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ORIGINAL ARTICLE

Synthesis and biological evaluation of 1,2,4-triazolidine-3-thiones as potent acetylcholinesterase inhibitors: in vitro and in silico analysis through kinetics, chemoinformatics and computational approaches

Prasad G. Mahajan[1] · Nilam C. Dige[2] · Balasaheb D. Vanjare[1] · Hussain Raza[2] · Mubashir Hassan[3] · Sung-Yum Seo[2] · Chong- Hyeak Kim[4] · Ki Hwan Lee[1]

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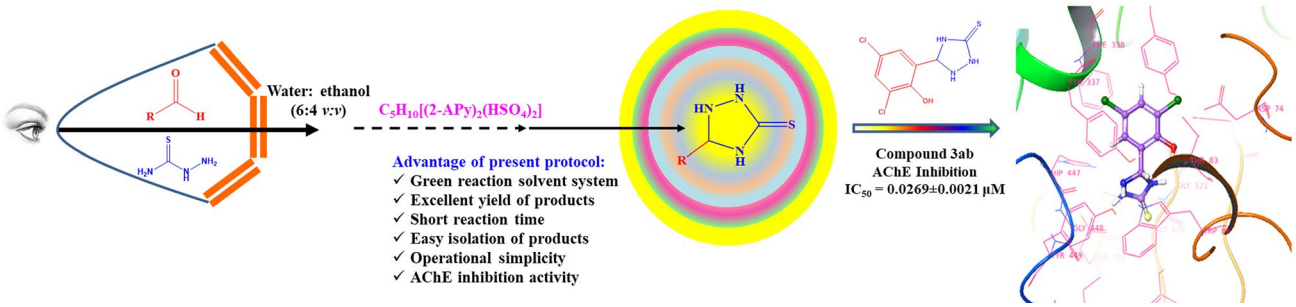
Abstract

We have designed and synthesized a novel acidic ionic liquid and explored its catalytic efficiency for the synthesis of 1,2,4-triazolidine-3-thione derivatives. A simple reaction between aldehydes and thiosemicarbazide for short time in 60:40 *v*/*v* water/ethanol at room temperature offers target 1,2,4-triazolidine-3-thione derivatives. The formation of target compounds is confirmed by NMR, IR and ESI–MS analysis. Pleasingly, synthesized compounds show noteworthy acetylcholinesterase (AChE) inhibitory activity with much lower IC_{50} values 0.0269 ± 0.0021–1.1725 ± 0.0112 µM than standard Neostigmine methylsulphate. In addition, synthesized 1,2,4-triazolidine-3-thiones exhibits significant free radical scavenging activity as compared to standard vitamin C. The studies on validation of Lipinski's rule through chemoinformatics properties and molecular docking analysis are in support of in vitro analysis. Therefore, overall present study illustrates synthesis of some new 1,2,4-triazolidines-3-thiones which can serve as a template for drug designing such as AChE inhibitors.

Graphic abstract

Herein, we proposed ionic liquid-catalyzed ease of synthetic approach for medicinally important 1,2,4-triazolidine-3-thiones and their bio-evaluations.



Keywords Ionic liquid · 1,2,4-triazolidine-3-thiones · Acetylcholinesterase inhibition · Lipinski rule · Molecular docking

Introduction

Being eco-friendly catalyst, versatile reaction medium and safe solvent for the synthetic methodology enhance the utility of ionic liquids (ILs) in organic chemistry. ILs shows unique properties such as low vapour pressure, high chemical, thermal as well as electrochemical stability, significant viscosity and low flammability. Such a characteristics of ILs,

Prasad G. Mahajan and Nilam C. Dige have contributed equally for this research work.

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Extended author information available on the last page of the article

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increases their significance in industrial area for the development of science and engineering field [1–4]. The distinct structure, unique physicochemical properties and ionic interactions of ILs were widely explored in organic synthesis [5], catalysis [6], extraction [7] and CO_2/SO_2 capture [8, 9]. In addition, non-volatile and non-corrosive nature, air stability, simple recovery and recycling process have gained significant research of interest in sulphate functionalized ILs and their application for synthetic organic procedures [10, 11].

Triazole derivatives are one of the important compounds in heterocyclic chemistry due to their extensive medicinal and pharmaceutical properties. Various reports are accessible for the synthesis of triazoles employing diverse synthetic approaches [12–14]. Triazoles were classified according to the position of nitrogen atoms in the ring viz. 1,2,3- and 1,2,4-triazoles. Interestingly, out of these two different types of triazoles, 1,2,4-triazoles derivatives are investigated thoroughly in the field of pharmacology and agrochemical [15–23]. The biological activities posed by the class of 1,2,4-triazoles or related compounds in this family mainly include studies on activities such as antibacterial [24, 25], analgesic [26], anti-cancer [27], anti-inflammatory [28], antifungal [29, 30] cytotoxic [31] antidepressant [32] and antitumor [33]. Remarkably, number of reports witnessed for biological effects of 1,2,4-triazolidine-3-thiones or their correlated compounds showed acetylcholinesterase inhibition [34–37], anti-cancer [38, 39], anti-HIV [40], antimycobacterial [41], anti-viral [42], antiepileptic [43], anti-allergic [44], antidepressant [45], carbonic anhydrase [37], and analgesic [46] activities. Due to their plant growth regulatory action, they have been used in the production of agrochemicals [47]. Nowadays, design and synthesis of 1,2,4-triazolidine-3-thiones for their biological activities is challenging, demanding and advantageous research interest before the scientific community. Therefore, synthesis of 1,2,4-triazolidine-3-thiones by using novel ionic liquid for their bio-evaluation against acetylcholinesterase inhibition activity was the main motivation to perform the present research work.

Literature survey guided us for the investigation towards finding out the attempts made by various research groups in designing and synthesizing 5-aryl-1,2,4-triazolidine-3-thiones [48–50]. It was realized that use of hazardous chemicals, longer reaction time, unsatisfactory yield of final products, difficulties in product isolation and absence of studies on biological evaluation were the main drawbacks in most of the reported studies. Therefore, our aim was to overcome these weaknesses through performing the ionic liquid-catalyzed reaction between variety of aldehydes and thiosemicarbazide in ethanol/water mixture as eco-friendly solvent at room temperature. The synthesized compounds were screened against acetylcholine-esterase (AChE) enzyme to examine biological activity. Delightfully, some of synthesized compounds found to be more potent against AChE enzyme as compared to standard Neostigmine methylsulphate.

Result and discussion

Synthesis of library of 1,2,4-triazolidine-3-thiones

Initially, we focused our attention towards design and synthesis of eco-friendly and homogenous Brønsted acidic ionic liquid (IL), 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(hydrogen sulphate), i.e. $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$. The synthesis was carried out in two steps. In the first step, two moles of 2-aminopyrimidine were reacted with one mole of 1,5 dibromopentane in DMF at 80 °C for 18 h to form 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-dibromide, i.e. $C_5H_{10}[(2\text{-}APy)_2(Br)_2]$. In the second step, the resulting dibromide form of ionic liquid was further treated with an equimolar amount of sulphuric acid in methanol at room temperature for 4 h. The expected product was 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(hydrogen sulphate), i.e. $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$. The synthetic route for Brønsted acidic ionic liquid is shown in Scheme 1.

After successful synthesis of IL, the attention was focused towards optimize the reaction conditions for the synthesis of novel 1,2,4-triazolidine-3-thiones. Initially, the reaction of benzaldehyde (1 mmol) and thiosemicarbazide (1 mmol) was performed as a model reaction to optimize the reaction conditions in the proposed methodology. The screening and choice of catalyst was done by using various catalysts such as tripotassium phosphate (K_3PO_4), *p*-toluenesulphonic acid (*p*-TSA), aluminium chloride ($AlCl_3$), ceric ammonium nitrate (CAN), ammonium acetate (NH_4OAc) and synthesized ionic liquid (IL)-$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ (Table 1, Entries 1–6). Pleasingly, it was noticed that synthesized ionic liquid showed excellent catalytic activity (Table 1, Entry 9). Furthermore, attempts were made towards optimization of IL loading by changing its quantity (Table 1, Entries 6–13). It was found that 5 mol% of synthesized IL was enough to drive the reaction forward with excellent product yield in short reaction time (Table 1, Entry 9). When the catalytic amount of IL was chosen to below 5 mol%, then there was a slight decrease in the product yield and more time was taken to complete the transformation (Table 1, Entries 10–13). However, a slight increase in product yield was observed if there was increase in the amount of IL catalyst from 5–20 mol% with no change in reaction time for present transformation (Table 1, Entries 6–9). Hence, we concluded that 5 mol% of synthesized IL can be used as optimized catalytic amount to perform the present organic transformation. After successful screening of catalyst, the efforts were done towards optimization of solvent system. As water is eco-friendly solvent and easily available resource, we performed the model reaction

Scheme 1 Synthetic route for Brønsted acidic ionic liquid

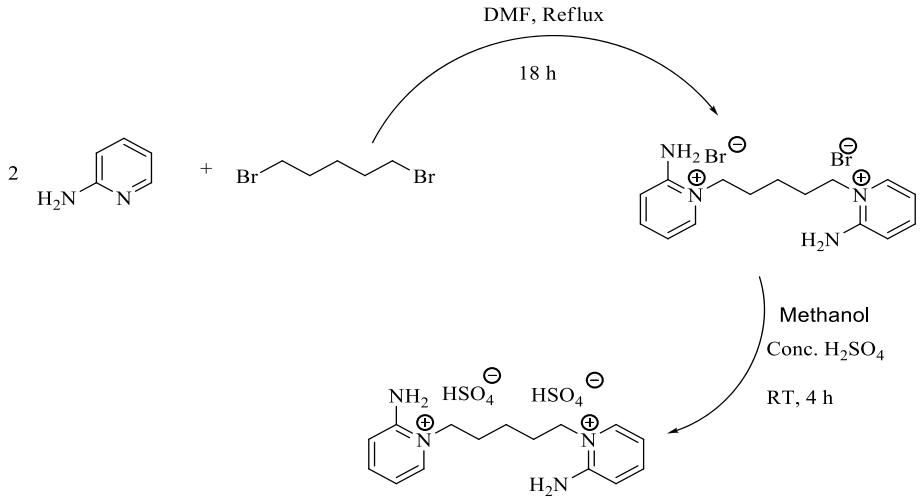


Table 1 Screening of catalyst and it's amount for the synthesis of 1,2,4-triazolidine-3-thione

S. no.	Catalyst	Loading (mol%)	Time (min)	Yield (%)
1	K_3PO_4	20	10	82
2	*p*-TSA	20	25	78
3	$AlCl_3$	20	35	79
4	CAN	20	40	77
5	NH_4OAc	20	20	83
6	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	20	3	88
7	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	15	3	88
8	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	10	3	87
9	**$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$**	**5**	**3**	**87**
10	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	4	5	85
11	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	3	8	84
12	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	2	10	84
13	$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$	1	13	80

Reaction condition: benzaldehyde (1 mmol), thiosemicarbazide (1 mmol), solvent-ethanol (10 mL), catalyst, room temperature

Bold indicates the most favorable conditions or suitable entries of compounds wherever necessary

Table 2 Screening of solvent for the synthesis of 1,2,4-triazolidine-3-thione

S. no.	Solvent	Time in min	Yield (%)
1	Water	25	77
2	Water/ethanol (9:1)	12	79
3	Water/ethanol (8:2)	14	81
4	Water/ethanol (7:3)	9	68
5	**Water/ethanol (6:4)**	**3**	**95**
6	Water/ethanol (5:5)	6	92
7	Water/ethanol (4:6)	6	91
8	Water/ethanol (3:7)	10	90
9	Water/ethanol (2:8)	15	88
10	Water/ethanol (1:9)	12	87
11	Ethanol	10	87

Reaction condition: benzaldehyde (1 mmol), thiosemicarbazide (1 mmol), specified solvent (10 mL), catalyst—$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ = 5 mol%, room temperature

Bold indicates the most favorable conditions or suitable entries of compounds wherever necessary

using water as a solvent. Unfortunately, the less solubility of reactants in the water may be responsible for low product yield and longer reaction time (Table 2, entry 1). Hence, we decided to scrutinize the reaction using ethanol/water mixture. Satisfyingly, we were found that water/ethanol (6:4 v:v) solvent mixture furnished exceptional product yield within very short reaction time and served as the best solvent for present transformation (Table 2, entry 5). Thus, 5 mol% of $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ as catalytic amount, water/ethanol (6:4 v:v) as solvent system and room temperature are the optimized reaction conditions for the present transformation.

With these optimized reaction conditions in hand, we have generated a library of 1,2,4-triazolidine-3-thiones through reaction of thiosemicarbazide with various aromatic aldehydes bearing electron-donating as well as electron-withdrawing groups (Table 3, entries **3a–3h**) and polycyclic aromatic aldehydes (Table 3, entries **3i–3j**) at room temperature. The synthetic route for 1,2,4-triazolidine-3-thiones is shown in Scheme 2. In addition to this, the scope of the reaction was extended using variety of heterocyclic aldehydes. All reactions were completed efficiently with satisfactory product yields in shorten reaction time (Table 3, entries **3k–3r**). Encouraged by this success, flexibility of method was also scrutinized for various substituted salicylaldehydes. Pleasingly, all reactions were completed smoothly as per expectations (Table 3, entries **3s–3ac**). Therefore, the ease of present synthetic methodology can be adaptable for the swift and economical synthesis of 1,2,4-triazolidine-3-thiones at room temperature.

Table 3 $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ catalyzed synthesis of 1,2,4-triazolidine-3-thione compounds library

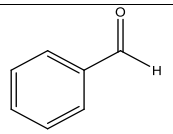
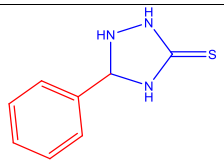
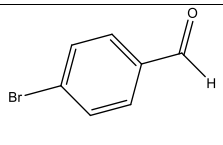
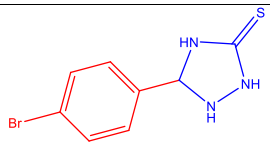
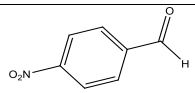
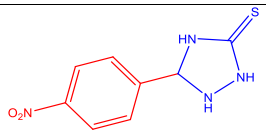
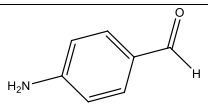
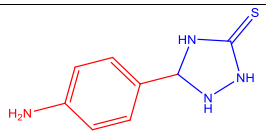
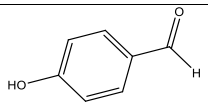
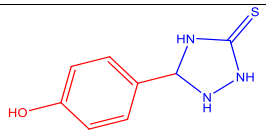
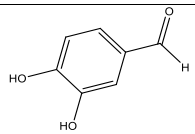
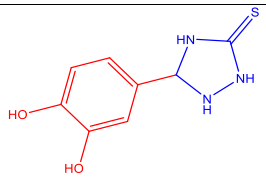
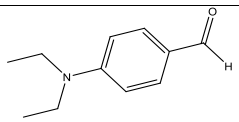
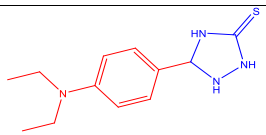
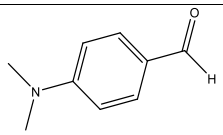
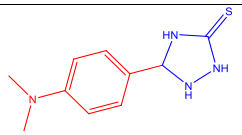
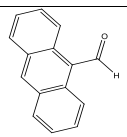
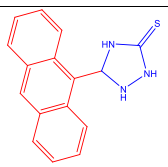
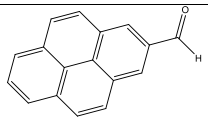
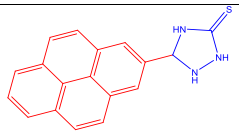
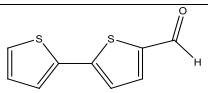
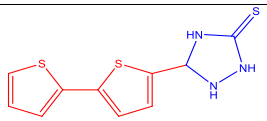
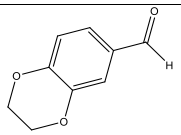
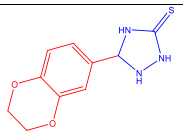
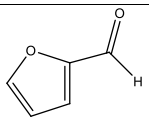
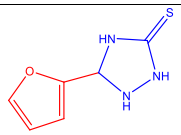
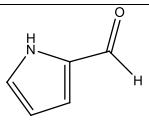
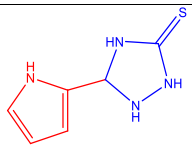
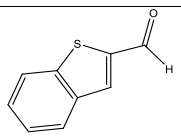
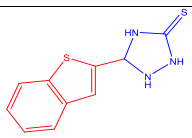
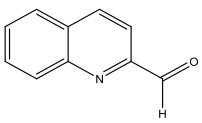
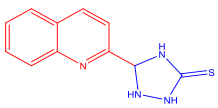
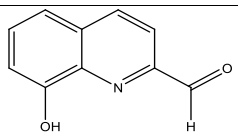
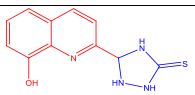
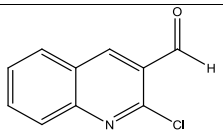
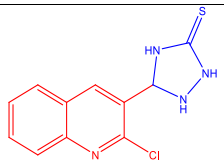
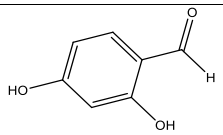
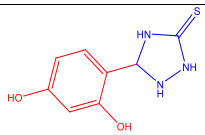
Entry	Aldehyde (1)	Product (3)	Time min	Yield %	M.P. obs. (Lit) ◦C Refs.
3a			3	95	154 (154) [41]
3b			5	91	204 (202–204) [14]
3c			10	96	227 (226–228) [14]
3d			15	81	198
3e			3	88	237
3f			3	81	241 (240–241) [13]
3g			3	79	178
3h			2	96	201
3i			2	97	216

Table 3 (continued)

Entry	Aldehyde (1)	Product (3)	Time min	Yield %	M.P. obs. (Lit) ◦C Refs
3j			3	97	255
3k			5	96	213
3l			10	86	209
3m			7	85	157
3n			3	88	210
3o			3	96	233
3p			2	84	246
3q			2	97	254
3r			2	97	235
3s			3	95	245

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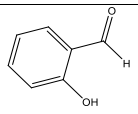
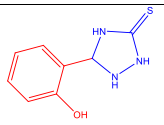
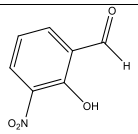
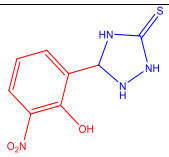
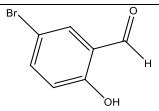
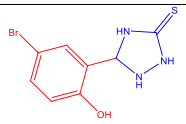
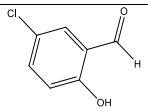
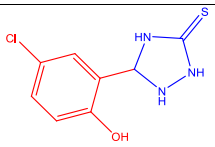
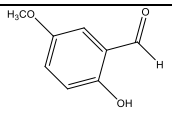
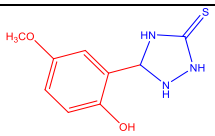
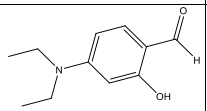
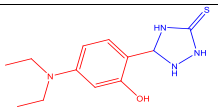
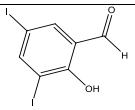
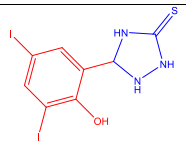
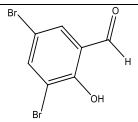
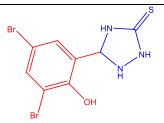
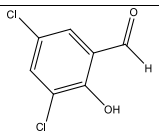
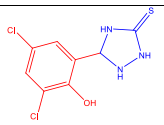
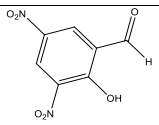
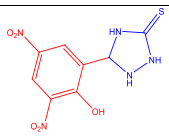
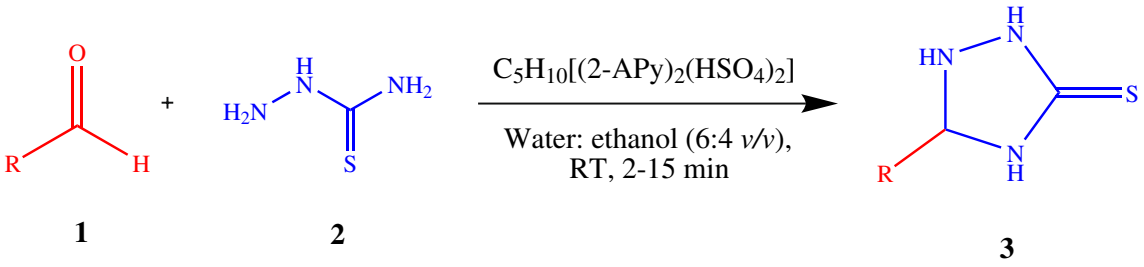
3t			10	92	218 (216–218) [14]
3u			8	95	240
3v			6	96	246
3w			5	96	272
3x			6	93	228
3y			2	94	233
3z			2	93	250
3aa			2	95	247
3ab			2	96	256
3ac			2	95	230

Reaction condition: aldehyde (1 mmol), thiosemicarbazide (1 mmol), Solvent = water/ethanol (6:4, v/v) (10 mL), catalyst–$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ = 5 mol%, room temperature

Bold indicates the most favorable conditions or suitable entries of compounds wherever necessary



Scheme 2 Synthesis of library for compounds of 1,2,4-triazolidine-3-thiones

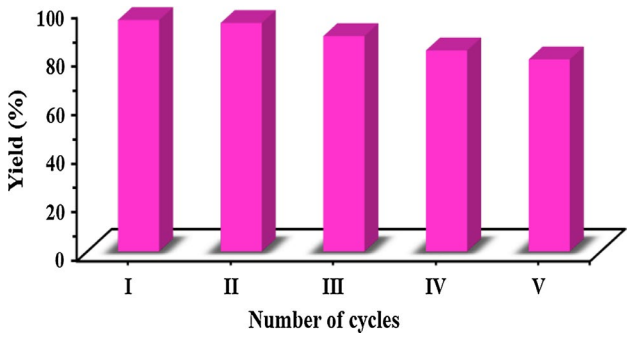


Fig. 1 Recycling of catalyst—$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ (5 mol%) for synthesis of 1,2,4-triazolidine-3-thiones

Reusability of synthesized IL-$C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$

The reusability of IL is a significant and fundamental characteristic property. Hence, attempts were made on the recovery and reuse of synthesized IL. The recovery and reusability of IL were performed with model reaction using $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$ (5 mol%) in water/ethanol (6:4 v:v, 5 mL) at room temperature. After completion of reaction, the reaction mixture was simply filtered off to separate solid compound and water/ethanol mixture. The collected solvent was further extracted with ethyl acetate (10 mL) because the product was soluble in ethyl acetate and can be readily separated by extraction from the aqueous catalytic system. While IL was soluble in water, it remains in the aqueous phase. The product dissolved in upper organic phase of ethyl acetate and IL dissolved in lower aqueous system were easily separated using simple liquid–liquid extraction. Thus, the residual IL contained aqueous phase was reused for the next run under the same reaction conditions. We were found that extracted IL can be reusable for 5 subsequent runs without the loss of catalytic activity and product yields which are graphically shown in Fig. 1. Thus, the use of this catalytic system and reaction conditions used in this studies follows the required trends and fundamental principles of green chemistry such as no side products, achievement of maximum final yield from reactants, utility of safe products, rule out the use of toxic solvents in huge quantity, use of mixed solvent system, reusable catalyst, reactions at ambient temperature and minimization of harmful chemicals [51].

Table 4 IC_{50} values of 1,2,4-triazolidine-3-thione compounds for AChE inhibition

Compound	AChE IC_{50} (μM)	Compound	AChE IC_{50} (μM)
3a	86.6436 ± 0.4511	3p	13.4829 ± 0.9851
3b	26.4585 ± 0.2611	3q	17.3251 ± 0.4397
3c	18.7433 ± 0.5146	3r	N.A
3d	37.9748 ± 0.9281	3s	3.3289 ± 1.2299
3e	61.6676 ± 1.1504	3t	0.7391 ± 0.0611
3f	104.0522 ± 1.3427	3u	0.5411 ± 0.0496
3g	12.8694 ± 0.9436	3v	0.6792 ± 0.0131
3h	22.4686 ± 0.1522	3w	0.4491 ± 0.0722
3i	N.A	3x	0.7344 ± 0.0204
3j	N.A	3y	0.8023 ± 0.0283
3k	6.7541 ± 0.1271	3z	0.2943 ± 0.0348
3l	38.8317 ± 0.4763	3aa	0.2179 ± 0.0229
3m	1.1725 ± 0.0112	3ab	0.0269 ± 0.0021
3n	199.9167 ± 3.8888	3ac	2.5858 ± 0.3121
3o	41.1167 ± 0.8964	N.M	2.0366 ± 0.0581

AChE acetylcholinesterase; values are expressed as mean ± SEM; *SEM* standard error of mean; *N.A* no activity; *N.M* Neostigmine methylsulphate

In vitro analysis

Acetylcholinesterase inhibition and structure–activity relationship

All the synthesized 1,2,4-triazolidine-3-thiones were tested against the acetylcholinesterase (AChE) inhibition activity. The compounds were shown varying degrees of inhibition activity against acetylcholinesterase enzyme. Table 4 illustrates IC_{50} values of 1,2,4-triazolidine-3-thione compounds for AChE inhibition. Significantly, some compounds exhibited excellent activity with much lower IC_{50} values than the standard Neostigmine methylsulphate. In the present

investigations, compounds **3u**, **3v**, **3w**, **3x**, **3y**, **3z**, **3aa**, **3ab** and **3ac** displayed much lower IC_{50} values than the standard Neostigmine methylsulphate which signifies their potency for acetylcholinesterase inhibition. The presence of different substituents on the phenyl moiety of 1,2,4-triazolidine-3-thiones might be the reason behind varying degrees in the IC_{50} values for all compounds within the range of 0.0269 ± 0.0021–199.9167 ± 3.8888 µM as compared to the IC_{50} value 2.0366 ± 0.0581 µM of standard Neostigmine methylsulphate. From the screening results, it was found that 1,2,4-triazolidine-3-thione compounds bearing substituted aromatic aldehydes and heterocyclic aldehydes show higher IC_{50} values than the compounds possessing hydroxyl (–OH) and another alkyl or halogen group on phenyl moiety. The electron-donating and electron-withdrawing groups present at different positions in all these structures play an important role in efficient interaction with the enzyme. For the instance, the compound **3s**, **3t**, **3x** and **3y** basically bearing the donating substituents on –OH containing phenyl side of 1,2,4-triazolidine-3-thione showed typical IC_{50} values from 3.3289 ± 1.2299 to 0.8023 ± 0.0283 µM as compared to that of standard drug used in this study (2.0366 ± 0.0581 µM). However, the compounds having –OH on phenyl side along with –nitro (NO_2), bromine (Br) and chlorine (Cl) group were exhibited excellent and much lower IC_{50} values against AChE inhibition (compounds **3u**, **3v** and **3w** with IC_{50} = 0.5411 ± 0.0496, 0.6792 ± 0.0131 and 0.4491 ± 0.0722 µM), while the compounds bearing di-substituted withdrawing groups on –OH containing phenyl side showed further lower IC_{50} values (**3z**, **3aa** and **3ab** with IC_{50} = 0.2943 ± 0.0348, 0.2179 ± 0.0229 and 0.0269 ± 0.0021 µM) than standard Neostigmine methylsulphate (IC_{50} = 2.0366 ± 0.0581 µM) except compound **3ac** (IC_{50} = 2.5858 ± 0.3121 µM). Amongst the all synthesized and screened compounds, **3ab** found to be the most active compound against the acetylcholinesterase inhibition. Therefore, it seems that active site of 1,2,4-triazolidine-3-thione compounds is phenyl moiety bearing electron-withdrawing groups on its structure, which possibly interacts more with the enzyme. However, the presence of highly withdrawing groups on the phenyl moiety of 1,2,4-triazolidine-3-thione (compound **3ac)** failed to produce much lower IC_{50} value is because of the bulky nature of -NO_2 groups interacting poorly with target enzyme. The previous research reports [34, 35] demonstrates that IC_{50} values against AChE inhibition were found to be 375 ± 27 µg/mL, 507 ± 39 µg/mL, 16.42 µM, 0.9–19.5 µM and 87 nM using Hypsiboas cordobae extract [34], Pseudis minuta extract [34], phthalimides [52], isoindoline-1,3-diones [53] and 2-(5-(2-fluorobenzylamino)-pentyl)isoindoline-1,3-dione [54], respectively. From significantly low and comparable IC_{50} values for some of synthesized 1,2,4-triazolidine-3-thiones in present investigations than earlier reports show potency of these compounds against AChE inhibition. Figure 2 displays the general structural features with structure–activity relationship of 1,2,4-triazolidine-3-thiones.

Kinetic mechanism

The AChE inhibition kinetic study was performed to understand the presence of the kinetics mechanism behind the inhibitory action. Being the much lower IC_{50} value

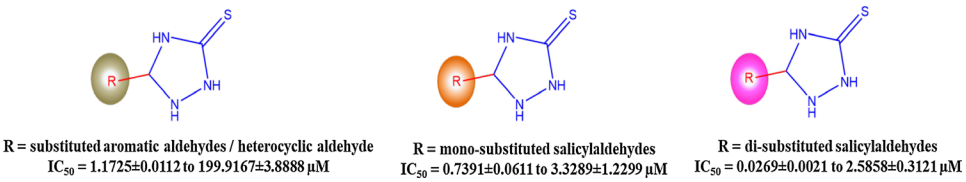


Fig. 2 General structural features with structure–activity relationship of 1,2,4-triazolidine-3-thiones and IC_{50}

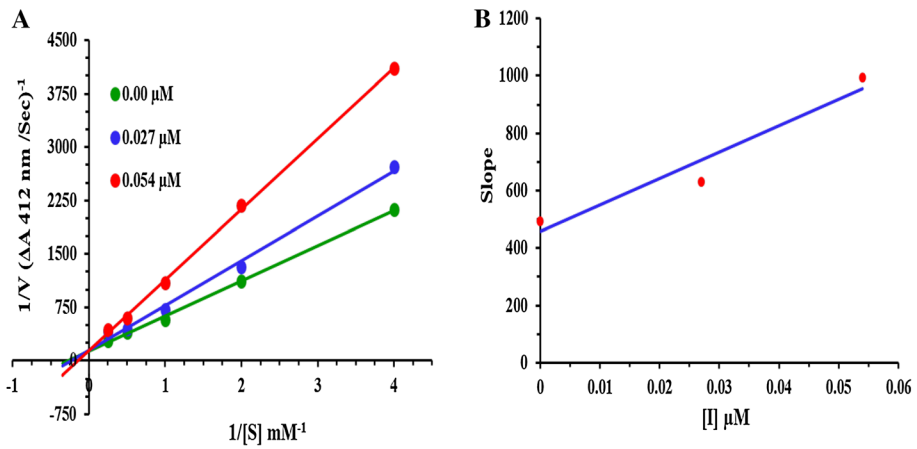


Fig. 3 **A** Lineweaver–Burk plots for inhibition of acetylcholinesterase from human erythrocytes in the presence of inhibitor **3ab** and **B** Plot of inhibitor **3ab** concentration versus slope value; [**3ab**] = 0.00, 0.027 and 0.054 µM, [Substrate acetylthiocholine iodide] = 4, 2, 1, 0.5 and 0.25 mM

possessed by compound **3ab**, it was chosen to investigate inhibition type and inhibition constant. The kinetic mechanism study involves examination of Lineweaver–Burk plot of 1/*V* against 1/[*S*] given as Fig. 3A Where, *V* and [*S*] represents reaction velocity and substrate concentration. The plot gave straight lines for the compound **3ab** and showed V_{max} remains the same with a change in the slope values. While, K_m increased with increasing concentration of **3ab**. The experimental results interpret that compound **3ab** inhibits the enzymes in competitive manner. The second plot given as Fig. 3B showed slope against concentration of **3ab**. The dissociation constant for inhibitor denoted as K_i, which was estimated from the slope of the graph and found to be 0.05 µM.

Free radical scavenging

All the synthesized 1,2,4-triazolidine-3-thiones were evaluated for DPPH free radical scavenging activity using vitamin C as control. All synthesized 1,2,4-triazolidine-3-thione compounds showed excellent free radical scavenging activity than standard vitamin C except **3a**, **3b**, **3c**, **3d**, **3l**, **3o**, **3p** and **3r**. These compounds did not show noteworthy activity even at its high concentration (100 µg/mL). The results are presented in Fig. 4.

In silico analysis

AChE structural assessment

The hydrolase protein which involves two chains (A,B) with comprises of 542 amino acids is the main core structure found in the human AChE protein. The presence of α-helices (33%), β-sheets (24%), coils (41%) and turns (21%) was analysed by using VADAR 1.8. The 93.50% of protein amino acids occurred in ideal region and 99% residues in permitted region concurred with analysis of Ramachandran plot. Most of the residues were existing in acceptable region with good precision of phi (φ) and psi (ψ) angles. The overall AChE protein structure and Ramachandran graph are given in supporting information.

Chemoinformatics properties and Lipinski's rule of five (RO5) validation

The chemoinformatics properties of all the synthesized compounds (**3a–3ac**) were predicted by using computational tools. The synthesized compounds (**3a–3ac**) were validated through RO5 analysis. It is well known that in order to follow the Lipinksi's rule of five compounds must have molecular mass and log*P* less than 500 g/mol and 5, respectively. Moreover, the compounds should possess no greater than 10 HBA and 5 HBD. The exceed values of HBA and HBD

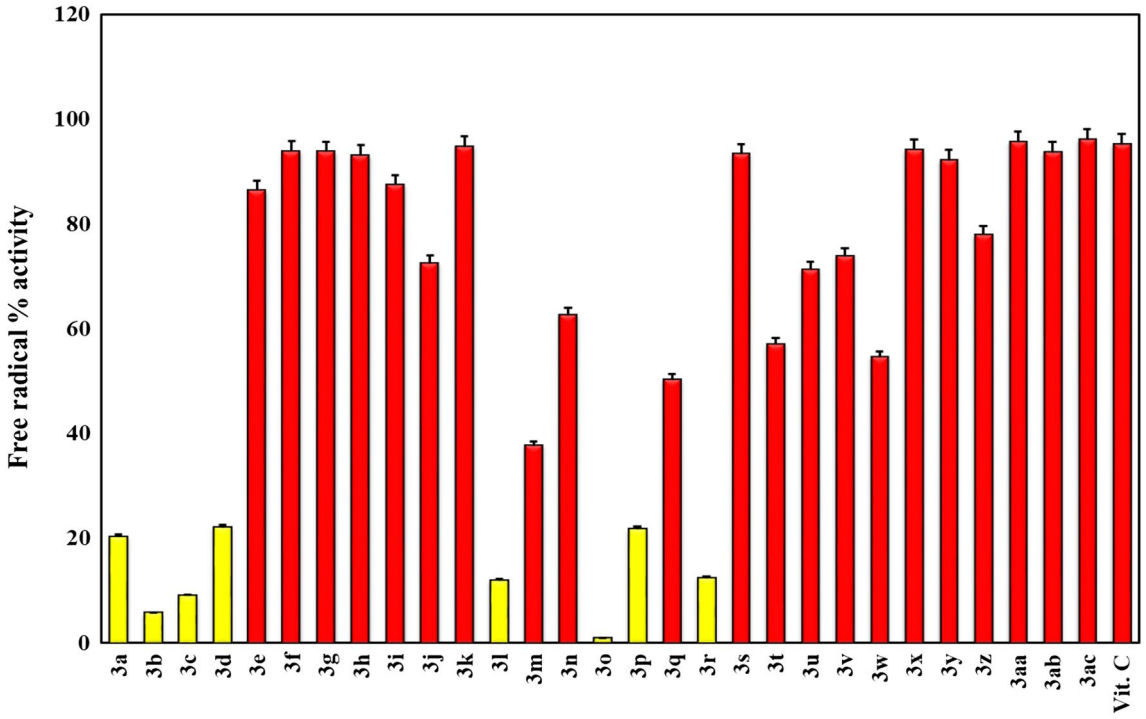


Fig. 4 Free radical % scavenging activity of synthetic compounds. (Values were represented as mean ± SEM (standard error of the mean). All compounds concentrations were 100 µg/mL

results in poor permeation. Our results showed that all of synthesized compounds possess < 10 HBA and < 5 HBD values which are comparable with standard values. Polar surface area (PSA) is also considered as good descriptor for characterizing the drug absorption, intestinal absorption, bioavailability, cell permeation ability and blood–brain barrier penetration. The predicted results showed that almost all of synthesized compounds possess less values of PSA than standard (89 $Å^2$) except compound **3d** and **3ac**. Thus, predicted cheminformatics parameters for all compound and their AChE inhibition activity suggest the molecular flexibility of synthesized compounds viz. **3u**, **3v**, **3w**, **3z**, **3aa** and **3ab**. The estimated chemoinformatics properties of synthesized 1,2,4-triazolidine-3-thiones (**3a–3ac**) listed in the tabular form and provided as supporting information.

Molecular docking analysis

Glide energy evaluation of synthesized compounds To understand the binding conformational performance of each ligand with the target protein, the well-known computational-based molecular docking analysis was performed [55, 56]. To predict the conformational position of synthesized compounds, ligands **3a–3h**, **3k–3q**, **3s–3ac** were docked against AChE separately. The predicted docked complexes were examined based on glide docking energy values (kcal/mol) and binding interaction (hydrogen/hydrophobic) pattern. The docking results showed that **3a–3h**, **3k–3q**, **3s–3ac** ligands were binds within the active region of target protein with different conformational poses (Fig. 5A). The glide docking energy values fluctuated from the highest energy value of − 9.22 kcal/mol to the lowest energy value of − 6.35 kcal/mol. Moreover, **3a–3h**, **3k–3q**, **3s–3ac** compounds exhibited good docking energy values as − 7.15, − 8.31, − 8.58, − 6.53, − 7.68, − 6.35, − 7.81, − 7.24, − 7.80, − 7.27, − 9.22, − 7.4, − 7.82, − 7.45, − 7.46, − 8.27, − 7.36, − 7.58, − 8.79, − 9.10, − 7.64, − 7.61, − 6.48, − 6.91, − 7.96 and − 7.09 kcal/mol, respectively. The basic skeleton of ligands is similar in all synthesized compounds and therefore the docking energy values were fall within the same region of value for all docking complexes. Figure 5B illustrates graphical presentation of docking energy values for each synthesized ligand against target protein.

Ligand-binding analysis of AChE docked complexes The in vitro study guided us to choose compound **3ab** from the synthesized library of 1,2,4-triazolidine-3-thiones to analyse the detailed binding interaction with target protein. The structure–activity relationship (SAR) study showed that couple of π–π interactions were observed in **3ab** docking complex. The phenyl ring structure is directly involved with Tyr337 and 1,2,4-triazolidine-3-thione ring having triazole moiety forms π–π interactions with Trp86. The Tyr337 and Trp86 are aromatic amino acids of target AChE protein which are involved in the binding interactions with the compound **3ab**. Figure 6 shows 3D (A) and 2D (B) binding interactions of **3ab** against AChE protein.

Thus, in vitro analysis and SAR are in combination with the literature survey ensured the importance of these residues in bonding with other AChE inhibitors and supports our docking results [55, 57]. The docking complexes for remaining compounds (**3a–3h**, **3k–3q**, **3s–3aa**, **3ac**) are mentioned in supporting data.

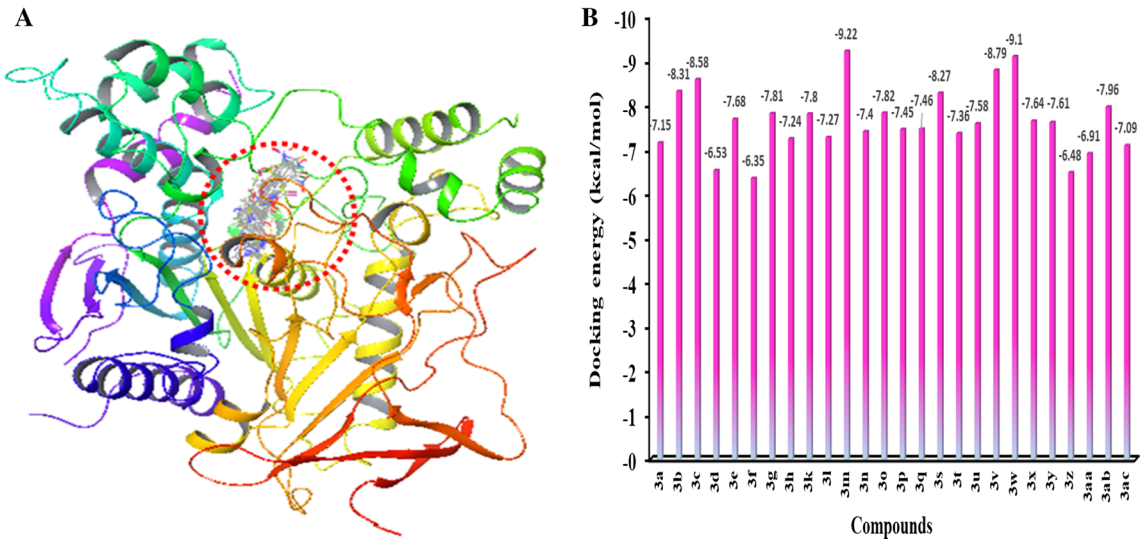


Fig. 5 Docking complexes of compounds **3a–3h, 3k–3q and 3s–3ac** with target protein (**A**) and their docking energy values (**B**)

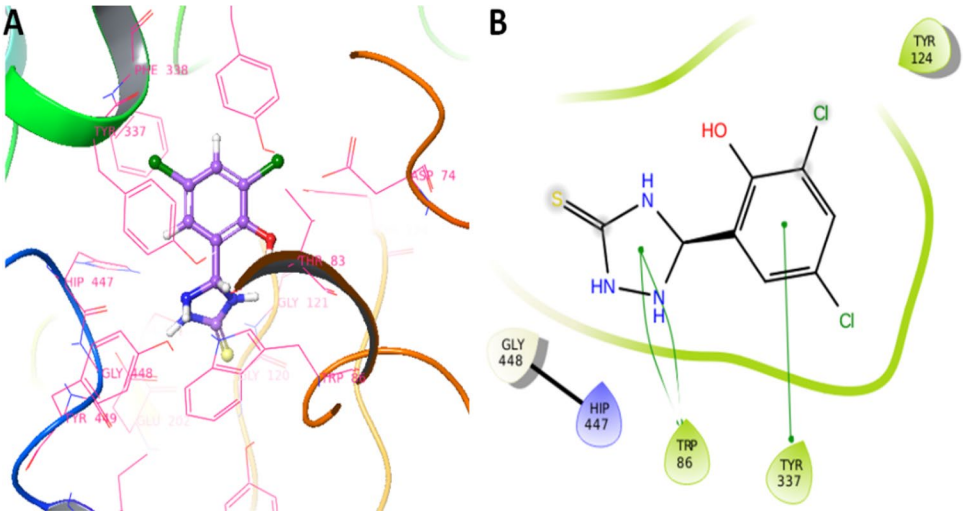


Fig. 6 3D (**A**) and 2D (**B**) binding interaction of **3ab** against AChE protein

Conclusion

A novel ionic liquid $C_5H_{10}[(2\text{-APy})_2(HSO_4)_2]$ was designed and synthesized from easily available precursors. The catalytic efficiency of synthesized ionic liquid was explored for the synthesis of 1,2,4-triazolidine-3-thiones using various aldehydes and thiosemicarbazide in water/ethanol (6:4 v:v) mixed solvent system at room temperature. Employment of mild reaction conditions, reactions at ambient temperature, use of water/ethanol as a eco-friendly solvent system, operational simplicity, excellent practical yield of products, short reaction time, easy isolation of products through simple filtration and reusability of ionic liquid are the remarkable advantages of the present synthetic methodology. In addition, the advantage of proposed compounds includes active biological effect against AChE inhibition and serves as template in drug design in medicinal field. The synthesized compounds were shown varying degree of IC_{50} values within the range 0.0269 ± 0.0021–199.9167 ± 3.8888 µM as compared to standard Neostigmine methylsulphate. While, compounds bearing hydroxyl and di-substituted halogen groups in their structures are found to be more potent AChE inhibitor. The overall, in vitro and in silico analysis suggests that the compound **3ab** possessing $IC_{50} = 0.0269 \pm 0.0021$ µM can be the effective and competent therapeutic agent for the AChE inhibition.

Experimental section

General

Various substituted aldehydes, salicylaldehydes (Alfa Aesar), thiosemicarbazide, *p*-Toluenesulphonic acid (*p*-TSA) (spectrochem), 2-aminopyridine, sulphuric acid and 1,5-dibromopentane (Sigma-Aldrich, Korea) were used as received without further purification. Tripotassium phosphate (K_3PO_4), aluminium chloride ($AlCl_3$), ceric ammonium nitrate (CAN) and ammonium acetate (NH_4OAc) were purchased from Sigma-Aldrich, Korea. The melting point for each synthesized compound was recorded on Digimelt (SRS, USA) melting point apparatus. IR spectra were recorded on a Frontier IR Perkin–Elmer spectrophotometer. NMR spectra were recorded on a Bruker AC-400 spectrometer using tetramethylsilane as an internal standard. The mass analysis (LC–MS) was recorded using 2795/ZQ2000 (waters) spectrometer.

General procedure for multi-component synthesis of IL

Synthesis of 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(bromide), i.e. $C_5H_{10}[(2\text{-APy})_2(Br)_2]$

In a 100-mL round bottom flask, 2-amino pyridine (5.0 g, 53.11 mmol) was mixed with 1,5-dibromopentane (6.10 g, 26.56 mmol) in DMF at 80 °C for 18 h. After the completion of reaction, the reaction mixture was cooled to room temperature and filtered. The obtained white crystals were then washed with DMF (10 mL), ethyl acetate (20 mL) and diethyl ether (20 mL) to remove traces of starting materials. Further, it was dried under reduced pressure to afford 83% yield of white crystals for 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(bromide), i.e. $C_5H_{10}[(2\text{-APy})_2(Br)_2]$.

Synthesis of 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(hydrogen sulphate), i.e. $C_5H_{10}[(2\text{-APy})_2(HSO_4)_2]$

To the 100 mL round bottom flask, dicationic 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(bromide), i.e. $C_5H_{10}[(2\text{-APy})_2(Br)_2]$ (3.0 g, 7.17 mmol) was added along with methanol (20 mL). Then, an equivalent amount of

concentrated sulphuric acid (1.41 g, 14.35 mmol) was added in it with constant stirring. The mixture was then stirred at 40 °C for 4 h at room temperature. On completion of reaction, the solvent was evaporated on rotary evaporator and sequentially dried under reduced pressure to afford 95% of 1,1′-(pentane-1,5-diyl)bis(2-aminopyridinium)-di(hydrogen sulphate), i.e. $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$.

Spectral characteristics of $C_5H_{10}[(2\text{-}APy)_2(HSO_4)_2]$

IR: 3331, 3022, 2949, 2836, 1668, 1590, 1529, 1395, 1202, 1192, 1143, 1133, 1121, 1004, 903, 764, 682 cm^{-1}; ^{1}H-NMR(400 MHz, CD_3OD): δ 7.97 (d, 2H, $J = 4$ Hz), 7.83–7.86 (m, 2H), 7.08 (d, 2H, $J = 8$ Hz), 6.90–6.93 (m, 2H), 4.20 (t, 4H, $J = 8$ & 4 Hz), 3.30–3.31(m, 2H), 1.86–1.92 (m, 6H), 1.50–1.57 (m, 2H) ppm; ^{13}C-NMR(100 MHz, CD_3OD): δ 154.05, 142.47, 139.67, 115.06, 113.42, 52.98, 26.69, 22.37 ppm; MS (ESI): 258 (M^+) *m/z*.

General procedure for multi-component synthesis of 1,2,4-triazolidine-3-thiones

The 50 ml round bottom flask was equipped with 1 mmol of corresponding aldehyde and 1 mmol of thiosemicarbazide in water/ethanol (6:4, v/v) (10 mL) as mixed solvent followed by addition of 5 mol% synthesized ionic liquid. The whole mixture was stirred at room temperature for the time mentioned in Table 3. The progress of the reaction was monitored on TLC. After completion of reaction, the mixture was filtered to separate solid compound and reaction solvent system. The filtered crude solid compound was poured in the ice water and simple filtration method was used to obtain solid compound. The purified form of synthesized compound was achieved by using recrystalization process in hot ethanol. Finally, the structures of all compounds were confirmed by IR, ^{1}H and ^{13}C NMR and mass analysis.

Spectral characteristics of newly synthesized 1,2,4-triazolidine-3-thiones

Table 3, entry 3d: 5-(4-aminophenyl)-1,2,4-triazolidine-3-thione Brown powder; M.P.: 198 °C; IR: 3429, 3266, 3154, 3022, 1590, 1539, 1386, 1286, 1202, 1192, 1134, 1118, 967, 903, 819, 729, 682 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.51 (s, 1H, –NH), 11.22 (s, 1H, –NH), 8.98 (s, 1H), 7.56–8.02 (m, 5H), 6.79 (s, 1H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.98, 143.45, 129.33, 120.11, 117.22 ppm; MS (ESI): 195 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_{10}N_4S$: C 49.46; H 5.19; N 28.84; S 16.51; found: C 49.45; H 5.21; N 28.82; S 16.52.

Table 3, entry 3e: 5-(4-hydroxyphenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 237 °C; IR: 3435, 3267, 3152, 3022, 1588, 1537, 1512, 1284, 1203, 1192, 1134, 1118, 903, 821, 692 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.23 (s, 1H, –NH), 9.85 (s, 1H, –OH), 8.04 (s, 1H), 7.93 (s, 1H), 7.81 (s, 1H), 7.59 (d, 2H), 6.75 (d, 2H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.89, 159.61, 143.07, 129.48, 125.54, 116.00 ppm; MS (ESI): 196 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_9N_3OS$: C 49.21; H 4.65; N 21.52; O 8.19; S 16.43; found: C 49.23; H 4.66; N 21.50; O 8.19; S 16.42.

Table 3, entry 3f: 5-(3,4-dihydroxyphenyl)-1,2,4-triazolidine-3-thione Pale Brown powder; M.P.: 241 °C; IR: 3436, 3268, 3154, 3022, 1590, 1525, 1506, 1281, 1192, 1117, 967, 903, 837, 803, 693 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.18 (s, 1H, –NH), 9.45 (s, 1H, –OH), 8.97 (s, 1H, –OH), 8.02 (s, 1H), 7.86 (s, 1H), 7.70, (s, 1H), 6.98–7.00 (m, 1H), 6.73 (d, 1H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.93, 148.45, 146.01, 143.94, 125.93, 120.70, 116.09, 114.44 ppm; MS (ESI): 212 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_9N_3O_2S$: C 45.49; H 4.29; N 19.89; O 15.15; S 15.18; found: C 45.48; H 4.27; N 19.91; O 15.14; S 15.20.

Table 3, entry 3g: 5-(4-(diethylamino)phenyl)-1,2,4-triazolidine-3-thione Yellow powder; M.P.: 178 °C; IR: 3437, 3268, 3154, 3022, 1590, 1525, 1460, 1388, 1282, 1192, 1117, 967, 903, 837, 819, 712, 693 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.12 (s, 1H, –NH), 7.95 (s, 1H, –NH), 7.87 (s, 1H, –NH), 7.69 (s, 1H, –CH), 7.52 (d, 2H, ArH), 6.62 (d, 2H, Ar H), 3.33–3.37 (q, 4H, $–NCH_2$), 1.08 (t, 6H, $–CH_3$) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.34, 149.30, 144.10, 129.23, 120.97, 111.56, 44.30, 13.03 ppm; MS (ESI): 251 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{12}H_{18}N_4S$: C 57.57; H 7.25; N 22.38; S 12.80; found: C 57.56; H 7.26; N 22.37; S 12.81.

Table 3, entry 3h: 5-(4-(dimethylamino)phenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 201 °C; IR: 3435, 3254, 3153, 3021, 1589, 1522, 1386, 1288, 1202, 1185, 1117, 968, 814, 692 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.15 (s, 1H, –NH), 7.97 (s, 1H, –NH), 7.91 (s, 1H, –NH), 7.73 (s, 1H, –CH), 7.55 (d, 2H, ArH), 6.68(d, 2H, Ar H), 2.94 (s, 6H, $–NCH_3$) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.59, 151.98, 143.92, 129.20, 122.01, 112.28, 40.37 ppm; MS (ESI): 223 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{10}H_{14}N_4S$: C 54.03; H 6.35; N 25.20; S 14.42; found: C 54.05; H 6.34; N 25.21; S 14.40.

Table 3, entry 3i: 5-(anthracen-9-yl)-1,2,4-triazolidine-3-thione Yellow powder; M.P.: 216 °C; IR: 3422, 3264, 3154, 3022, 1591, 1540, 1457, 1385, 1287, 1202, 1192, 1118, 967, 903, 836, 729 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.63 (s, 1H, –NH), 9.31(s, 1H), 8.70 (s,

1H), 8.56 (s, 1H), 8.55 (s, 1H), 8.30 (s, 1H), 8.13 (d, 2H), 7.70 (s, 1H), 7.54–7.64 (m, 4H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.60, 142.75, 131.46, 130.22, 130.04, 129.55, 127.92, 126.18, 125.64, 125.37 ppm; MS (ESI): 280 (M + 1), *m/z*. Elemental analysis calcd (%) for $C_{16}H_{13}N_3S$: C 68.79; H 4.69; N 15.04; S 11.48; found: C 68.78; H 4.69; N 15.02; S 11.51.

Table 3, entry 3j: 5-(pyren-2-yl)-1,2,4-triazolidine-3-thione Yellow powder; M.P.: 255 °C; IR: 3440, 3273, 3157, 1604, 1592, 1543, 1461, 1383, 1291, 1186, 1117, 926, 837, 753, 712 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.54 (s, 1H, –NH), 9.25(s, 1H), 8.87 (d, 1H), 8.48 (d, 1H), 8.08–8.35 (m, 10H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.38, 140.84, 132.34, 131.48, 130.61, 129.29, 128.83, 128.06, 126.32, 124.82, 122.22 ppm; MS (ESI): 304 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{18}H_{13}N_3S$: C 71.26; H 4.32; N 13.85; S 10.57; found: C 71.29; H 4.33; N 13.83; S 10.55.

Table 3, entry 3k: 5-(2,2′-bithiophen-5-yl)-1,2,4-triazolidine-3-thione Yellow powder; M.P.: 213 °C; IR: 3404, 3157, 1603, 1586, 1521, 1501, 1456, 1376, 1348, 1304, 1277, 1241, 1087, 1055, 921, 835, 799, 692 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.48 (s, 1H, –NH), 8.20 (s, 1H), 8.17 (s, 1H), 7.54–7.59(t, 2H), 7.37 (s, 2H), 7.28 (d, 1H), 7.10 (t, 1H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 124.76, 125.32, 126.87, 132.28, 136.71, 137.66, 137.87, 139.05, 177.91 ppm; MS (ESI): 268 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{10}H_9N_3S_3$: C 44.92; H 3.39; N 15.71; S 35.98; found: C 44.93; H 3.40; N 15.69; S 35.98.

Table 3, entry 3l: 5-(2,3-dihydrobenzo[b][1, 4]dioxin-6-yl)-1,2,4-triazolidine-3-thione White powder; M.P.: 209 °C; IR: 3410, 3268, 3159, 1606, 1587, 1523, 1502, 1405, 1304, 1278, 1203, 1097, 921, 836, 799, 693 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.28 (s, 1H, –NH), 8.07 (s, 1H), 7.94 (s, 1H), 7.89 (s, 1H), 7.39 (s, 1H), 7.16–7.18 (d, 1H), 6.83–6.85 (d, 1H), 4.24 (s, 4H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 64.42, 64.74, 115.68, 117.62, 121.80, 128.07, 142.42, 144.11, 145.57, 178.12 ppm; MS (ESI): 238 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{10}H_{11}N_3O_2S$: C 50.62; H 4.67; N 17.71; O 13.49; S 13.51; found: C 50.60; H 4.68; N 17.70; O 13.48; S 13.54.

Table 3, entry 3m: 5-(furan-2-yl)-1,2,4-triazolidine-3-thione Pale Brown powder; M.P.: 157 °C; IR: 3436, 3285, 3166, 1600, 1524, 1486, 1362, 1286, 1192, 1104, 925, 811, 695 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.43 (s, 1H, –NH), 8.21 (s, 1H), 7.96 (s, 1H), 7.81(d, 1H), 7.63 (s, 1H), 6.97 (d, 1H), 6.63 (t, 1H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.33, 149.87, 145.55, 132.95, 113.35, 112.76 ppm; MS (ESI): 170 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_6H_7N_3OS$: C 42.59; H 4.17; N 24.83; O 9.46; S 18.95; found: C 42.58; H 4.15; N 24.85; O 9.45; S 18.97.

Table 3, entry 3n: 5-(1H-pyrrol-2-yl)-1,2,4-triazolidine-3-thione White powder; M.P.: 210 °C; IR: 3438, 3270, 3153, 1590, 1527, 1459, 1389, 1284, 1202, 1192, 1116, 966, 903, 837, 817, 737, 694 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.33 (s, 1H, –NH), 11.24 (s, 1H, –NH), 8.05 (s, 1H), 7.93 (s, 1H), 7.81(s, 1H), 6.95 (s, 1H), 6.37–6.38 (m, 1H), 6.08 (t, 1H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.79, 134.40, 127.97, 122.18, 113.54, 109.78 ppm; MS (ESI): 169 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_6H_8N_4S$: C 42.84; H 4.79; N 33.31; S 19.06; found: C 42.83; H 4.80; N 33.29; S 19.08.

Table 3, entry 3o: 5-(benzo[b]thiophen-2-yl)-1,2,4-triazolidine-3-thione Pale yellow powder; M.P.: 233 °C; IR: 3437, 3266, 3152, 3022, 1590, 1538, 1460, 1388, 1285, 1192, 1118, 967, 903, 820, 754, 682 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.61 (s, 1H, –NH), 8.34(s, 1H), 8.31 (s, 1H), 7.92 (d, 1H), 7.35–7.83 (m, 5H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.39, 139.96, 139.65, 138.36, 128.37, 126.49, 125.42, 124.88, 123.16 ppm; MS (ESI): 236 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{10}H_9N_3S_2$: C 51.04; H 3.85; N 17.86; S 27.25; found: C 51.05; H 3.84; N 17.83; S 27.28.

Table 3, entry 3p: 5-(quinolin-2-yl)-1,2,4-triazolidine-3-thione Pale Yellow powder; M.P.: 246 °C; IR: 3437, 3258, 3152, 3021, 1590, 1523, 1456, 1387, 1284, 1192, 1117, 967, 903, 837, 814, 693 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.78 (s, 1H, –NH), 8.44 (d, 2H), 8.33 (t, 2H), 8.00 (s, 1H), 7.97 (t, 2H), 7.73–7.77 (m, 1H), 7.58–7.61 (t, 1H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 179.86, 154.62, 147.92, 143.11, 136.88, 130.42, 129.26, 128.43, 127.52, 118.88 ppm; MS (ESI): 230 (M +) *m/z*. Elemental analysis calcd (%) for $C_{11}H_{10}N_4S$: C 57.37; H 4.38; N 24.33; S 13.92; found: C 57.39; H 4.37; N 24.33; S 13.91.

Table 3, entry 3q: 5-(8-hydroxyquinolin-2-yl)-1,2,4-triazolidine-3-thione White powder; M.P.: 254 °C; IR: 3436, 3266, 3153, 3021, 1590, 1535, 1505, 1463, 1323, 1281, 1250, 1192, 1106, 919, 837, 815, 751, 720, 694 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.83 (s, 1H, –NH), 9.83 (s, 1H, –OH), 8.42(d, 2H, ArH), 8.30 (s, 1H, –NH), 8.26 (s, 1H, –NH), 8.24 (s, 1H, –CH), 7.37–7.43 (m, 2H, ArH), 7.07–7.35(m, 1H, ArH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 179.04, 154.02, 152.38, 143.04, 138.74, 136.74, 129.38, 128.80, 118.23, 112.43 ppm; MS (ESI): 247 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{11}H_{10}N_4OS$: C 53.64; H 4.09; N 22.75; O 6.50; S 13.02; found: C 53.66; H 4.10; N 22.72; O 6.49; S 13.03.

Table 3, entry 3r: 5-(2-chloroquinolin-3-yl)-1,2,4-triazolidine-3-thione Yellow powder; M.P.: 235 °C; IR: 3436, 3265, 3149, 1590, 1525, 1460, 1280, 1192, 1116, 903, 838, 816, 760, 694 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.79 (s, 1H, –NH), 9.31 (s, 1H, –NH), 8.49 (s, 1H, –NH), 8.46 (s, 1H, –CH), 8.26 (s, 1H), 7.93–7.99 (m, 2H, ArH), 7.81–7.85(m, 1H, ArH), 7.67–7.70 (m, 1H, ArH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.99, 149.05, 147.55, 137.34, 136.65, 132.18, 129.09, 128.52, 128.36, 127.62, 126.73 ppm; MS (ESI): 265 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{11}H_9ClN_4S$: C 49.91; H 3.43; Cl 13.39; N 21.16; S 12.11; found: C 49.90; H 3.45; Cl 13.40; N 21.15; S 12.10.

Table 3, entry 3s: 5-(2,4-dihydroxyphenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 245 °C; IR: 3434, 3267, 3154, 3022, 1590, 1525, 1388, 1284, 1192, 1118, 967, 903, 836, 820, 693 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.16 (s, 1H, –NH), 9.73 (s, 2H, –OH), 8.22 (s, 1H), 7.93 (s, 1H), 7.93, (s, 1H), 7.73 (s, 1H), 7.65 (d, 1H), 6.22–6.28 (m, 2H) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 177.59, 161.03, 158.78, 141.27, 129.04, 112.22, 108.49, 102.74 ppm; MS (ESI): 212 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_9N_3O_2S$: C 45.49; H 4.29; N 19.89; O 15.15; S 15.18; found: C 45.51; H 4.28; N 19.91; O 15.13; S 15.17.

Table 3, entry 3u: 5-(2-hydroxy-3-nitrophenyl)-1,2,4-triazolidine-3-thione Yellow powder; M.P.: 240 °C; IR: 3437, 3264, 3151, 3021, 1591, 1525, 1461, 1365, 1230, 1192, 1116, 925, 835, 820, 740, 712, 694 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.58 (s, 1H, –NH), 10.73 (s, 1H, –OH), 8.41(d, 2H, ArH), 8.24 (s, 1H, –NH), 8.11 (s, 1H, –NH), 7.99–8.01 (m, 1H, ArH), 7.06 (t, 1H, ArH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.99, 149.05, 147.55, 137.34, 136.65, 132.18, 129.09, 128.52, 128.36, 127.62, 126.73 ppm; MS (ESI): 241 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_8N_4O_3S$: C 40.00; H 3.36; N 23.32; O 19.98; S 13.34; found: C 40.02; H 3.37; N 23.30; O 19.96; S 13.35.

Table 3, entry 3v: 5-(5-bromo-2-hydroxyphenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 246 °C; IR: 3436, 3257, 3153, 3000, 1592, 1542, 1478, 1360, 1284, 1263, 1192, 1117, 915, 835, 819, 693 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.40 (s, 1H, –NH), 10.20 (s, 1H, –OH), 8.27 (s, 1H, –NH), 8.19 (s, 1H, –NH), 8.13–8.14 (d, 1H, ArH), 7.30–7.32 (t, 1H, ArH), 6.80 (d, 1H, ArH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.64, 155.98, 137.91, 134.03, 129.21, 123.52, 118.71, 111.59 ppm; MS (ESI): 276 (M + 2) *m/z*. Elemental analysis calcd (%) for $C_8H_8BrN_3OS$: C 35.05; H 2.94; Br 29.15; N 15.33; O 5.84; S 11.69; found: C 35.06; H 2.92; Br 29.14; N 15.34; O 5.86; S 11.68.

Table 3, entry 3w: 5-(5-chloro-2-hydroxyphenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 272 °C; IR: 3437, 3258, 3149, 3022, 1591, 1525, 1507, 1282, 1192, 1115, 924, 837, 819, 750, 713, 694 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.40 (s, 1H, –NH), 10.19 (s, 1H, –OH), 8.28 (s, 1H, –NH), 8.13 (s, 2H, –NH&–CH), 8.07 (s, 1H, ArH), 7.18–7.20 (m, 1H, ArH), 6.84 (d, 1H, ArH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 178.44, 155.72, 137.90, 130.70, 126.02, 124.02, 122.94, 118.52 ppm; MS (ESI): 229 (M +) *m/z*. Elemental analysis calcd (%) for $C_8H_8ClN_3OS$: C 41.83; H 3.51; Cl 15.44; N 18.29; O 6.97; S 13.96; found: C 41.82; H 3.50; Cl 15.46; N 18.28; O 6.99; S 13.95.

Table 3, entry 3x: 5-(2-hydroxy-5-methoxyphenyl)-1,2,4-triazolidine-3-thione Pale yellow powder; M.P.: 228 °C; IR: 3429, 3266, 3154, 3022, 1590, 1539, 1486, 1458, 1386, 1363, 1286, 1202, 1192, 1142, 1134, 1118, 967, 913, 903, 836, 819, 783, 753, 729, 692, 682 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.88 (s, 1H, –NH), 11.47 (s, 1H, –OH), 9.97 (s, 1H, –NH), 8.52 (s, 1H, –CH), 7.94–7.92 (d, 1H, ArH), 7.49–7.30 (m, 2H, ArH), 6.33 (s, 1H, –NH), 3.81 (s, 3H, –OCH_3) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 160.27, 137.54, 130.32, 129.25, 124.11, 120.18, 115.80, 107.81, 103.45 ppm; MS (ESI): 226 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_9H_{11}N_3O_2S$: C 47.99; H 4.92; N 18.65; O 14.21; S 14.23; found: C 47.98; H 4.93; N 18.67; O 14.20; S 14.22.

Table 3, entry 3y: 5-(4-(diethylamino)-2-hydroxyphenyl)-1,2,4-triazolidine-3-thione Pale Yellow powder; M.P.: 233 °C; IR: 3438, 3258, 3153, 3021, 1591, 1524, 1507, 1360, 1281, 1181, 1115, 925, 839, 819, 713, 694 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.50 (s, 1H, –NH), 9.47 (s, 1H, –OH), 8.16 (s, 1H, –NH), 7.86 (s, 1H, –NH), 7.64 (s, 1H, –CH), 7.49 (d, 1H, ArH), 6.17–6.19 (m, 1H, ArH), 6.06 (d, 1H, ArH), 3.27–3.31 (q, 4H, –NCH_2), 1.07 (t, 6H, –CH_3) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 176.97, 158.72, 150.53, 142.84, 129.35, 108.02, 104.53, 97.90, 44.21, 13.15 ppm; MS (ESI): 267 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_{12}H_{18}N_4OS$: C 54.11; H 6.81; N 21.03; O 6.01; S 12.04; found: C 54.10; H 6.82; N 21.01; O 6.02; S 12.05.

Table 3, entry 3z: 5-(2-hydroxy-3,5-diiodophenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 250 °C; IR: 3435, 3255, 3153, 3022, 1591, 1537, 1507, 1357, 1282, 1114, 925, 831, 819, 750 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.58 (s, 1H, –NH), 9.93 (s, 1H, –OH), 8.21 (s, 2H, –ArH), 8.18 (s, 1H, –NH), 8.06 (s, 1H, –NH), 7.99 (s, 1H, –CH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 176.49, 146.84, 137.73, 137.07, 114.87, 105.96, 81.61, 60.88 ppm; MS (ESI): 447 (M +) *m/z*. Elemental analysis calcd (%) for $C_8H_7I_2N_3OS$:

C 21.49; H 1.58; I 56.78; N 9.40; O 3.58; S 7.17; found:: C 21.51; H 1.57; I 56.75; N 9.38; O 3.60; S 7.19.

Table 3, entry 3aa: 5-(3,5-dibromo-2-hydroxyphenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 247 °C; IR: 3439, 3358, 3260, 3151, 2999, 1590, 1534, 1450, 1359, 1281, 1116, 922, 837, 815, 713, 691 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 11.51 (s, 1H, –NH), 9.96 (s, 1H, –OH), 8.27 (s, 1H, –NH), 8.22 (s, 2H, –ArH), 8.13 (s, 1H, –NH), 7.73 (s, 1H, –CH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ178.80, 152.48, 138.77, 135.62, 129.53, 113.55, 112.92 ppm; MS (ESI): 355 (M + 2), 353 (M^+) *m/z*. Elemental analysis calcd (%) for $C_8H_7Br_2N_3OS$: C 27.22; H 2.00; Br 45.27; N 11.90; O 4.53; S 9.08; found: C 27.23; H 2.01; Br 45.26; N 11.88; O 4.54; S 9.08.

Table 3, entry 3ab: 5-(3,5-dichloro-2-hydroxyphenyl)-1,2,4-triazolidine-3-thione White powder; M.P.: 256 °C; IR: 3461, 3347, 3148, 2990, 1612, 1595, 1527, 1451, 1358, 1283, 1218, 1144, 927, 860, 817, 752, 732, 682 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 10.63 (s, 1H, –NH), 8.42 (s, 1H, –OH), 7.70 (s, 1H, –NH), 7.47 (s, 2H, –ArH), 7.46 (s, 2H, –NH & –CH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 179.52, 151.15, 140.97, 130.27, 127.14, 124.68, MS (ESI): 265(M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_7Cl_2N_3OS$: C 36.38; H 2.67; Cl 26.84; N 15.91; O 6.06; S 12.14; found: C 36.40; H 2.66; Cl 26.82; N 15.93; O 6.06; S 12.13.

Table 3, entry 3ac: 5-(2-hydroxy-3,5-dinitrophenyl)-1,2,4-triazolidine-3-thione Red–Orange powder; M.P.: 230 °C; IR: 3465, 3356, 3154, 3009, 1611, 1592, 1531, 1454, 1357, 1285, 1202, 1192, 1133, 1117, 904, 840, 818, 735, 714 cm^{-1}; ^{1}H-NMR(400 MHz, DMSO-d_6): δ 10.80 (s, 1H, –NH), 9.09 (s, 2H, –NH), 8.92 (s, 1H, –OH), 8.92 (s, 1H, –CH), 8.62 (s, 2H, –ArH) ppm; ^{13}C-NMR(100 MHz, DMSO-d_6): δ 180.08, 156.46, 140.05, 134.84, 126.57, 121.34 ppm; MS (ESI): 286 (M + 1) *m/z*. Elemental analysis calcd (%) for $C_8H_7N_5O_5S$: C 33.69; H 2.47; N 24.55; O 28.05; S 11.24; found: C 33.70; H 2.45; N 24.54; O 28.04; S 11.27.

In vitro methodology

Acetylcholinesterase inhibition assay

The inhibitory activity of 1,2,4-triazolidine-3-thiones was determined spectrophotometrically using reported methods with some modifications and acetylthiocholine iodide as substrate [58, 59]. Briefly, the assay solution consists of increasing concentrations of test inhibitor compounds (10 µL) with the composition of 180 µL of 50 mM Tris HCl buffer having pH 7.7 and 20 µL of enzyme AChE, EC 3.1.1.7. Further, the prepared solutions were pre-incubated for 30 min at 4 °C followed by addition of 0.3 mM 20 µL of 5,5′-dithiobis-(2-nitrobenzoic acid) and 1.8 mM of 20 µL acetylthiocholine iodide which were incubated at 37 °C for the time of 10 min. The assays were executed with blank solution holding each compound without acetylcholinesterase for the investigation of non-enzymatic reaction. The absorbance of each solution thus measured and recorded at 412 nm within the wavelength ranging from 340 to 850 nm using a microplate reader (OPTI Max, Tunable). The reaction rates were compared and the per cent inhibition was calculated using Eq. 1. Neostigmine methylsulphate was used as standard reference inhibitor.

$$\text{Inhibition } (\%) = \frac{(B - S)}{B} \times 100 \quad (1)$$

Here, the B and S are the absorbance for the blank and sample under studies, respectively. The three independent experiments were executed and IC_{50} values were calculated by nonlinear regression using GraphPad Prism 5.0.

Kinetic analysis

A series of experiments were performed to determine the inhibition kinetics of **3ab** by following the already reported method [60]. Kinetics were carried out by varying the concentration of acetylthiocholine iodide against various concentrations of inhibitor **3ab** (0.00, 0.027 and 0.054 µM). The procedure involves the change in concentration of acetylthiocholine iodide (4, 2, 1, 0.5 and 0.25 mM) for the investigation of kinetics effect and remaining procedure was same for all kinetic studies as described in protocol used for acetylcholine-esterase inhibition assay. The linear portion of absorbances thus measured was used to evaluate the maximum initial velocities up to 5 min with addition of enzyme in the interval of each 30 s. The Lineweaver–Burk plot was used to determine the enzyme inhibition type for the present bio-evaluation. Further, the inhibitor dissociation constant (K_i) was also determined.

Free radical scavenging assay

2, 2-diphenyl-1 picrylhydrazyl (DPPH) assay was used to determine the radical scavenging activity [61, 62]. In this assay, solution consists of 100 µL of DPPH (150 µM) and 20 µL of increasing concentration of tested compounds with final volume of 200 µL in each well using methanol. The assay solutions were allowed to incubate for 30 min at ambient temperature. The reference inhibitor used for this study was Vitamin C. The microplate reader was used to measure the absorbance of each assay at 517 nm ($OPTI_{Max}$, Tunable). The reaction rates were compared and the per cent inhibition for tested inhibitors was calculated. Thus, whole experiment was carried out three times to check the output consistency.

Computational methodology

Retrieval of human acetylcholinesterase in protein preparation wizard

The human acetylcholinesterase (AChE) structure was obtained from Protein Data Bank (PDB) (http://www.rcsb.org/structure/4EY7) with PDBID 4EY7 [63]. The Maestro interface was used for the adjustment in pre-process and minimization of AChE protein structure.

Designing of ligands in ACD/ChemSketch

The ACD/ChemSketch was used to sketch the synthesized ligands (**3a–ac**) and further retrieved in mol format. The chemoinformatics properties and validation of Lipinski's rule for all synthesized compounds (**3a–ac**) were examined using online computational tools namely Molinspiration and Molsoft, respectively.

Grid generation and molecular docking

The protein preparation wizard workflow in Schrödinger Suite Release 2019-1 was used for the preparation of optimized AChE structure and which was further used in molecular docking studies. The active site of the enzyme was defined from the co-crystallized ligands from Protein Data Bank and literature survey [55, 56]. Moreover, synthesized ligands (**3a–3h**, **3k–3q**, **3s–3ac**) sketched using 2D sketcher in Maestro was examined for the docking studies against target protein drawn by using Glide docking protocol [64]. The predicted binding energies (docking scores) and conformational positions of ligands within active region of protein were also performed using Glide experiment. Throughout the docking simulations, both partial flexibility and full flexibility around the active site residues were performed by Glide/SP/XP and induced fit docking (IFD) approaches [65].

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Compliance with ethical standards

Conflicts of interest The authors declare no conflict of interests.

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Affiliations

Prasad G. Mahajan[1] · Nilam C. Dige[2] · Balasaheb D. Vanjare[1] · Hussain Raza[2] · Mubashir Hassan[3] · Sung-Yum Seo[2] · Chong- Hyeak Kim[4] · Ki Hwan Lee[1]

✉ Sung-Yum Seo
dnalove@kongju.ac.kr

✉ Ki Hwan Lee
khlee@kongju.ac.kr

[1] Department of Chemistry, Kongju National University, Gongju, Chungnam 32588, Republic of Korea

[2] Department of Biological Sciences, Kongju National University, Gongju, Chungnam 32588, Republic of Korea

[3] Institute of Molecular Biology and Biotechnology, The University of Lahore, Defence Road, Lahore 54590, Pakistan

[4] Center for Chemical Analysis, Korea Research Institute of Chemical Technology, Yuseong, Daejeon 34114, Republic of Korea