 6.53	52.59 ± 1.98	27.76 ± 1.48

Abbreviations: BHA, butylated hydroxyanisole; BHT, butylated hydroxytoluene; CUPRAC, cupric reducing antioxidant capacity; DMSO, dimethyl sulfoxide, SD, standard deviation.

[a]IC_{50} and $A_{0.50}$ values expressed are means ± SD of three parallel measurements ($p < 0.05$).

[b]Reference compounds.

However, only compound **7l** exhibited a strong antibacterial effect against *S. epidermidis* (17–18 mm) and a good antibacterial effect against *S. aureus* (13–12 mm) among Gram-positive bacteria. Compound **7l** exhibited a higher inhibition zone than standard antibiotic, tetracycline (12 mm) against *S. epidermidis* but it exhibited a lower inhibition zone than standard antibiotic, erytromycin (45 mm) against *S. epidermidis*. Besides this, the inhibition zone of compound **7l** (13–12 mm) against *S. aureus* was much lower than that of the antibiotics, tetracyline (33 mm) and erytromycin (32 mm) against *S. aureus*. Regarding the antibacterial activity, results against *S. epidermidis* and *S. aureus* show compound **7l** can be a candidate antimicrobial agent for the treatment of implanted prostheses infections in human body and for the treatment of various localized and diffused skin infections, respectively.

3 | CONCLUSION

In the present study, we report the development of a novel library of substituted bis(4-amino-5-cyano-pyrimidines) as multitargeting agents that exhibit cholinesterase inhibition as well as antioxidant properties. Ten of the target compounds **7** exhibited varying levels of dual ChE inhibitions, whereas the other derivatives exhibited good antioxidant properties.

Compound **7c** exhibited high AChE inhibition (IC_{50} = 5.72 ± 1.53 μM); compound **7h** (IC_{50} = 12.19 ± 0.57 μM) was considered as the most potent BChE inhibitor, whereas compound **7f** exhibited dual BChE inhibition (IC_{50} = 9.09 ± 1.66 μM) and very high antioxidant activity (AA) in the β-carotene assay (IC_{50} = 4.91 ± 0.32 μM). Molecular modeling study revealed that compounds **7c**, **7f**, and **7b** showed a higher inhibitory activity than the galantamine against both AChE and BChE. Among all tested compounds for AA in the superoxide–DMSO alkaline assay, compound **7j** showed very high inhibition (IC_{50} = 0.37 ± 0.28 μM). Besides this, only compound **7l** showed strong and good antibacterial effects against *S. epidermidis* and *S. aureus* among all tested compounds. These comprehensive studies demonstrated the potential application of 4-disubstituted pyrimidines as a suitable template to develop multitargeting agents for the treatment of AD.

4 | EXPERIMENTAL

4.1 | Chemistry

4.1.1 | General

All the chemicals were purchased from the Sigma-Aldrich and used without further purification. All solvents used for spectroscopic and synthesis studies were of reagent grade and further purified by literature methods. Thin-layer chromatography (TLC) was performed using a mixture of EtOAc and hexane (1:2) as eluents and silica gel 60 F_{254} (Merck) plates. Melting points (mp) were determined on an Electrothermal capillary fine-control apparatus. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 instrument at 400 and 100 MHz respectively, in $CDCl_3$ or DMSO-d_6. Chemical shifts (δ) are given in parts per million downfield from TMS as an internal standard for ^{1}H and ^{13}C NMR. Coupling constants (J) values were indicated in Hz. The chemicals were used as obtained commercially. High-resolution mass spectra (HRMS) were recorded with a high-resolution time-of-flight mass spectrometer coupled with a liquid chromatography system (Agilent 1200/6210) or MicroTof–Q 98. Elemental analyses were carried out on a Microanalyzer Flash EA1112 CHNS/O Thermo Electron.

The InChI codes of the investigated compounds together with some biological activity data are provided as Supporting Information.

4.1.2 | General procedure for the preparation of 2,2′-(alkane-1,3-diyl)bis(thiouronium) (3)

A mixture of thiourea (2 mmol) and alkyl bromide (1.2 mmol) in EtOH (5 ml) was stirred overnight under reflux, the mixture was cooled to

TABLE 4 Antibacterial activities of selected bis(4-amino-5-cyano-pyrimidines) **7**

Mean diameter of inhibitory zones[a]									
	Compounds						Positive controls		Negative control
Bacteria	7a	7d	7g	7i	7l	7o	Erythromycin	Tetracycline	(dimethyl sulfoxide)
Escherichia coli	–	–	–	–	–	–	15	30	–
Pseudomonas aeruginosa	–	–	–	–	–	–	–	16	–
Salmonella typhimurium	–	–	–	–	–	–	12	21	–
Serratia marcescens	–	–	–	–	–	–	12	17	–
Proteus vulgaris	–	–	–	–	–	–	12	27	–
Enterobacter cloacae	–	–	–	–	–	–	–	30	–
Klebsiella pneumoniae	–	–	–	–	–	–	14	27	–
Staphylococcus aureus	–	–	–	–	12–13	–	32	33	–
Staphylococcus epidermidis	–	–	–	–	17–18	–	45	12	–
Streptococcus pyogenes	–	–	–	–	–	–	–	–	–

[a]Data presented as zone of inhibition of bacterial growth in mm.

room temperature and evaporated. The final product was used in the next step without further purification.

*Propane-1,3-diyldicarbamimidothioate dihydrobromide (**3a**)*
Compound **3a** was obtained as a white solid (95%). Mp: 218°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.11 (s, 8H, 4NH_2), 3.28 (t, J = 7.3 Hz, 2H, CH_2), and 1.94 (q, J = 7.3 Hz, 4H, 2CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 170.0, 29.2, and 29.0.

*Butane-1,4-diyldicarbamimidothioate dihydrobromide (**3b**)*
Compound **3b** was obtained as a white solid (97%). Mp: 232°C. ^{1}H NMR (300 MHz, DMSO-d_6) δ (ppm): 9.08 (s, 8H, 4NH_2), 3.24–3.21 (m, 4H, 2CH_2), and 1.70–1.68 (m, 4H, 2CH_2). ^{13}C NMR (75 MHz, DMSO-d_6) δ (ppm): 170.3, 29.9, and 27.7.

4.1.3 | General procedure for the preparation of bis (4-amino-5-cyano-pyrimidines) (7)

In a 100 ml flask were introduced 2-alkylthiouronium (1 mmol) and 2-(arylidene)malononitrile derivative (2 mmol) in the presence of K_2CO_3 (2 mmol). The mixture was refluxed in 10 ml of isopropanol. The progress of the reaction was monitored by TLC. After completion of the reaction, the mixture was cooled to room temperature and was poured into 100 ml of water. The precipitate was obtained by vacuum filtration. The resulting solid residue was recrystallized from ethanol.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-phenylpyrimidine-5-carbonitrile) (**7a**)*
Compound **7a** was obtained as a white solid (65%). Mp: 228–230°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.81 (dd, J = 6.8, 1.6 Hz, 4H), 7.57–7.48 (m, 6H), 3.23 (t, J = 6.8 Hz, 4H, 2CH_2), and 2.10 (qt, J = 6.8 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.9, 167.7, 164.0, 136.4, 131.1, 128.9, 128.8, 116.7, 83.9, and 29.7. Anal. calcd for $C_{25}H_{20}N_8S_2$: C, 60.46; H, 4.06; N, 22.56; S, 12.91; Found: C, 59.86; H, 4.05; N, 22.07, S, 12.30.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-p-tolylpyrimidine-5-carbonitrile) (**7b**)*
Compound **7b** was obtained as a beige solid (58%). Mp: >250°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 7.89 (d, J = 8.2 Hz, 4H), 7.23 (d, J = 8.2 Hz, 4H), 3.23 (t, J = 5.9 Hz, 4H, 2CH_2), 2.23 (s, 6H, 2CH_3), and 2.14 (qt, J = 5.9 Hz, 2H, CH_2). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.7, 167.2, 164.1, 141.3, 135.9, 135.5, 135.1, 133.4, 129.1, 128.7, 119.8, 116.9, 82.6, 29.6, 29.4, and 20.9. HRMS (APCI$^-$) m/z 523.15653 [M−H]$^+$. Anal. calcd for $C_{27}H_{23}N_8S_2$: 523.15379.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(3-methoxyphenyl)pyrimidine-5-carbonitrile) (**7c**)*
Compound **7c** was obtained as a white solid (67%). Mp: 222°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 7.75–7.72 (m, 6H), 7.14–7.12 (m, 2H), 6.0 (s, 4H, 2NH_2), 3.79 (s, 6H, 2CH_3-O), 3.23 (t, J = 6.2 Hz, 4H, 2CH_2), and 2.13 (qt, J = 6.2 Hz, 2H, CH_2). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.5, 167.2, 163.5, 159.2, 137.4, 129.9, 116.9, 116.5, 113.9, 82.8, 55.5, 29.5, and 29. HRMS (APCI$^-$) m/z 555.14636 [M−H]$^+$. Anal. calcd for $C_{27}H_{23}N_8O_2S_2$: 555.13685.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile) (**7d**)*
Compound **7d** was obtained as a white solid (64%). Mp: >250°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.85 (dd, J = 6.8, 2 Hz, 4H), 7.03 (dd, J = 6.8, 2 Hz, 4H), 3.79 (s, 6H, 2CH_3-O), 3.23 (t, J = 6.8 Hz, 4H, 2CH_2), and 2.09 (qt, J = 6.8 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.4, 166.7, 163.9, 162.1, 130.7, 128.4, 117.1, 114.3, 81.9, 55.9, and 29.6. HRMS (ESI$^+$) m/z 595.1121 [M+K]$^+$. Anal. calcd for $C_{27}H_{24}N_8O_2S_2K$: 595.1101.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(4-nitrophenyl)-pyrimidine-5-carbonitrile) (**7e**)*
Compound **7e** was obtained as a brown solid (64%). Mp: >250°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 8.28 (d, J = 8.7 Hz, 4H), 8.05 (d, J = 8.7 Hz, 4H), 5.79 (s, 4H, 2NH_2), 3.20 (t, J = 6.1 Hz, 4H, 2CH_2), and 2.11 (qt, J = 6.1 Hz, 2H, CH_2). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.9, 165.2, 163.2, 148.7, 141.9, 129.9, 123.5, 119.5, 115.8, 83.3, 29.4, and 29.1. HRMS (APCI$^-$) m/z 585.09539 [M−H]$^+$. Anal. calcd for $C_{25}H_{17}N_{10}O_4S_2$: 585.09021.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(3-nitrophenyl)-pyrimidine-5-carbonitrile) (**7f**)*
Compound **7f** was obtained as a brown solid (79%). Mp: 238°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 8.63 (t, J = 8.3 Hz, 2H), 8.37 (d, J = 8.0 Hz, 2H), 8.34 (d, J = 7.7 Hz, 2H), 7.79 (t, J = 8.0 Hz, 2H), 6.02 (s, 4H, 2NH_2), 3.09 (t, J = 6.2 Hz, 4H, 2CH_2), and 2.17 (qt, J = 6.2 Hz, 2H, CH_2). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.9, 164.5, 163.2, 147.6, 137.3, 134.5, 129.1, 125.3, 116.1, 115.8, 82.8, 29.3, and 29.1. HRMS (APCI$^-$) m/z 585.09539 [M−H]$^+$. Anal. calcd for $C_{25}H_{17}N_{10}O_4S_2$: 585.09214.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(4-chlorophenyl)-pyrimidine-5-carbonitrile) (**7g**)*
Compound **7g** was obtained as a white solid (70%). Mp: >250°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.83 (dd, J = 6.8, 2 Hz, 4H), 7.56 (dd, J = 6.8, 2.0 Hz, 4H), 3.22 (t, J = 6.8 Hz, 4H, 2CH_2), and 2.08 (qt, J = 6.8 Hz, 2H, CH_2). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.8, 166.4, 163.6, 136.4, 135.1, 130.8, 129.0, 116.5, 82.9, 29.7, and 29.4. HRMS (ESI$^+$) m/z 603.0128 [M+K]$^+$. Anal. calcd for $C_{25}H_{18}Cl_2N_8S_2K$: 603.0110.

*2,2′-(Propane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(4-bromophenyl) pyrimidine-5-carbonitrile) (**7h**)*
Compound **7h** was obtained as a white solid (69%). Mp: >250°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 7.77 (d, J = 8.6 Hz, 4H), 7.61 (d, J = 8.6 Hz, 4H), 5.71 (br s, 4H, 2NH_2), 3.22 (t, J = 6.8 Hz, 4H, 2CH_2), and 2.12 (qt, J = 6.8 Hz, 2H, CH_2). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.5, 165.8, 163.3, 134.9, 131.3, 130.2, 124.9, 116.0, 82.3, 29.2, and 29. HRMS (APCI$^-$) m/z 650.94626 [M−H]$^+$. Anal. calcd for $C_{25}H_{17}Br_2N_8S_2$: 650.93832.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-phenylpyrimidine-5-carbonitrile) (**7i**)*

Compound **7i** was obtained as a white solid (63%). Mp: >250°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.79 (d, J = 6.8 Hz, 4H), 7.54–7.47 (m, 6H), 3.12 (s, 4H), and 1.78 (s, 4H). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.9, 166.4, 163.8, 136.4, 135.4, 130.5, 128.9, 116.3, 82.7, 30.2, 28.9, and 26.0. HRMS (ESI$^+$) m/z 549.1074 [M+K]$^+$. Anal. calcd for $C_{26}H_{22}N_8S_2K$: 546.1046.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-p-tolylpyrimidine-5-carbonitrile) (**7j**)*

Compound **7j** was obtained as a beige solid (62%). Mp: 140°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 7.87 (d, J = 8.0 Hz, 4H), 7.44 (d, J = 8.0 Hz, 4H), 3.23–3.18 (m, 4H, 2CH$_2$), 2.31 (s, 6H, 2CH$_3$), and 1.82–1.79 (m, 4H, 2CH$_2$). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.5, 166.5, 161.3, 145.8, 130.7, 130.2, 129.3, 128.8, 116.3, 82.8, 29.9, 29.1, and 21.5. HRMS (APCI$^-$) m/z 537.17218 [M−H]$^+$. Anal. calcd for $C_{28}H_{25}N_8S_2$: 537.16936.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-(3-methoxyphenyl)pyrimidine-5-carbonitrile) (**7k**)*

Compound **7k** was obtained as a white solid (73%). Mp: 226°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 7.49–7.43 (m, 6H), 7.15–7.12 (m, 2H), 5.97 (s, 4H, 2NH$_2$), 3.92 (s, 6H, 2CH$_3$-O), 3.14–3.11 (m, 4H, 2CH$_2$), and 1.81–1.78 (m, 4H, 2CH$_2$). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.6, 167.2, 163.5, 159.2, 137.4, 129.9, 120.9, 119.8, 116.9, 116.5, 114, 82.7, 55.4, 29.9, and 28.5. HRMS (APCI$^-$) m/z 569.16201 [M−H]$^+$. Anal. calcd for $C_{28}H_{25}N_8O_2S_2$: 569.15243.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-(4-methoxyphenyl)pyrimidine-5-carbonitrile) (**7l**)*

Compound **7l** was obtained as a white solid (68%). Mp: >250°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.82 (d, J = 6.8 Hz, 4H), 7.01 (d, J = 6.8 Hz, 4H), 3.87 (s, 6H, 2CH$_3$-O), 3.12 (s, 4H, 2CH$_2$), and 1.78 (s, 4H, 2CH$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 173.9, 166.5, 163.7, 162.5, 130.3, 128.2, 117.5, 114.4, 82.0, 55.8, 30.2, 28.9, and 25.8. HRMS (ESI$^+$) m/z 609.1284 [M+K]$^+$. Anal. calcd for $C_{28}H_{26}N_8O_2S_2K$: 609.1257.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-(4-nitrophenyl)-pyrimidine-5-carbonitrile) (**7m**)*

Compound **7m** was obtained as a brown solid (68%). Mp: >250°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 8.33 (d, J = 8.7 Hz, 4H), 8.06 (d, J = 8.7 Hz, 4H), 6.09 (s, 4H, 2NH$_2$), 3.06 (m, 4H, 2CH$_2$), and 1.80 (m, 4H, 2CH$_2$). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.9, 165.1, 163.2, 148.8, 137.6, 135.1, 130.2, 123.8, 119.6, 83.2, 30.2, and 28.7. HRMS (APCI$^-$) m/z 599.11104 [M−H]$^+$. Anal. calcd for $C_{26}H_{19}N_{10}O_4S_2$: 599.10129.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-(3-nitrophenyl)-pyrimidine-5-carbonitrile) (**7n**)*

Compound **7n** was obtained as a brown solid (69%). Mp: >250°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 8.63 (t, 2H), 8.37 (d, J = 7.7 Hz, 2H), 8.29 (d, J = 7.7 Hz, 2H), 7.80 (t, J = 8.0 Hz, 2H), 6.05 (br s, 4H, 2NH$_2$), 3.23 (m, 4H, 2CH$_2$), and 1.84 (m, 4H, 2CH$_2$). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 174.3, 165.1, 163.5, 148.0, 137.6, 135.1, 130.7, 126.0, 123.5, 83.2, 30.1, and 28.9. HRMS (APCI$^-$) m/z 599.11104 [M−H]$^+$. Anal. calcd for $C_{26}H_{19}N_{10}O_4S_2$: 599.10126.

*2,2′-(Butane-1,4-diylbis(sulfanediyl))bis(4-amino-6-(4-chlorophenyl)-pyrimidine-5-carbonitrile) (**7o**)*

Compound **7o** was obtained as a white solid (60%). Mp: >250°C. ^{1}H NMR (400 MHz, DMSO-d_6) δ (ppm): 7.84 (d, J = 6.8 Hz, 4H), 7.56 (d, J = 6.8 Hz, 4H), 3.13 (s, 4H, 2CH$_2$), and 1.78 (s, 4H, 2CH$_2$). ^{13}C NMR (100 MHz, DMSO-d_6) δ (ppm): 174.1, 166.5, 163.6, 136.3, 135.2, 130.8, 129.0, 116.6, 82.9, 62.4, 30.1, 28.7, and 25.9. HRMS (ESI$^+$) m/z 617.0297 [M+K]$^+$. Anal. calcd for $C_{26}H_{20}Cl_2N_8S_2K$: 617.0266.

*2,2′-(Butane-1,3-diylbis(sulfanediyl))bis(4-amino-6-(4-bromophenyl)-pyrimidine-5-carbonitrile) (**7p**)*

Compound **7p** was obtained as a white solid (72%). Mp: 160°C. ^{1}H NMR (250 MHz, DMSO-d_6) δ (ppm): 7.87 (d, J = 8.4 Hz, 4H), 7.42 (d, J = 8.4 Hz, 4H), 5.86 (br s, 4H, 2NH$_2$), 3.87–3.84 (m, 4H), and 1.83–1.78 (m, 4H). ^{13}C NMR (62.9 MHz, DMSO-d_6) δ (ppm): 173.1, 165.6, 164.1, 133.6, 132.1, 130.5, 126.2, 124.9, 116.8, 81.9, 29.5, and 29.2. HRMS (ESI$^+$) m/z 666.94830 [M+H]$^+$. Anal. calcd for $C_{26}H_{21}Br_2N_8S_2$: 666.96191.

4.2 | Method for cholinesterase activity assay

AChE and BChE inhibitory activity was measured by the spectrophotometric method developed by Ellman et al.[18] Briefly, 150 µl of 100 mM sodium phosphate buffer (pH 8.0), 10 µl of a sample solution dissolved in methanol at different concentrations and 20 µl AChE (5.32×10^{-3} units) or BChE (6.85×10^{-3} units) solutions were mixed and incubated for 15 min at 25°C, and 10 µl of 0.5 mM 5,5′-dithio-bis-(2-nitrobenzoic) acid (DTNB) were added. The reaction was then initiated by the addition of 10 µl of acetylthiocholine iodide (0.71 mM) or butyrylthiocholine chloride (0.2 mM). The hydrolysis of these substrates were monitored spectrophotometrically by the formation of the yellow 5-thio-2-nitrobenzoate anion, as the result of the reaction of DTNB with thiocholine, released by the enzymatic hydrolysis of acetylthiocholine iodide or butyrylthiocholine chloride, respectively, at a wavelength of 412 nm, every 5 min for 15 min, utilizing a 96-well microplate reader (Multimode Plate Reader EnSpire; Perkin Elmer) in triplicate experiments. Galantamine was used as a reference compound. The results were given as 50% inhibition concentration (IC_{50}) and the percentage of inhibition of AChE or BChE was determined by the comparison of reaction rates of samples relative to the blank sample (methanol in phosphate buffers, pH 8) using the formula

$$\text{Inhibition of AChE or BChE (\%)} = \frac{E - S}{E} \times 100,$$

where E is the activity of an enzyme without test samples and S is the activity of an enzyme with test samples.

4.3 | Molecular docking procedures

The three-dimensional (3D) coordinates of *h*AChE (ID: 4M0E)[25] and *h*BChE (ID: 5NN0)[19] were retrieved from the Protein Data Bank (https://www.rcsb.org), and prepared using the LeadIT 2.1.8 package (www.biosolveit.com). For each enzyme, only the catalytic chain A was kept. Then, their active sites were defined by selecting all residues within a radius of 6.5 Å around the inhibitors in the crystal structures. Thereafter, the protonation state and the side chain orientations of their active site residues were inspected. On the contrary, the 3D structures of the designed compounds were drawn, minimized, and exported as mol2 files using Chem3D 16.0 (http://www.cambridgesoft.com).

Molecular docking calculations of the designed compounds into the active sites of AChE and BChE were performed using FlexX 2.1.8,[26] which is based on an incremental construction of ligands. The selection of the base fragments was set to automatic mode and the fragment placement used the standard algorithm. FlexX scoring function, which gives scores as ΔG in kJ/mol, was used for ranking of molecules.

4.4 | Method for antioxidant activity assays

4.4.1 | Superoxide radical scavenging assay

Superoxide was generated according to the alkaline DMSO method,[27] the reduction of NBT by superoxide was determined in the presence and absence of extracts. The reaction mixture containing NBT (1 mg/ml solution in distilled water) and 40 µl of sample at various concentrations was added to 130 µl alkaline DMSO (1.0 ml DMSO containing 5 mM NaOH in 0.1 ml water) to give a final volume of 200 µl and the absorbance was measured at 560 nm using a microplate reader. The decrease in the absorbance at 560 nm with antioxidants indicated the consumption of the generated superoxide. The experiment was repeated in triplicate and the percentage scavenging was calculated by using the formula:

$$\text{Inhibition } (\%) = \frac{A_{\text{sample}} - A_{\text{control}}}{A_{\text{sample}}} \times 100.$$

4.4.2 | Reducing power assay

The reducing power of the tested compounds was determined according to the previously described method.[28] To determine the reducing power activity, 10 µl of serial diluted sample were added into a 96-well round-bottom plate. Following this, 40 µl of 0.2 M phosphate buffer (pH 6.6) and 50 µl of potassium ferricyanide (1%) were added to each well and the plate was incubated at 50°C for 20 min. Finally, 50 µl of TCA (10%) and distilled water (40 µl) and 10 µl of ferric chloride (0.1%) were added into each well to measure the reducing power activity. Afterward, the absorbance was measured in a microplate reader at 700 nm. Higher absorbance of the reaction mixture indicates greater reducing power.

4.4.3 | Cupric reducing antioxidant capacity

The cupric reducing antioxidant capacity was determined according to the CUPRAC method.[29] In each well, to the reaction mixture containing 40 µl of sample solution and 50 µl of a copper(II) chloride solution, 50 µl of neocuproine alcoholic solution, and 60 µl of ammonium acetate aqueous buffer at pH 7 was added to give a final volume of 200 µl. After 30 min, the absorbance was measured at 450 nm. Results were recorded as absorbance compared with the absorbance of BHA and BHT, which were used as antioxidant standards.

4.4.4 | Antioxidant capacity by the β-carotene bleaching assay

The β-carotene bleaching activity of our synthetic compounds was evaluated using the β-carotene-linoleic acid system described by Marco.[30] Thus, a solution of β-carotene (0.5 mg) in 1 ml of chloroform was added to 25 µl of linoleic acid and 200 µl of Tween 40. After evaporation in vacuum of the chloroform, 50 ml of hydrogen peroxide (H_2O_2) was added under vigorous agitation. The absorbance of the solution was adjusted to 0.8–09 nm. Amounts of 160 µl of this solution were added to 40 µl of solution of the studied synthetic compounds at different concentrations. The absorbance was measured at 470 nm using a 96-well microplate reader. The emulsion system was incubated for 2 hr at 50°C. A blank, devoid of β-carotene, was prepared for background subtraction. BHA and BHT were used as standards.

The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = \frac{\ln\frac{a}{b}}{t},$$

where ln is the natural log, a is the absorbance at time zero, and b is the absorbance at time t (120 min).

The AA was calculated in terms of percent of inhibition relative to the control, using the following equation:

$$\text{AA } (\%) = \frac{R_{\text{sample}} - R_{\text{control}}}{R_{\text{sample}}} \times 100.$$

4.5 | Statistical analysis

All data on AA test were the average of triplicate analyses. Data were recorded as the mean ± standard deviation. Significant differences between means were determined using Student's t test; $p < 0.05$ were regarded as significant.

4.6 | Method for antibacterial activity assay

The antibacterial activity of the selected compounds (**7a**, **7e**, **7i**, **7k**, **7n**, **7r**) was determined by using the disc-diffusion assay. Agar culture plates were prepared as previously described.[31] Each bacterial

strain was grown on Tryptic Soy Agar plates and incubated for 2 days at 37°C. The turbidity of each bacterial broth culture was adjusted to equal that of the 0.5 McFarland standard and then the broth cultures adjusted were separately inoculated on Mueller Hinton Agar plates using cotton swabs. Thirty to thirty-five milligrams of each compound (**7**) was weighed and dissolved in DMSO to a final concentration of 100,000 ppm. Thirteen microliters of each solution was applied to sterile filter paper discs (6 mm in diameter; Glass Microfiber filters, Whatman®). Standard antibiotic disks (tetracycline [30 µg], erythromycin [15 µg]) were used. DMSO was used as a negative control since the compounds were dissolved in DMSO. Inoculated plates with discs were incubated at 37°C for 24 hr. After incubation, the inhibition zone diameter (mm) was measured. Three independent experiments were performed at different times.

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ORCID

Raouf Boulcina http://orcid.org/0000-0001-9102-3854

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