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Full Paper

Synthesis, Acetylcholinesterase and Alkaline Phosphatase Inhibition of Some New 1,2,4-Triazole and 1,3,4-Thiadiazole Derivatives

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A new series of 4,5-disubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**6a**–**s**) and 2,5-disubstituted-1,3,4-thiadiazoles (**7a**–**h**) was synthesized by intramolecular dehydrocyclization of various 1,4-disubstituted thiosemicarbazide derivatives (**5a**–**s**) by refluxing in 4 N aqueous sodium hydroxide and by overnight stirring with polyphosphoric acid, respectively. The structures of these compounds were characterized by IR, ^{1}H and ^{13}C NMR, elemental analysis and mass spectroscopic studies. All the synthesized compounds were screened for their acetylcholinesterase and alkaline phosphatase inhibition studies. Most of the tested compounds showed promising activities, amongst them (**6k**) and (**6q**) showed excellent acetylcholinesterase inhibitory activity with IC_{50} 0.241 ± 0.012 and 0.260 ± 0.013 μM, respectively, as compared with those of standard drug whereas the compound (**6p**), with IC_{50} 0.044 ± 0.001 μM, was found to be the most potent inhibitor of alkaline phosphatase.

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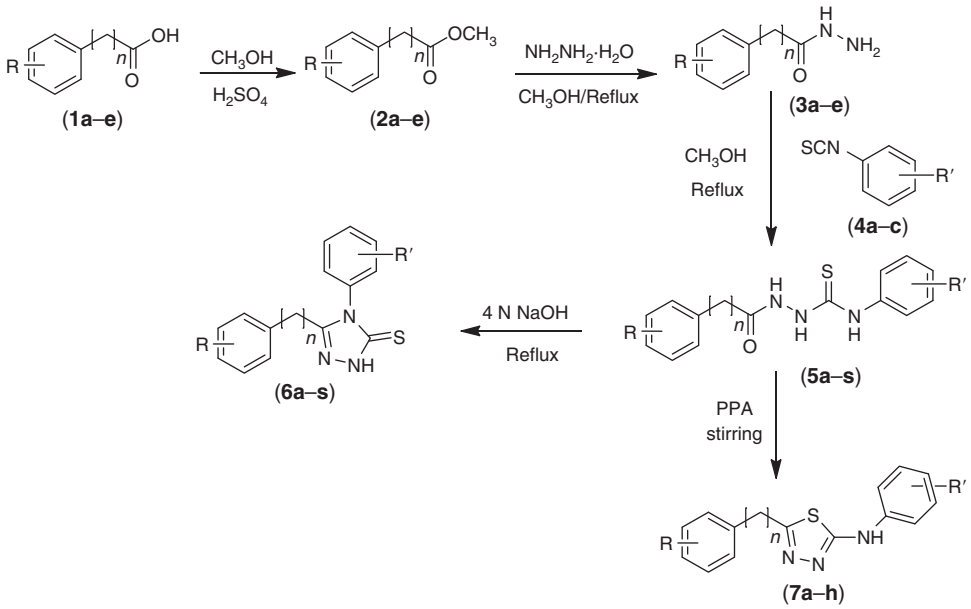
Introduction

Acetylcholinesterase is a serine hydrolase (AChE, acetylcholine hydrolase, EC 3.1.1.7) that plays an essential role in the cholinergic synapses. Hydrolysis of the neurotransmitter acetylcholine (ACh) in the nervous system by acetylcholinesterase is known to be one of the most efficient enzyme catalytic reactions. The basis of this high efficiency has been sought by means of ligand-binding studies using various substrates and has led to the suggestion that the active centre is composed of a cationic esteratic subsite containing the active serine, an anionic site which accommodates the choline moiety of ACh and a peripheral anionic site (PAS).[1,2] The primary physiologic role of the AChE peripheral site is to accelerate the hydrolysis of acetylcholine at low substrate concentrations.[3,4]

The role of the cholinergic system has been an intensive issue of interest in Alzheimer's disease, which is a neurodegenerative disorder causing deterioration of memory and other cognitive functions.[5,6] In Alzheimer's disease, a cholinergic deficiency in the brain has been reported.[7,8] Therefore, the synthesis and study of inhibitors of acetylcholinesterase may aid to the development of therapeutically useful compounds to treat such neurological disorders. Acetylcholinesterase inhibitors donepezil hydrochloride, galantamine hydrobromide and rivastigmine tartrate are the current approved drugs for the treatment of Alzheimer's patients.[9] Apart from marketed drugs, several acetylcholinesterase inhibitors such as cambogin (**A**) and guttiferone F (**B**) have been isolated from *Symphonia globulifera* leaves possessing acetylcholinesterase inhibitory activity with IC_{50} values of 1.13 ± 0.06 and 0.95 ± 0.01 μM, respectively.[10] However, acetylcholinesterase inhibitors present some limitations, such as their short half-lives and excessive side effects caused by activation of peripheral cholinergic systems, as well as hepatotoxicity, which is the most frequent and important side effects of these drug therapies.[11–13] For this reason, alternative and complementary therapies need to be developed.

Alkaline phosphatase (ALP, E.C.3.1.3.1.) is a non-specific phosphomonoester hydrolase that catalyzes the hydrolysis and transphosphorylation of a wide variety of organic monophosphates and regulates the functions of many biological systems.[14–16] The widespread occurrence of ALP in nature suggests its involvement in fundamental biochemical processes, however, there is no positive evidence regarding its physiological function, or the nature of the natural substrates. Hydrolysis of phosphoesters, phosphate transferase activity, protein phosphatase activity, phosphate transport, modulation of organic cation transport, and involvement in cell proliferation have been



	R	R′	*n*		R	R′	*n*		R	R′	*n*
6a	3-OCH_3	4-Cl	1	**6j**	4-CH_3	2,6-diCH_3	0	**7a**	4-CH_3	2,6-diCH_3	0
b	3-OCH_3	4-OCH_3	1	**k**	2-Cl	2,6-diCH_3	0	**b**	2-Cl	2,6-diCH_3	0
c	3-OCH_3	4-Cl	2	**l**	4-Cl	2,3-diCH_3	0	**c**	4-Cl	2,3-diCH_3	0
d	3-OCH_3	4-OCH_3	2	**m**	3-NO_2	2,4-diCH_3	0	**d**	3-NO_2	2,4-diCH_3	0
e	3,4-diOCH_3	4-Cl	0	**n**	H	2,3-diCH_3	0	**e**	H	2,3-diCH_3	0
f	3,4-diOCH_3	4-OCH_3	0	**o**	4-Cl	2,6-diCH_3	0	**f**	4-Cl	2,6-diCH_3	0
g	3-OCH_3	2-OCH_3	1	**p**	3-Cl	2,4-diCH_3	0	**g**	3-Cl	2,4-diCH_3	0
h	3-OCH_3	2-OCH_3	2	**q**	2-CH_3	2,3-diCH_3	0	**h**	4-NO_2	2,4-diCH_3	
i	3,4,5-triOCH_3	3-OCH_3	2	**r**	3-Cl	2,6-diCH_3	0				
				s	4-Cl	2,4-diCH_3	0				

Scheme 1.

suggested as possible functions of ALP.[17,18] The biological action of the alkaline phosphatase in serum is associated with metabolic bone (hypophosphatasia) and liver diseases and also is used as a marker of osteoblastic differentiation.[19] Alkaline phosphatases may potentially be employed as therapeutic agents and therapeutic targets and show several uses in clinical medicine and in biotechnology. Literature investigation has revealed that the theophylline (**C**) and pyrazole (**D**) are the widely used alkaline phosphatase inhibitors.

Five-membered heterocyclic compounds, particularly azoles, occupy a unique place in the realm of natural and synthetic organic chemistry.[20] 1,2,4-Triazoles represent an overwhelming and rapid developing field in modern heterocyclic chemistry. From literature, it is predictable that 1,2,4-triazoles represent important pharmacophores, and have a wide range of therapeutic properties. They play a vital role as medicinal agents owing to different biological activities and an extensive exploration on this moiety can be helpful for obtaining better therapeutic molecules.[21]

In view of above facts and in continuation of our previous studies,[22,23] it was contemplated to evaluate some new triazole derivatives with different substitution patterns for acetyl cholinesterase and alkaline phosphatase inhibitory activities. This paper describes the synthesis of nine triazole derivatives (**6a**–**i**) and enzyme inhibitory activities of nineteen triazoles (**6a**–**s**) and eight thiadiazoles (**7a**–**h**). The synthesis of ten triazoles (**6j**–**s**) and eight thiadiazoles (**7a**–**h**) is described elsewhere.[22]

Results and Discussion

Chemistry

The synthetic route for the target compounds 4,5-disubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**6a**–**i**) is illustrated in Scheme 1. Substituted aromatic esters (**2a**–**e**) were synthesized by the reaction of the corresponding substituted aromatic acids (**1a**–**e**) with methanol in the presence of catalytic amount of sulfuric acid. Esters (**2a**–**e**) were converted to the corresponding acid hydrazides (**3a**–**e**) by refluxing with hydrazine hydrate (80 %) in methanol.[24–27] Different substituted anilines were converted into corresponding dithiocarbamate salts by stirring with concentrated ammonia solution and pure carbon disulfide in methanol. The intermediate dithiocarbamate salts were not isolated but converted directly to substituted phenylisothiocyanates (**4a**–**e**) by overnight stirring with lead nitrate solution.[28] The compounds (**4a**–**e**) were isolated by steam distillation. Thiosemicarbazide derivatives (**5a**–**i**) were synthesized by the condensation of the corresponding acid hydrazides (**3a**–**e**) and phenylisothiocyanates (**4a**–**c**). These compounds were used as key intermediate for the synthesis of 1,2,4-triazole derivatives. The 4,5-disubstituted-2,4-dihydro-3*H*-1,2,4-triazole-3-thiones (**6a**–**i**) were synthesized by intramolecular dehydrative cyclization of thiosemicarbazides (**5a**–**i**) when refluxed in 4 N sodium hydroxide solution, followed by neutralization with concentrated HCl.[22] The structure elucidations of the newly synthesized compounds were carried out by FTIR, ^{1}H NMR and ^{13}C NMR spectroscopy. Further confirmations of

Table 1. Acetylcholinesterase inhibition activities of the synthesized compounds (6a–i) and literature compounds 6j–s and 7a–h

Compound	IC_{50}^{A} [μM] ± s.e.m.[B] or (% Inhibition)[C]	K_i [μM]
6a	2.89 ± 0.41	2.63 ± 0.31
6b	(19 ± 3)[c]	–
6c	1.78 ± 0.16	1.62 ± 0.14
6d	4.68 ± 0.54	4.27 ± 0.44
6e	(22 ± 4)[c]	–
6f	–	–
6g	4.76 ± 0.14	4.34 ± 0.13
6h	–	–
6i	4.34 ± 0.42	3.96 ± 0.32
6j	(0)[c]	–
6k	0.241 ± 0.012	0.221 ± 0.011
6l	(6.2 ± 2) [c]	–
6m	(14 ± 5)[c]	–
6n	(0.5 ± 0.1)[c]	–
6o	(10 ± 2)[c]	–
6p	(11 ± 3)[c]	–
6q	0.260 ± 0.013	0.242 ± 0.012
6r	0.314 ± 0.015	0.286 ± 0.013
6s	21.9 ± 5.2	20.1 ± 4.2
7a	(2 ± 0.1)[c]	–
7b	(8.4 ± 2)[c]	–
7c	(4 ± 1)[c]	–
7d	–	–
7e	2.22 ± 0.75	2.03 ± 0.55
7f	27.1 ± 4.3	24.75 ± 3.3
7g	0.351 ± 0.013	0.320 ± 0.012
7h	(14 ± 4)[c]	–
Neostigmine methylsulfate	69.1 ± 8.2	63.1 ± 7.1
Donepezil	0.021 ± 0.004	0.019 ± 0.004

–, not determined
[A]The IC_{50} presented here is the concentration that inhibited 50 % of the enzyme activity.
[B]s.e.m. = Standard mean error of 3 experiments.
[C]The % inhibition of the enzyme activity caused by 1 mM of the tested compounds, given in parentheses.

Table 2. Alkaline phosphatase inhibition activities of the synthesized compounds (6a–i) and literature compounds 6j–s and 7a–h

Compounds	IC_{50}^{A} [μM] ± s.e.m[B] or (% Inhibition)[C]	K_i [μM]
6a	(19)[c]	–
6b	(15)[c]	–
6c	(11)[c]	–
6d	(18)[c]	–
6e	(11)[c]	–
6f	(8)[c]	–
6g	(11)[c]	–
6h	(26)[c]	–
6i	(5)[c]	–
6j	(8)[c]	–
6k	(13)[c]	–
6l	(19)[A]	–
6m	(9)[c]	–
6n	(6)[c]	–
6o	6.7 ± 5.7	4.92 ± 4.61
6p	0.061 ± 0.001	0.044 ± 0.001
6q	10 ± 2	7.3 ± 2
6r	(16)[c]	–
6s	(16)[c]	–
7a	(15)[c]	–
7b	(17)[c]	–
7c	–	–
7d	–	–
7e	71 ± 8	52 ± 7
7f	0.15 ± 0.02	0.11 ± 0.02
7g	20 ± 2	14 ± 2
7h	(19)[c]	–
KH_2PO_4	3.11 ± 0.03	1.5
Theophylline	47 ± 0.51	91 ± 0.51

–, not determined
[A]The IC_{50} presented here is the concentration that inhibited 50 % of the enzyme activity.
[B]s.e.m. = standard mean error of 3 experiments.
[C]The % inhibition of the enzyme activity caused by 0.1 mM of the tested compounds, given in parentheses.

the compounds were carried out by mass spectrometry and microanalysis.

Pharmacology

Acetylcholinesterase Inhibition

The acetylcholinesterase (E.C. 3.1.1.7 from Rabbit brain) inhibition activity of the synthesized compounds (**6a**–**s**) was evaluated quantitatively by Ellman's method.[29] The tested compounds showed acetylcholinesterase inhibition activity and K_i value ranging from IC_{50} 0.241 ± 0.012 to 27.1 ± 4.3, and 0.22 ± 0.011 to 24.75 ± 3.3 μM respectively, whereas standard drug neostigmine methylsulfate and Donepezil showed IC_{50} value 69.1 ± 8.2 μM and K_i value 63.1 ± 7.1 μM and 0.021 ± 0.004 μM and K_i value 0.019 ± 0.004 μM respectively. Results of acetylcholinesterase inhibitory activity of the synthesized compounds (**6a**–**s**) and (**7a**–**h**) are given in Table 1. It is clear from the results that the activity of compounds is associated with the position of the substituents on both the rings A and B. The compound (**6k**) having *ortho*-chloro group at aryl ring A with an IC_{50} value 0.241 ± 0.012 μM showed maximum inhibitory activity. Similarly, the compounds (**6q**–**r**) possessing methyl and chloro groups at the *ortho* and *para* position at ring A were found to be more active than the standard neostigmine methylsulfate. The replacement of this group by *meta*-methoxy group at aryl ring A and *para*-chloro group at aryl ring B resulted in the slight decrease in the acetylcholinesterase inhibition (IC_{50} 1.78 ± 0.16 μM) and K_i value 1.62 ± 0.14 μM as shown by compound (**6c**). By increasing the number of substituents in the aryl ring A, for instance 3,4-dimethoxy groups, resulted in the reduction of acetylcholinesterase inhibitory activity and by increasing the length of the carbon chain between the positions 5 of triazole ring and methoxyphenyl group from 0 to 2, would result in the increase in acetylcholinesterase inhibition potential of the compounds. The triazole class of compounds was found to be significantly active against acetylcholinesterase. In the thiadiazole class, the presence of the *meta*-chloro group at aryl ring A resulted in the maximum inhibitory activity as exhibited by compound (**7g**).

In general, the triazole class of compounds showed maximum acetylcholinesterase inhibitory activity as compared with thiadiazoles.

Alkaline Phosphatase Inhibition

A spectrophotometric method by Iqbal[30] was used to carry out the inhibitory studies. Some of the triazole derivatives

showed excellent inhibitory activity in the micromolar range towards ALP, with (**6p**) being the most potent among them showing maximum inhibitory activity as compared with the standard drug. The results of alkaline phosphatase inhibition studies of the synthesized compounds are given in Table 2.

Among the synthesized compounds (**6a**–**s**) and (**7a**–**h**), only four were potent inhibitors of alkaline phosphatase. Others either precipitated out or had low inhibitory activity. In the triazole series, compound (**6p**) bearing a chloro group at the *meta* position showed maximum potency having $IC_{50} = 0.061 \pm 0.001\,\mu M$ and K_i value $0.044 \pm 0.001\,\mu M$, whereas for (**6o**) the inhibition decreases ($IC_{50} = 6.7 \pm 5.7\,\mu M$) upon shifting the chloro substituent to the *para* position.

In the thiadiazole series, the potent compound (**7f**) has IC_{50} $0.15 \pm 0.02\,\mu M$ as the *para* position bears a chloro group in comparison to (**7g**) ($IC_{50} = 20 \pm 2\,\mu M$), which exhibits chloro functionality at the *meta* position. So, the pattern suggests that inhibition increases when the chloro is substituted at the *para* position. Other compounds such as (**6q**) and (**7e**) have rather less inhibitory activity, which suggests that other substituents are rather unfavourable for inhibition.

Conclusions

In the present study, we reported a new series of triazole derivatives. The acetylcholinesterase inhibitory activity of the synthesized compounds was evaluated. Some of the synthesized compounds were found to be significant inhibitors of acetylcholinesterase. The compounds (**6k**), (**6q**,**r**) and (**7g**) showed excellent acetylcholinesterase inhibitory activity as compared with those of standard drug.

The synthesized compounds were also evaluated for alkaline phosphatase inhibition. The compounds (**6p**) and (**7f**) were found to be the most promising inhibitors of alkaline phosphatase.

Experimental

General

The purity of the synthesized compounds were ascertained by thin-layer chromatography and the R_f values were determined by employing pre-coated silica gel aluminium plates, Kieslgel 60 F_{254} from Merck (Germany), using petroleum ether : ethyl acetate (8 : 2 as an eluent. Melting points were measured on a Gallenkamp melting point apparatus (MP-D) and are uncorrected. The IR spectra were recorded on FTS 3000 MX, Bio-Rad Merlin (Excalibur model) spectrophotometer.

NMR spectra were recorded on a Bruker Avance 300 MHz spectrometer using DMSO-d_6 solution with TMS as an internal standard. The multiplicities were expressed as s = singlet, d = doublet, t = triplet, q = quartet, dt = doublet of triplet and coupling constant (*J*) in Hertz (Hz). Mass spectra were recorded on Agilent Technologies 6890N gas chromatograph and an inert mass selective detector 5973 mass spectrometer. The elemental analyses were performed on Leco CHNS-932 Elemental Analyzer, Leco Corporation (USA).

General Procedure for the Synthesis of Aromatic Esters (**2a**–**e**) and Acid Hydrazides (**3a**–**e**)

Substituted aromatic esters (**2a**–**e**) were synthesized by the reaction of corresponding substituted aromatic acids (**1a**–**e**) in the presence of catalytic amount of sulfuric acid, the esters (**2a**–**e**) were converted into corresponding aromatic acid hydrazides (**3a**–**e**) by refluxing with hydrazine hydrate (80 %) in methanol as described in literature.[24–27]

General Procedure for the Synthesis of Substituted Phenylisothiocyanates (**4a**–**e**)

Substituted anilines were converted to their dithiocarbamate salts, which were oxidized to corresponding phenylisothiocyanates (**4a**–**e**) by the standard procedure.[28]

General Procedure for the Synthesis of 1,4-Disubstituted Thiosemicarbazides (**5a**–**i**)

The corresponding acid hydrazide (**3**) (0.0068 mol) was dissolved in methanol (30 mL) and added slowly to the solution of substituted isothiocyanate (0.0066 mol) in methanol (10 mL). The reaction mixture was refluxed for 10–12 h and monitored by TLC. After consumption of the starting materials, the mixture was cooled to room temperature. Evaporation of solvent under reduced pressure left crude 1,4-disubstituted thiosemicarbazide (**5**) as an oil, which solidified on cooling.[22] It was purified by recrystallization from a mixture of ethyl acetate and petroleum ether to yield thiosemicarbazide (**5**).

4-(4-Chlorophenyl)-1-(2-(3-methoxyphenyl)acetyl) thiosemicarbazide **5a**

White solid (1.59 g, 67 %), mp 156–157°C. R_f 0.25 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3321, 3208, 1661, 1619, 1595, 1557, 1258. δ_H (300 MHz, DMSO-*d*6)10.10 (s, 1H, N*H*-C=O), 9.86 (s, 1H, N*H*-C=S), 9.74 (s, 1H, N*H*-C=S), 7.50 (d, *J* 8.7, 2H, Ar-*H*), 7.41 (d, *J* 8.7, 2H, Ar-*H*), 7.20 (t, *J* 7.8, 1H, Ar-*H*), 6.91–6.79 (m, 3H, Ar-*H*), 3.54 (s, 3H, OCH_3), 3.40 (s, 2H, CH_2). δ_C 172.11, 169.91, 159.61, 138.60, 137.97, 137.33, 129.65, 128.48, 122.07, 121.67, 115.40, 115.17, 55.44, 41.48. Anal. Calc. for $C_{16}H_{16}ClN_3O_2S$: C 54.93, H 4.61, N 12.01, S 9.17. Found: C 55.21, H 4.52, N 12.12, S 9.15 %.

4-(4-Methoxyphenyl)-1-(2-(3-methoxyphenyl)acetyl) thiosemicarbazide **5b**

White solid (1.53 g, 65 %), mp 149–150°C. R_f 0.34 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3359, 3206, 1682, 1593, 1542, 1262. δ_H (300 MHz, DMSO-*d*6) 10.11 (s, 1H, N*H*-C=O), 9.76 (s, 1H, N*H*-C=S), 9.50 (s, 1H, N*H*-C=S), 7.30–7.11 (m, 3H, Ar-*H*), 6.99–6.72 (m, 5H, Ar-*H*), 3.74 (s, 3H, OCH_3), 3.44 (s, 2H, CH_2), 3.31 (s, 3H, OCH_3). δ_C 174.31, 159.16, 138.60, 137.37, 136.11, 129.65, 129.01, 128.48, 125.37, 122.07, 115.39, 112.47, 55.17, 55.21, 42.75. Anal. Calc. for $C_{17}H_{19}N_3O_3S$: C 59.11, H 5.54, N 12.17, S 9.28. Found: C 59.01, H 5.65, N 12.21, S 9.12 %.

4-(4-Chlorophenyl)-1-(3-(3-methoxyphenyl)propanoyl) thiosemicarbazide **5c**

White solid (1.53 g, 62 %), mp 173–175°C. R_f 0.29 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3300, 3179, 1671, 1593, 1542, 1252. δ_H (300 MHz, DMSO-*d*6) 9.94 (s, 1H, N*H*-C=O), 9.69 (s, 1H, N*H*-C=S), 9.54 (s, 1H, N*H*-C=S), 7.47 (d, *J* 8.7, 1H, Ar-*H*), 7.38 (d, *J* 8.7, 1H, Ar-*H*), 7.20 (t, *J* 8.1, 1H, Ar-*H*), 7.01 (t, *J* 2.1, 1H, Ar-*H*), 6.81–6.70 (m, 4H, Ar-*H*), 3.41 (s, 3H, OCH_3), 2.82 (t, *J* 6.9, 2H, CH_2), 2.54 (t, *J* 6.9, 2H, CH_2). δ_C 172.82, 159.47, 139.89, 138.17, 137.03, 129.65, 128.52, 123.17, 121.54, 115.30, 115.11, 111.41, 55.36, 37.43, 33.24. Anal. Calc. for $C_{17}H_{18}ClN_3O_2S$: C 56.12, H 4.99, N 11.55, S 8.81. Found: C 55.98, H 4.85, N 11.32, S 8.73 %.

4-(4-Methoxyphenyl)-1-(3-(3-methoxyphenyl) propanoyl)thiosemicarbazide ***5d***

White solid (1.61 g, 66 %), mp 180–182°C. R_f 0.28 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3327, 3269, 1680, 1593, 1542, 1257. δ_H (300 MHz, DMSO-*d*6) 10.18 (s, 1H, N*H*-C=O), 9.73 (s, 1H, N*H*-C=S), 9.71 (s, 1H, N*H*-C=S), 7.48 (d, *J* 8.4, 1H, Ar-*H*), 7.40 (d, *J* 8.4, 1H, Ar-*H*), 7.23 (t, *J* 7.8, 1H, Ar-*H*), 7.03 (t, *J* 2.1, 1H, Ar-*H*), 6.89–6.71 (m, 4H, Ar-*H*), 3.72 (s, 3H, OCH_3), 3.69 (s, 3H, OCH_3), 2.78 (t, *J* 6.8, 2H, CH_2), 2.54 (t, *J* 6.8, 2H, CH_2). δ_C 171.56, 159.61, 138.60, 137.34, 133.46, 130.71, 129.65, 128.48, 126.54, 122.07, 115.39, 112.47, 55.24, 53.37, 36.14, 34.75. Anal. Calc. for $C_{18}H_{21}N_3O_3S$: C 60.15, H 5.89, N 11.69, S 8.92. Found: C 59.85, H 6.05, N 11.38, S 8.72 %.

4-(4-Chlorophenyl)-1-(3,4-dimethoxybenzoyl) thiosemicarbazide ***5e***

White solid (1.84 g, 74 %), mp 165–167°C. R_f 0.24 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3400, 3180, 1668, 1596, 1510, 1262. δ_H (300 MHz, DMSO-*d*6) 10.30 (s, 1H, N*H*-C=O), 9.61 (s, 1H, N*H*-C=S), 9.54 (s, 1H, N*H*-C=S), 7.59 (dd, *J* 8.4, 1.8, 1H, Ar-*H*), 7.54 (d, *J* 1.8, 1H, Ar-*H*), 7.32 (d, *J* 9.0, 2H, Ar-*H*) 7.05 (d, *J* 8.4, 1H, Ar-*H*), 6.93 (d, *J* 9.0, 2H, Ar-*H*), 3.81 (s, 3H, OCH_3), 3.71 (s, 3H, OCH_3). δ_C 173.71, 166.10, 152.19, 148.56, 137.44, 132.57, 127.99, 125.16, 121.85, 113.64, 111.62, 111.26, 53.60, 52.81. Anal. Calc. for $C_{16}H_{16}ClN_3O_3S$: C 52.53, H 4.41, N 11.49, S 8.76. Found: C 52.75, H 4.69, N 11.77, S 8.50 %.

1-(3,4-Dimethoxybenzoyl)-4-(4-methoxyphenyl) thiosemicarbazide ***5f***

White solid (1.84 g, 75 %), mp 174–175°C. R_f 0.36 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3364, 3185, 1664, 1599, 1511, 1270. δ_H (300 MHz, DMSO-*d*6) 10.42 (s, 1H, N*H*-C=O), 9.83 (s, 1H, N*H*-C=S), 9.78 (s, 1H, N*H*-C=S), 7.60–7.33 (m, 6H, Ar-*H*), 7.06 (d, *J* 8.4, 1H, Ar-*H*), 3.82 (s, 3H, OCH_3), 3.72 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3). δ_C 172.42, 166.03, 152.20, 148.56, 136.89, 132.57, 128.04, 125.16, 121.86, 113.66, 111.62, 111.26, 54.28, 53.32, 52.72. Anal. Calc. for $C_{17}H_{19}N_3O_4S$: C 56.50, H 5.30, N 11.63, S 8.87. Found: C 56.22, H 5.07, N 11.48, S 8.69 %.

4-(2-Methoxyphenyl)-1-(2-(3-methoxyphenyl)acetyl) thiosemicarbazide ***5g***

White solid (1.60 g, 68 %), mp 189–191°C. R_f 0.23 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3412, 3146, 1691, 1614, 1546, 1258. δ_H (300 MHz, DMSO-*d*6) 10.37 (s, 1H, N*H*-C=O), 9.68 (s, 1H, N*H*-C=S), 9.57 (s, 1H, N*H*-C=S), 7.10–6.99 (m, 4H, Ar-*H*), 7.01 (t, *J* 7.2, 1H, Ar-*H*), 6.47 (dd, *J* 8.4, 2.1, 1H, Ar-*H*), 6.41 (dd, *J* 8.4, 2.1, 1H, Ar-*H*), 6.39 (s, 1H, Ar-*H*), 3.71 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.37 (s, 2H, CH_2); δ_C 173.21, 165.17, 154.35, 147.51, 137.21, 133.67, 128.14, 126.34, 125.47, 122.16, 120.39, 114.61, 111.63, 111.03, 55.47, 53.31, 43.52. Anal. Calc. for $C_{17}H_{19}N_3O_3S$: C 59.11, H 5.54, N 12.17, S 9.28. Found: C 59.22, H 5.66, N 12.30, S 9.45 %.

4-(2-Methoxyphenyl)-1-(3-(3-methoxyphenyl) propanoyl)thiosemicarbazide ***5h***

White solid (1.78 g, 73 %), mp 186–187°C. R_f 0.26 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3425, 3212, 1682, 1612, 1585, 1251. δ_H (300 MHz, DMSO-*d*6) 10.47 (s, 1H, N*H*-C=O), 9.83 (s, 1H, N*H*-C=S), 9.78 (s, 1H, N*H*-C=S), 7.27 (t, *J* 7.6, 1H, Ar-*H*), 7.12 (d, *J* 2.1, 1H, Ar-*H*), 7.20–6.82 (m, 4H, Ar-*H*), 6.74 (dd, *J* 8.4, 2.4, 1H, Ar-*H*), 6.67 (d, *J* 7.8, 1H, Ar-*H*), 3.68 (s, 3H, OCH_3), 3.65 (s, 3H, OCH_3), 3.71 (t, *J* 6.9, 2H, CH_2), 3.40 (t, *J* 6.9, 2H, CH_2). δ_C 172.03, 165.17, 152.56, 149.31, 137.43, 132.55, 129.34, 125.77, 121.86, 118.73, 115.24, 113.65, 111.14, 110.21, 55.72, 53.44, 42.12, 38.60. Anal. Calc. for $C_{18}H_{21}N_3O_3S$: C 60.15, H 5.89, N 11.69, S 8.92. Found: C 59.86, H 5.69, N 11.51, S 9.14 %.

4-(3-Methoxyphenyl)-1-(3-(3,4,5-trimethoxyphenyl) propanoyl)thiosemicarbazide ***5i***

White solid (2.02 g, 71 %), mp 155–156°C. R_f 0.40 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3385, 3216, 1675, 1625, 1605, 1496, 1255. δ_H (300 MHz, DMSO-*d*6) 11.02 (s, 1H, N*H*-C=O), 9.88 (s, 1H, N*H*-C=S), 9.75 (s, 1H, N*H*-C=S), 7.44 (s, 2H, Ar-*H*), 7.26 (t, *J* 8.1, 1H, Ar-*H*), 7.13 (s, 1H, Ar-*H*), 7.01 (d, *J* 7.2, 1H, Ar-*H*), 6.80 (d, *J* 7.1, 1H, Ar-*H*), 3.81 (s, 9H, OCH_3), 3.72 (s, 3H, OCH_3), 3.41 (t, *J* 6.9, 2H, CH_2), 3.30 (t, *J* 6.9, 2H, CH_2). δ_C 187.01, 167.12, 156.33, 151.23, 146.33, 138.21, 134.22, 128.23, 125.67, 118.17, 114.87, 111.28, 110.31, 63.78, 55.47, 43.31, 35.29. Anal. Calc. for $C_{20}H_{25}N_3O_5S$: C 57.26, H 6.01, N 10.02, S 7.64. Found: C 57.21, H 5.89, N 10.21, S 7.41 %.

General Procedure for the Synthesis of 1,2,4-Triazol-3-thiones ***6a–i***

The corresponding thiosemicarbazide (**5**) (0.0014 mol) was refluxed in aqueous sodium hydroxide solution (4 N, 25 mL). The progress of the reaction was monitored by TLC. After completion of reaction (12–14 h), the reaction mixture was cooled to room temperature and filtered. The filtrate was neutralized with hydrochloric acid (4 N) to precipitate the 1,2,4-triazole-3-thione (**6**), which was filtered and recrystallized from aqueous ethanol.[22]

5-(3-Methoxybenzyl)-4-(4-chlorophenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6a***[31]

White solid (0.36 g, 78 %), mp 169–170°C. R_f 0.31 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3294, 1561, 1543, 1483, 1272. δ_H (300 MHz, DMSO-*d*6) 13.74 (s, 1H, N*H*), 7.14 (t, *J* 9.0, 1H, Ar-*H*), 7.13 (dt, *J* 9.0, 2.1, 2H, Ar-*H*), 7.01 (dt, *J* 9.0, 2.1, 2H, Ar-*H*), 6.75 (dd, *J* 9.0, 2.1, 1H, Ar-*H*), 6.53 (d, *J* 7.5, 1H, Ar-*H*), 6.46 (t, *J* 2.1, 1H, Ar-*H*), 3.67 (s, 3H, OCH_3), 3.43 (s, 2H, CH_2). δ_C 168.40, 159.62, 151.55, 136.28, 134.56, 132.93, 130.71, 129.88, 129.76, 121.25, 114.71, 113.05, 55.34, 31.88. *m/z* (GCMS, %) 331 (100), 316 (9), 298 (14), 283 (5), 121 (38), 111 (22), 91 (31), 77 (27), 51 (18). Anal. Calc. for $C_{16}H_{14}ClN_3OS$: C 57.91, H 4.25, N 12.66, S 9.66. Found: C 57.65, H 4.56, N 12.87, S 9.54 %.

5-(3-Methoxybenzyl)-4-(4-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6b***

White solid (0.33 g, 73 %), mp 171–173°C. R_f 0.30 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3289, 1605, 1574, 1517, 1267. δ_H (300 MHz, DMSO-*d*6) 13.68 (s, 1H, N*H*), 7.12 (dt, *J* 8.4, 2.1, 2H, Ar-*H*), 7.11 (d, *J* 1.8, 1H, Ar-*H*), 7.08 (dt, *J* 9.0, 2.1, 2H, Ar-*H*), 6.72 (dd, *J* 8.4, 2.1, 1H, Ar-*H*), 6.65 (d, *J* 7.2, 1H, Ar-*H*), 6.46 (t, *J* 2.1, 1H, Ar-*H*), 3.80 (s, 3H, OCH_3), 3.64 (s, 3H, OCH_3), 3.33 (s, 2H, CH_2). δ_C 168.64, 160.15, 154.87, 150.28, 138.91, 129.88, 129.11, 126.43, 122.20, 119.34, 114.21, 111.98, 55.78, 55.31, 31.92. *m/z* (GCMS, %) 327 (100), 312 (8), 294 (8), 279 (4), 146 (10), 121 (29), 91 (21), 77 (21), 51 (8). Anal. Calc. for $C_{17}H_{17}N_3O_2S$: C 62.36, H 5.23, N 12.83, S 9.79. Found: C 62.06, H 5.45, N 12.64, S 9.70 %.

5-{2-(3-Methoxyphenyl)ethyl}-4-(4-chlorophenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6c***

White solid (0.36 g, 75 %), mp 163–164°C. R_f 0.25 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3263, 1594, 1534, 1523, 1284. δ_H (300 MHz, DMSO-*d*6) 13.70 (s, 1H, N*H*), 7.62 (dt, *J* 8.7, 2.1, 2H, Ar-*H*), 7.40 (dt, *J* 6.6, 3.0, 2H, Ar-*H*), 7.15 (t, *J* 8.1, 1H, Ar-*H*), 6.74 (dd, *J* 9.3, 1.8, 1H, Ar-*H*), 6.63 (d, *J* 8.4, 1H, Ar-*H*), 6.61 (s, 1H, Ar-*H*), 3.65 (s, 3H, OCH_3), 3.30–2.75 (m, 4H, 2 × CH_2). δ_C 168.00, 159.73, 151.93, 141.96, 134.62, 132.02, 130.66, 129.95, 129.86, 120.87, 114.34, 112.18, 55.32, 37.48, 31.79. *m/z* (GCMS, %) 345 (92), 330 (4), 312 (8), 297 (2), 175 (10), 150 (6), 121 (100), 91 (65), 75 (25). Anal. Calc. for $C_{17}H_{16}ClN_3OS$: C 59.04, H 4.66, N 12.15, S 9.27. Found: C 59.33, H 4.36, N 12.39, S 9.46 %.

5-{2-(3-Methoxyphenyl)ethyl}-4-(4-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6d***

White solid (0.34 g, 71 %), mp 178–180°C. R_f 0.25 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3286, 1596, 1544, 1515, 1293. δ_H (300 MHz, DMSO-*d*6) 13.67 (s, 1H, N*H*), 7.27 (dt, *J* 8.7, 1.8, 2H, Ar-*H*), 7.14 (t, *J* 7.8, 1H, Ar-*H*), 7.07 (dt, *J* 9.0, 3.3, 2H, Ar-*H*), 6.73 (dd, *J* 8.7, 2.1, 1H, Ar-*H*), 6.63 (d, *J* 7.8, 1H, Ar-*H*), 6.60 (s, 1H, Ar-*H*), 3.55 (s, 3H, OCH_3), 3.52 (s, 3H, OCH_3), 2.70 (t, *J* 6.0, 2H, CH_2), 2.62 (t, *J* 6.0, 2H, CH_2). δ_C 168.24, 160.11, 159.72, 152.25, 142.07, 129.89, 126.58, 123.47, 120.86, 115.00, 114.33, 112.16, 55.92, 55.33, 37.41, 34.70. *m/z* (GCMS, %) 341 (100), 326 (5), 308 (6), 293 (3), 179 (12), 121 (46), 91 (26), 77 (16). Anal. Calc. for $C_{18}H_{19}N_3O_2S$: C 63.32, H 5.61, N 12.31, S 9.39. Found: C 63.09, H 5.66, N 12.51, S 9.30 %.

4-(4-Chlorophenyl)- 5-(3,4-dimethoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6e***

White solid (0.37 g, 76 %), mp 234–236°C. R_f 0.29 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3273, 1587, 1534, 1512, 1257. δ_H (300 MHz, DMSO-*d*6) 14.08 (s, 1H, N*H*), 7.60 (dt, *J* 8.7, 2.1, 2H, Ar-*H*), 7.42 (dt, *J* 8.7, 3.0, 2H, Ar-*H*), 6.95 (d, *J* 8.1, 1H, Ar-*H*), 6.87 (dd, *J* 6.1, 1.8, 1H, Ar-*H*), 6.83 (d, *J* 2.1, 1H, Ar-*H*), 3.65 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3). δ_C 168.79, 150.83, 148.70, 137.51, 134.48, 131.29, 129.86, 126.84, 118.12, 113.71, 111.99, 111.92, 55.70, 54.23. *m/z* (GCMS, %) 347 (100), 332 (12), 304 (10), 183 (8), 163 (14), 120 (12), 111 (12), 75 (18). Anal. Calc. for $C_{16}H_{14}ClN_3O_2S$: C 55.25, H 4.06, N 12.08, S 9.22. Found: C 55.44, H 4.36, N 12.00, S 9.35 %.

5-(3,4-Dimethoxyphenyl)-4-(4-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6f***

White solid (0.35 g, 73 %), mp 221–223°C. R_f 0.28 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3297, 1576, 1517, 1489, 1256. δ_H (300 MHz, DMSO-*d*6) 13.90 (s, 1H, N*H*), 7.27 (d, *J* 8.7, 2H, Ar-*H*), 7.15 (d, *J* 9.0, 2H, Ar-*H*), 7.11–6.70 (m, 3H, Ar-*H*), 3.65 (s, 3H, OCH_3), 3.63 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3). δ_C 169.12, 151.00, 151.00, 150.72, 148.63, 136.52, 130.49, 127.84, 121.60, 118.42, 111.88, 111.82, 55.96, 55.93, 53.67. *m/z* (GCMS, %) 343 (100), 328 (10), 179 (5), 163 (18), 147 (15), 120 (10), 92 (10), 77 (11), 51 (4). Anal. Calc. for $C_{17}H_{17}N_3O_3S$: C 59.46, H 4.99, N 12.24, S 9.34. Found: C 59.41, H 4.67, N 12.20, S 9.55 %.

5-(3-Methoxybenzyl)-4-(2-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6g***

White solid (0.32 g, 71 %), mp 156–158°C. R_f 0.24 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3225, 1591, 1523, 1498, 1289. δ_H (300 MHz, DMSO-*d*6) 13.74 (s, 1H, N*H*), 7.03 (t, *J* 8.7, 1H, Ar-*H*), 7.21–6.90 (m, 4H, Ar-*H*), 6.74 (dd, *J* 8.4, 3.0, 1H, Ar-*H*), 6.46 (d, *J* 8.3, 1H, Ar-*H*), 6.39 (t, *J* 1.9, 1H, Ar-*H*), 3.59 (s, 3H, OCH_3), 3.54 (s, 3H, OCH_3), 2.41 (s, 2H, CH_2). δ_C 168.63, 159.50, 154.98, 152.05, 136.39, 131.82, 130.53, 129.72, 122.10, 121.22, 120.98, 114.98, 112.95, 112.83, 55.48, 53.38, 35.91. *m/z* (GCMS, %) 327 (50), 312 (2), 294 (100), 161 (4), 146 (10), 121 (30), 91 (21), 77 (29), 51 (18). Anal. Calc. for $C_{17}H_{17}N_3O_2S$: C 62.36, H,5.23, N 12.83, S 9.79. Found: C 62.41, H 5.29, N 12.61, S 9.56 %.

5-{2-(3-Methoxyphenyl)ethyl}-4-(2-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6h***

White solid (0.35 g, 73 %), mp 120–122°C. R_f 0.36 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3286, 1586, 1509, 1501, 1230. δ_H (300 MHz, DMSO-*d*6) 13.69 (s, 1H, N*H*), 7.53 (t, *J* 8.7, 1H, Ar-*H*), 7.61–7.10 (m, 4H, Ar-*H*), 6.71 (dd, *J* 8.4, 2.9, 2H, Ar-*H*), 6.35 (t, *J* 1.9, 1H, Ar-*H*), 3.59 (s, 3H, OCH_3), 3.56 (s, 3H, OCH_3), 3.34 (t, *J* 7.4, 2H, CH_2), 3.31 (t, *J* 7.4, 2H, CH_2). δ_C 168.31, 152.49, 142.83, 131.94, 130.72, 129.88, 122.21, 121.32, 120.88, 116.64, 114.39, 113.29, 112.09, 111.00, 55.37, 53.44, 37.25, 34.63. *m/z* (GCMS, %) 341 (100), 326 (5), 308 (5), 293 (4), 175 (4), 121 (35), 91 (25), 77 (15). Anal. Calc. for $C_{18}H_{19}N_3O_2S$: C 63.32, H 5.61, N 12.31, S 9.39. Found: C 63.54, H 5.40, N 12.49, S 9.19 %.

5-{2-(3,4,5-Trimethoxyphenyl)ethyl}-4-(3-methoxyphenyl)-2,4-dihydro-1,2,4-triazole-3-thione ***6i***

White solid (0.41 g, 74 %), mp 208–210°C. R_f 0.23 (petroleum ether/ethyl acetate, 8 : 2). ν_{max} (KBr)/cm^{-1} 3200, 1595, 1534, 1528, 1289. δ_H (300 MHz, DMSO-*d*6) 13.73 (s, 1H, N*H*), 7.46 (t, *J* 8.1, 1H, Ar-H), 7.11 (dd, *J* 8.4, 2.4, 1H, Ar-*H*), 6.94 (t, *J* 1.8, 1H, Ar-*H*), 6.90 (dt, *J* 8.1, 1.6, 1H, Ar-*H*), 6.50–6.44 (m, 2H, Ar-*H*), 3.69–3.65 (m, 12H, OCH_3), 3.37 (t, *J* 7.2, 2H, CH_2), 3.31 (t, *J* 7.2, 2H, CH_2). δ_C 167.98, 151.43, 153.17, 152.06, 136.32, 136.18, 135.13, 130.65, 120.75, 115.39, 114.60, 105.89, 55.62, 54.25, 51.29, 36.82, 35.65. *m/z* (GCMS, %) 401 (38), 386 (8), 287 (13), 181 (100), 148 (12), 92 (6), 77 (6). Anal. Calc. for $C_{20}H_{23}N_3O_4S$: C 59.83, H 5.77, N 10.47, S 7.99. Found: C 59.54, H 5.51, N 10.40, S 8.09 %.

Pharmacological Procedures

Acetylcholinesterase Inhibition Assay

The inhibitory activities of newly synthesized novel compounds were determined spectrophotometrically using acetylthiocholine as substrate by modifying the method of Ellman.[29] The assay solution consisted of 20 µL of 50 mM Tris–hydrochloride buffer, containing 0.1 M sodium chloride, 0.02 M magnesium chloride (pH 8.0) and 50 µL of 3 mM 5,5′-dithio-bis(2-nitrobenzoic acid). Increasing concentration of test compounds (10 µL) were added to the assay solution and pre-incubated for 15 min at 25°C with the enzyme. The enzymatic reaction was started by adding 10 µL of acetylethiocholinchloride as a substrate and again incubated for 5 min. The hydrolysis of acetylthiocholine was determined by monitoring the formation of the yellow 5-thio-2-nitrobenzoate anion as a result of the reaction with 5,5′-dithio-bis(2-nitrobenzoic acid) with thiocholines, catalyzed by enzymes at a wavelength of 412 nm. For the non-enzymatic reaction, the assays were carried out with a blank containing all components except acetylcholinesterase. The reaction rates were compared and the percentage inhibition owing to the presence of tested inhibitors was calculated.

Neostigmine methylsulfate and Donepezil were used as standard inhibitors. Each concentration was analyzed in three independent experiments run in triplicate, The Cheng–Prusoff equation was used to calculate the K_i values from the IC_{50} values determined by the nonlinear curve-fitting program Prism 5.0 (GraphPad, San Diego, CA, USA).

Alkaline Phosphatase Inhibition Assay

Initial screening of the newly synthesized compounds was performed at a concentration of 0.1 mM of test compounds. For potentially active compounds, full concentration-inhibition curves were determined. To screen putative inhibitors, activity of calf intestinal alkaline phosphatase (CIALP) was measured by a spectrophotometric assay as previously described by J. Iqbal.[30] The reaction mixture comprised of 50 mM TRIS-HCl, 5 mM $MgCl_2$, 0.1 mM $ZnCl_2$ (pH 9.5), the inhibitors (0.1 mM with final DMSO 1 % (v/v) and the mixture was pre-incubated for 10 min by adding 5 µL of CIALP (0.025 U mL^{-1}). Then, 10 µL of substrate (0.5 mM *p*-NPP) was added to initiate the reaction and the assay mixture was incubated again for 30 min at 37°C. The change in absorbance of released *p*-nitrophenolate was monitored at 405 nm, using a 96-well microplate reader (Bio-Tek ELx 800™, Instruments, Inc. USA). The inhibitor activity of each sample containing the inhibitor was compared with the control sample (without inhibitor). The compounds that exhibited more than 50 % inhibitory activities at calf intestinal alkaline phosphatase were further evaluated for the determination of inhibition constants. Inhibitory activities of the potent compounds were determined by a range of concentrations of inhibitors spanning three orders of magnitude. All of the experiments were repeated three times in a triplicate manner. KH_2PO_4 and Theophylline were used as the standard inhibitors of calf ALP. The K_i values from the IC_{50} values determined by the nonlinear curve-fitting program Prism 5.0 (GraphPad, San Diego, CA, USA).

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References

[1] J. P. Changeux, *Mol. Pharmacol.* **1966**, *2*, 369.
[2] D. J. Epstein, H. A. Berman, P. Taylor, *Biochemistry* **1979**, *18*, 4749. doi:10.1021/BI00588A040
[3] W. D. Mallender, T. Szeglets, T. L. Rosenberry, *J. Biol. Chem.* **1999**, *274*, 8491. doi:10.1074/JBC.274.13.8491
[4] T. Szegletes, W. D. Mallender, P. J. Thomas, T. L. Rosenberry, *Biochemistry* **1999**, *38*, 122. doi:10.1021/BI9813577
[5] A. Y. Abramov, L. Canvari, M. R. Duchen, *J. Neurosci.* **2003**, *15*, 5088.
[6] D. A. Butterfield, *Chem. Res. Toxicol.* **1997**, *10*, 495. doi:10.1021/TX960130E
[7] R. M. Marchbanks, *J. Neurochem.* **1982**, *39*, 9. doi:10.1111/J.1471-4159.1982.TB04695.X
[8] P. Taylor, *Pharmacological basis of therapeutics*, **1985** (Eds A. G. Gilman, L. S. Goodman, F. Murad) (MacMillan: New York).
[9] J. M. Ellis, *J. Am. Osteopath. Assoc.* **2005**, *105*, 145.
[10] B. N. Lenta, C. Vonthron-Sénécheau, B. Weniger, K. P. Devkota, J. Ngoupayo, M. Kaiser, Q. Naz, M. I. Choudhary, E. Tsamo, N. Sewald, *Molecules* **2007**, *12*, 1548. doi:10.3390/12081548
[11] M. Farlow, S. I. Gracon, L. A. Hershey, K. W. Lewis, C. H. Sadowsky, J. Dolan-Ureno, *JAMA* **1992**, *268*, 2523. doi:10.1001/JAMA.1992.03490180055026
[12] D. K. Lahiri, M. R. Farlow, N. H. Grieg, K. Sambamurti, *Drug Dev. Res.* **2002**, *56*, 267. doi:10.1002/DDR.10081
[13] S. L. Rogers, M. R. Farlow, R. S. Doody, R. Mohs, L. T. Friedhoff, *Neurology* **1998**, *50*, 136. doi:10.1212/WNL.50.1.136
[14] M. H. Le Du, J. L. Millan, *J. Biol. Chem.* **2002**, *277*, 49808. doi:10.1074/JBC.M207394200
[15] L. Zhang, M. Balcerzak, J. Radisson, C. Thouverey, S. Pikula, G. Azzar, R. Buchet, *J. Biol. Chem.* **2005**, *280*, 37289. doi:10.1074/JBC.M504260200
[16] S. P. Coburn, J. D. Mahuren, M. Jain, Y. Zubovic, J. Wortsman, *J. Clin. Endocrinol. Metab.* **1998**, *83*, 3951. doi:10.1210/JC.83.11.3951
[17] D. Sarrouilhe, P. Lalegerie, M. Baudry, *Biochim. Biophys. Acta* **1992**, *1118*, 116. doi:10.1016/0167-4838(92)90137-3
[18] M. Muda, N. N. Rao, A. Torriani, *J. Bacteriol.* **1992**, *174*, 8057.
[19] M. Li, W. Ding, B. Baruah, D. C. Crans, R. Wang, *J. Inorg. Biochem.* **2008**, *102*, 1846. doi:10.1016/J.JINORGBIO.2008.06.007
[20] A. Katrizky, *Comprehensive Heterocyclic Chemistry*, **1984**, vol. 6, (Pergamon: Oxford, London).
[21] M. S. Karthikeyan, B. S. Holla, *Monatsh. Chem.* **2008**, *139*, 691. doi:10.1007/S00706-007-0794-Z
[22] I. Khan, S. Ali, S. Hameed, N. H. Rama, M. T. Hussain, A. Wadood, R. Uddin, Z. U. Haq, A. Khan, S. Ali, M. I. Choudhary, *Eur. J. Med. Chem.* **2010**, *45*, 5200. doi:10.1016/J.EJMECH.2010.08.034
[23] O. R. Abid, T. M. Babar, F. I. Ali, S. Ahmed, A. Wadood, N. H. Rama, Z. U. Haq, R. Uddin, A. Khan, M. I. Choudhary, *ACS Med. Chem. Lett* **2010**, *51*, 145.
[24] X. Zheng, Z. Li, Y. Wang, W. Chen, Q. Huang, C. Liu, G. Song, *J. Fluor. Chem.* **2003**, *123*, 163. doi:10.1016/S0022-1139(03)00168-4
[25] M. Moise, V. Sunel, L. Profire, M. Popa, J. Desbrieres, C. Peptu, *Molecules* **2009**, *14*, 2621. doi:10.3390/MOLECULES14072621
[26] W. Shi, X. Qian, R. Zhang, G. Song, *J. Agric. Food Chem.* **2001**, *49*, 124. doi:10.1021/JF0007941
[27] M. Amir, K. Shikha, *Eur. J. Med. Chem.* **2004**, *39*, 535. doi:10.1016/J.EJMECH.2004.02.008
[28] H. Gilman, A. H. Blatt, *Organic Synthesis Coll.*, **1967**, 2nd ed. (John Wiley Inc: New York).
[29] G. L. Ellman, K. D. Courtney, V. Andres, Jr, R. M. Feather-Stone, *Biochem. Pharmacol.* **1961**, *7*, 88. doi:10.1016/0006-2952(61)90145-9
[30] J. Iqbal, *Anal. Biochem.* **2011**, *414*, 226. doi:10.1016/J.AB.2011.03.021
[31] M. Hanif, G. Qadeer, N. H. Rama, S. Vuoti, J. Autio, *Acta Crystallogr.* **2007**, *E63*, o4754.