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# $SiO_2$-$H_3BO_3$ promoted solvent-free, green and sustainable synthesis of bioactive 1-substituted-1*H*-tetrazole analogues

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## Abstract

In the present study, a focused library of multi functionalized 1-substituted-1*H*-tetrazole analogues **4** (**a-o**) were synthesized, which were typically accessed *via* a facile, solvent-free and green synthetic protocol. The reaction involves expeditious reusable catalyst ($SiO_2$-$H_3BO_3$) promoted condensation between a variety of heterocyclic/aromatic amines, sodium azide and triethyl *ortho*-formate, eliminating the use of environmentally toxic solvent. This new eco-friendly and sustainable protocol resulted in a remarkable enhancement in the synthetic efficiency (90-97 %, yield) with high purity. This silica-boric acid catalyst is air and water stable and easy to prepare from cheap silica and boric acid. The present methodology is a green protocol offering several advantages such as excellent yield of products, simple operational procedure, minimizing production of chemical wastes, mild reaction conditions, shorter reaction profile, easy preparation of catalyst and its recyclability up to five cycles without any appreciable loss in catalytic activity. The optimization conditions achieved in the present study, revealed that 2.5 mol% of $SiO_2$-$H_3BO_3$ catalyst under solvent-free condition at 90 $^oC$ are the best suited conditions for the synthesis of tetrazole derivatives in excellent yields. The present protocol is applicable to a broader substrate scope (electron-rich and electron-deficient). Compounds possessing nicotinic acid nucleus (**4e**, **4f**, **4g**) displayed strongest inhibition against AChE with $IC_{50}$ values of of 0.43 μM, 0.21 μM and 0.26 μM, respectively. The results revealed that, electronic effect of substituents inflict prominent effect on AChE activity.

Key words: Solvent-free, $SiO_2$-$H_3BO_3$, Tetrazoles derivatives, Green protocol, AChE, BuChE

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## 1. Introduction

Tetrazoles belong to a class of 5-membered nitrogen containing heterocyclic compounds possessing highest nitrogen content among the known stable heterocycles. The first compound comprising a tetrazole ring is thought to be 2-phenyl-2*H*-tetrazole-5-carbonitrile, which was synthesized and characterized in 1885.[1] In the last few decades, the chemistry of *N*-containing heterocycles (tetrazoles), have received considerable attention due to their promising biological properties especially antiviral,[2] anticonvulsant,[3] antiinflammatory,[4] antimicrobial,[5] antinociceptive,[6] antifungal,[7] anti-HIV,[8] antiulcer,[9] antiallergic,[10] antiproliferative,[11] analgesic[12] and anticancer.[13] They have also been shown to be potential P2X7-antagonists,[14] TNF alpha inhibitors[15] and inhibitors of anandamide cellular uptake.[16] The importance of tetrazole and its derivatives in medicinal chemistry has augmented because of their use as lipophilic spacers and as metabolically stable surrogate for a carboxylic acid group in drug design.[17] A number of drugs approved by the Food and Drug Administration (FDA) which are medicinally important possess tetrazole moiety in their structures.[18] It has been reported, that the lipophilic nature of tetrazole moiety in a drug improves its oral bioavailability and cell penetration.[19] In this regard, tetrazole chemistry has gained so much interest due to their potential therapeutic profile. Valsartan, losartan and irbesartan, are well-known antihypertensive drugs belonging to the class of nonpeptide angiotensin-II inhibitors and possess biphenyl tetrazolyl moiety in their structure (Fig. 1). Similarly, TAK-456 a broad-spectrum antifungal drug also bears a tetrazole ring in its structure (Fig. 1).

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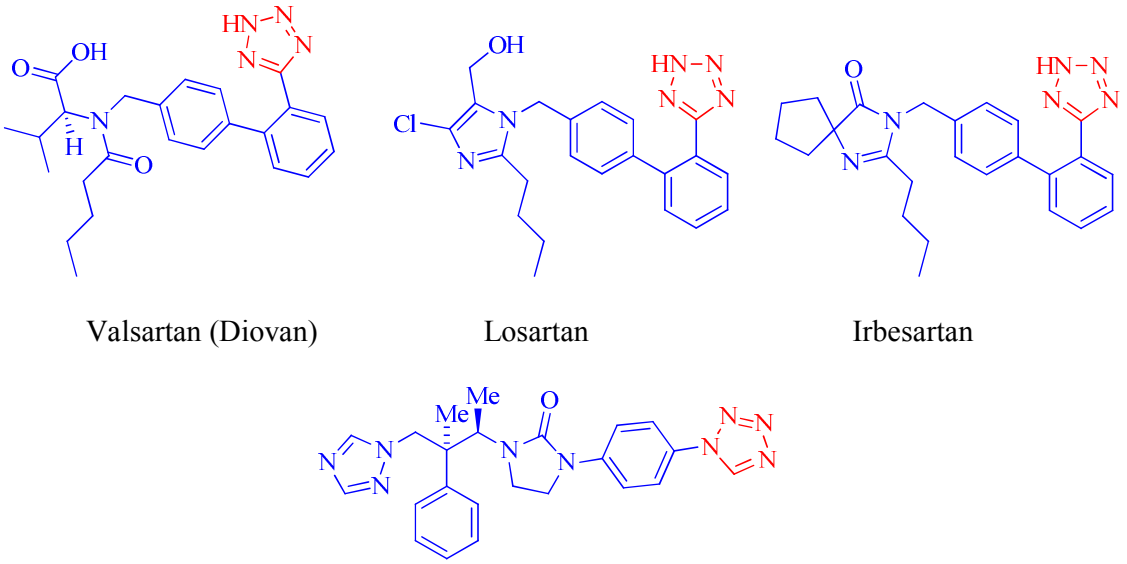


Valsartan (Diovan) Losartan Irbesartan

TAK-456

**Fig. 1** Drugs in the market with a tetrazole moiety

Tetrazoles have also found a wide range of applications in materials as specialty explosives,[20] information recording systems,[21] rocket propellants[22] and agrichemical applications.[23] They are endowed with befitting coordinating property as nitrogen-containing heterocyclic ligands are capable to form stable complexes with several metal ions and behave as precursors to a variety of nitrogen heterocycles in organic synthesis.[24,25] Moreover, tetrazole ring is a crucial intermediate in the synthesis of other more complex heterocycles, through various rearrangements.[26] It has been recently documented that nitrogen containing heterocyclic compounds possess inherent acetylcholinesterase inhibition potency and hence, have emerged as one of the most promising candidates for the treatment of Alzheimer's disease (AD).[27] As a consequence of such immense synthetic utility and inherent pharmacological applications of tetrazoles, there is a need for the development of efficient and elegant protocols for their synthesis.

Among tetrazoles, 1-substituted tetrazoles have received much attention because of their wide applications.[28] However, the lack of convenient methods for the synthesis of 1-

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substituted tetrazoles, strongly restricts their potential application in medical practice. Although many 5-substituted tetrazoles are known, yet only a very few 1-substituted tetrazoles have been described.

Several sophisticated methodologies have been postulated in the literature for the synthesis of tetrazole nucleus. The most convenient method to achieve 5-substituted 1*H*-tetrazoles is [3+2] cycloaddition between organic/inorganic azides or hydrazoic acid and corresponding nitriles in the presence of varied catalysts *viz*. $Cu_2O$,[29a] $Pd(OAc)_2/ZnBr_2$,[29b] $Zn(OTf)_3$,[29c] $ZrOCl_2$,[29d] Zn/Al hydrotalcite,[29e] $Pd(PPh_3)_4$[29f] and ZnS.[29g] However, 1-substituted tetrazole can be synthesized by acid-catalyzed cycloaddition between isocyanides and hydrazoic acid[30] or cyclization between primary amines, triethyl orthoformate, and sodium azide in presence of acetic acid[31] or by using different catalysts such as $In(OTf)_3$,[32a] $Yb(OTf)_3$,[32b] SSA,[32c] $[Hbim]BF_4$[32d] and recently natrolite zeolite.[32e] Although, all of these methods are worthwhile, however a number of them have several shortcomings such as, use of expensive and hazardous reagents, application of high boiling solvents, prolonged reaction times, tedious workup procedures, high temperature, toxic waste generation, difficulty in separation and recovery of the catalyst and the presence of hydrazoic acid which is highly toxic and explosive. Hence, it is of great practical importance to develop a more efficient and environmentally benign method that avoids or minimize all of these complications.

In recent years, the use of reusable heterogeneous catalysts has received considerable attention in organic synthesis as a result of their economic and environmental benefits. Such reagents not only simplify purification processes but also help in preventing the discharge of toxic reaction residues into the environment. The use of solid heterogeneous catalysts for the development of clean processes is a theme of extreme importance in the chemical industry. Solid support based heterogeneous catalysts are capable of driving several impracticable organic transformations faster and under mildest possible conditions. These catalysts make

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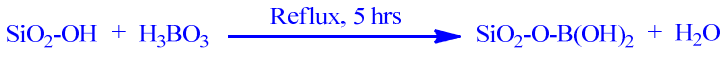
the synthetic process economically feasible as they can be easily recovered by simple filtration from the reaction mixture and can be reused several times to achieve very high turnover numbers and also reduces the risk of discharge of toxic reaction residues into the environment when compared with the conventional homogeneous catalysts. The development of selective and reusable solid support catalysts for organic reactions has been a very active area of research. During the last few decades, silica-supported catalysts have achieved considerable reputation because of their many advantageous properties such as higher mechanical and thermal stabilities, higher efficiency, larger surface area, better selectivity, cheap, lower toxicity, reusability and easy separation of the catalyst from the reaction mixture.[33,34]

Motivated by these findings, and in continuation of our ongoing efforts endowed with the finding of new synthetic protocols[35] under the principles of green chemistry, herein, we report for the first time silica-supported boric acid ($SiO_2$-$H_3BO_3$) catalyzed synthesis of 1-substituted 1*H*-tetrazoles under thermal solvent free condition. Silica-supported boric acid ($SiO_2$-$H_3BO_3$) catalyst was prepared by employing standard procedures depicted in the literature (Scheme 1).[36] It has emerged as a promising heterogeneous solid acid catalyst in many organic transformations[36-38] and possesses environmentally benign properties such as non-toxicity, biocompatibility, recyclability, physiological inertness, inexpensiveness and thermal stability. The structure and morphology of the catalyst was established on the basis of FT-IR, powder X-ray diffraction (XRD), scanning electron microscopy (SEM), energy-dispersive X-ray spectroscopy (EDX) and transmission electron microscopy (TEM). The synthesized compounds were screened for aetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition studies.




**Scheme: 1** Synthetic pathway for the synthesis of catalyst

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## 2. Results and discussions

In the present study a library of bioactive 1-substituted-1*H*-tetrazole derivatives **4(a-o)** have been synthesized by using environmentally benign heterogenous catalyst ($SiO_2$-$H_3BO_3$) under solvent free conditions. This protocol offers several advantages over other existing synthetic methodologies in terms of yield and purity of products, operational procedure, reaction times, catalyst stability and recyclability. This synthetic scheme possesses diverse applicability and is compatible to a range of functional groups (electron donating/ electron withdrawing). In context to the potential applications of nitrogen containing heterocyclic compounds against Alzheimer's disease (AD),[27] we have tested our synthesized compounds for the possible AChE and BuChE inhibition studies. The results revealed that, electronic effect of substituents inflict prominent effect on AChE activity.

### 2.1. Characterization of silica-supported boric acid catalyst

The FT-IR spectrum of the catalyst ($SiO_2$-$H_3BO_3$) is depicted in (Fig. 2) and was recorded using the KBr disk technique. The FT-IR spectrum displayed a stretching band of SiO-H at 3434 $cm^{-1}$, while the stretching band of BO-H appeared at 3227 $cm^{-1}$. The peak at 2260 $cm^{-1}$ was assigned to the B-OH combination and the peak at 1187 $cm^{-1}$ was attributed to the bending vibration of B-OH. The Si-O-Si asymmetric stretching vibration band appeared at 1091 $cm^{-1}$ and the low frequency modes at 800 $cm^{-1}$ were due to the symmetric Si-O-Si stretching vibrations. The peaks at 927 and 649 $cm^{-1}$ for the stretching and bending vibration of borosiloxane linkage (Si-O-B), respectively and a B-O stretching band of borosiloxane at 1435 $cm^{-1}$ observed in the FT-IR spectrum of the catalytic system, authenticates the loading of $H_3BO_3$ on the $SiO_2$ surface.

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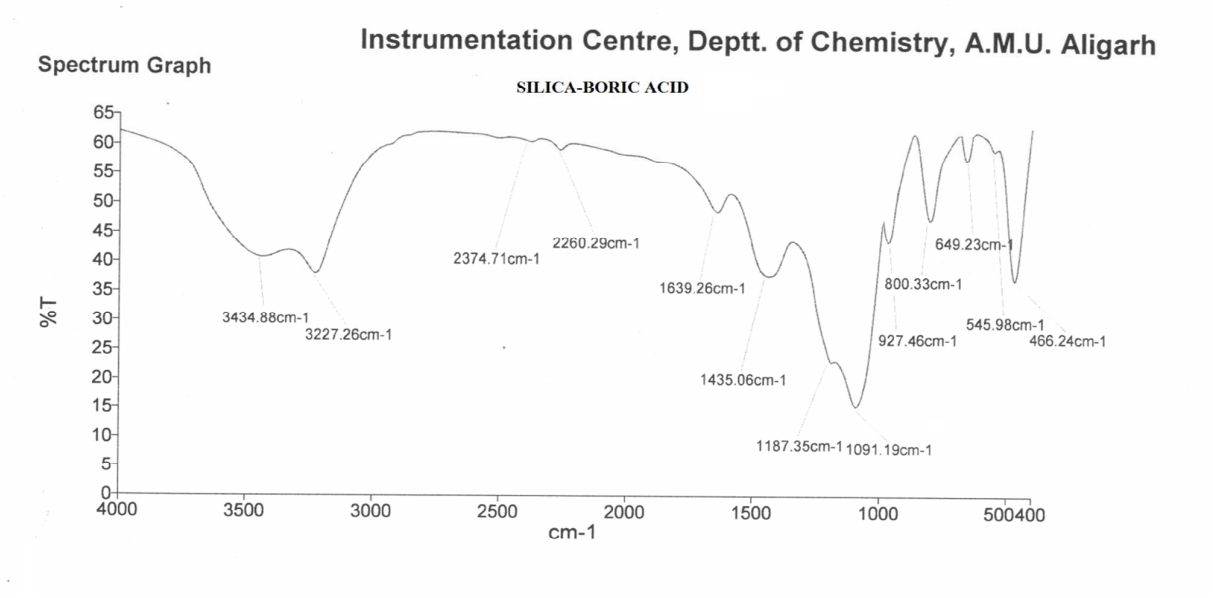


**Fig. 2** FT-IR spectrum of catalyst $SiO_2$-$H_3BO_3$

Formation of the catalytic system ($SiO_2$-$H_3BO_3$) was further confirmed by powder XRD analysis (Fig. 3). The XRD pattern showed a single broad peak at $2\theta = 22^o$ indicating fine dispersion of boric acid on the silica material and thus confirming the formation of amorphous $SiO_2$-$H_3BO_3$ matrix. The absence of reflection at $2\theta = 28^o$ for free boric acid[39,40] indicated that boron atoms are mainly engaged in the formation of borosiloxane bridges in the catalyst.

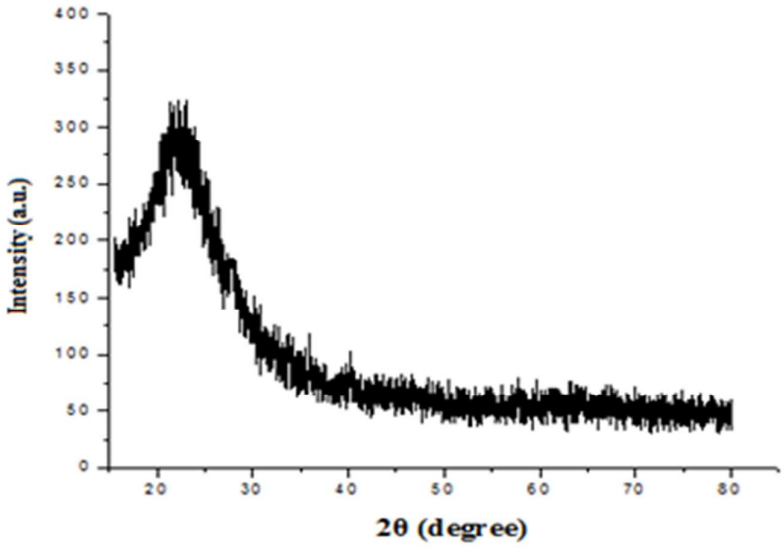


**Fig. 3** Powder XRD analysis of catalyst $SiO_2$-$H_3BO_3$

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SEM analysis was employed to study the surface morphology of the catalytic system (Fig. 4). SEM micrographs of the catalyst showed that $H_3BO_3$ particles were of uneven size and shape, well dispersed and no conglomeration of $H_3BO_3$ particles was found on the surface of the silica gel. The increased reactivity of boric acid supported on silica material is believed to be due to the catalyst-support interactions and the resultant changes in the surface properties of the reactive sites. TEM micrograph (Fig. 5) of the catalytic system further displayed uniform distribution of $H_3BO_3$ particles as black dots on the surface of silica, approving the formation of the expected catalytic system. EDX analysis (Fig. 6) of the catalyst showed the presence of Si, B and O elements suggesting the formation of $SiO_2$-$H_3BO_3$ catalytic system.

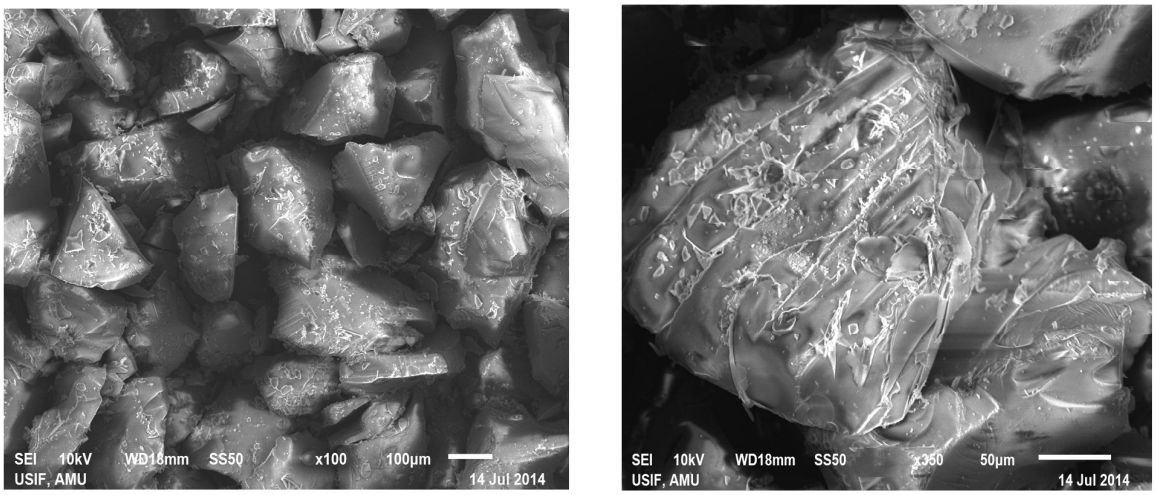


**Fig. 4** SEM image of catalyst $SiO_2$-$H_3BO_3$

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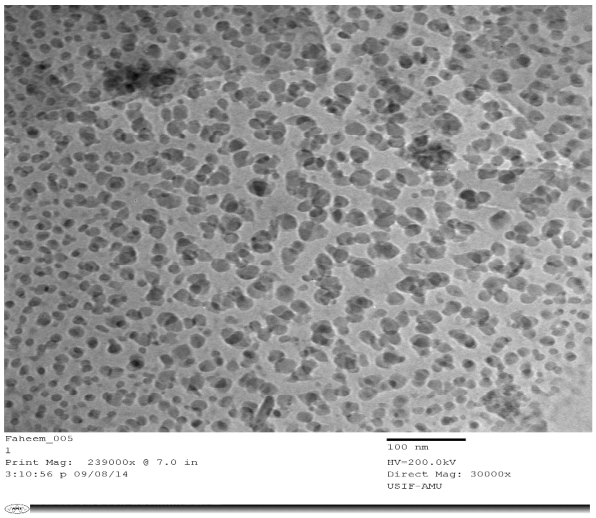


**Fig. 5** TEM image of catalyst $SiO_2$-$H_3BO_3$

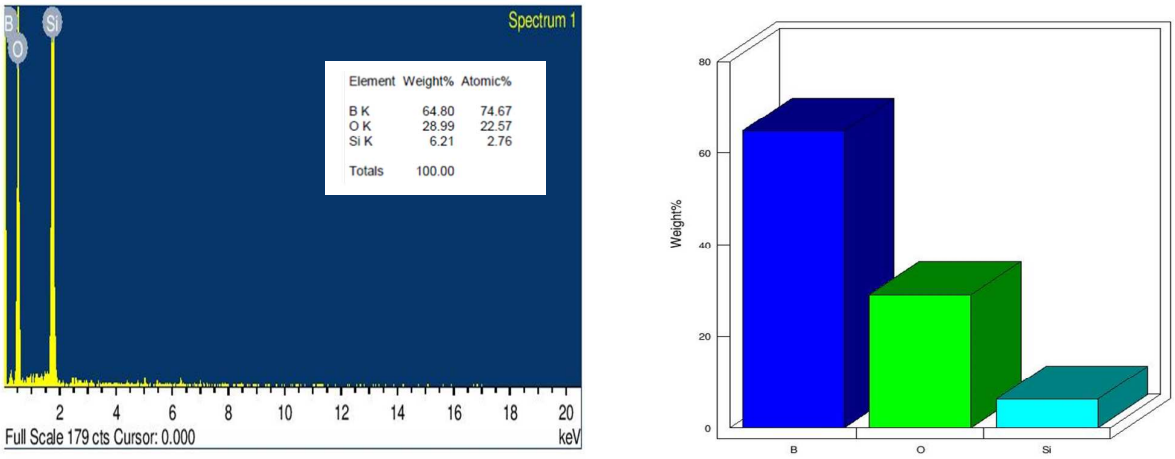


**Fig. 6** Energy dispersive X-ray (EDX) analysis of catalyst $SiO_2$-$H_3BO_3$

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## 2.2. Chemistry

The synthetic pathway of a series of 1-substituted-1*H*-tetrazoles **4(a-o)** is presented in Scheme 2. Herein, a series of compounds were typically accessed *via* a solvent free facile environmentally benign cyclization reaction, involving various heterocyclic/aromatic amines with sodium azide and triethyl *ortho*-formate to yield target 1-substituted-1*H*-tetrazoles in excellent yields (90-97 %) with high degree of purity.

The structural elucidation of all the synthesized compounds **4** (**a-o**) has been established on the basis of elemental analysis, IR, $^{1}$H NMR, $^{13}$C NMR and mass spectral studies. The analytical results for C, H and N were found to be within ± 0.3% of the theoretical values. The spectral analysis has been in good corroboration with the expected structural framework of the synthesized compounds. All the synthesized compounds showed the absence of -$NH_2$ peak in the IR spectrum, which confirmed the reaction at the target amine moiety of the substrates. Moreover, all the compounds displayed a characteristic peak for C=N and N=N group, resonating at around 1578-1603 $cm^{-1}$ and 1503-1514 $cm^{-1}$, respectively, which signifies the formation of tetrazole ring.

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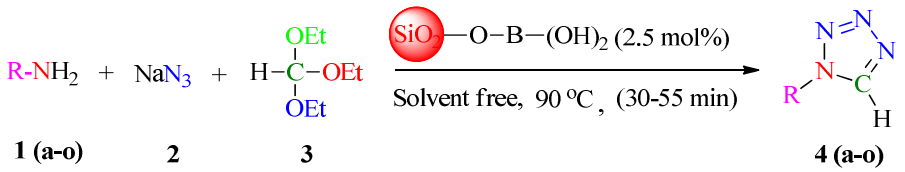
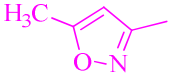
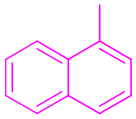
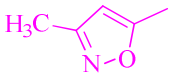
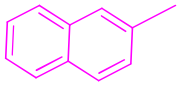
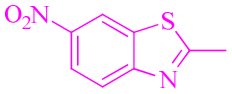
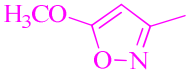
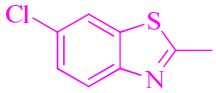
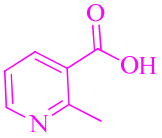
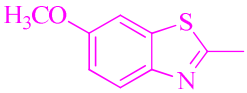
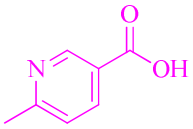
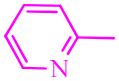
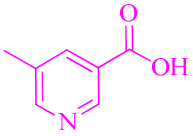
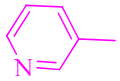
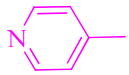



| Products | R | Products | R |
|---|---|---|---|
| 4a |  | 4h |  |
| 4b |  | 4i |  |
| 4c |  | 4j |  |
| 4d |  | 4k |  |
| 4e |  | 4l |  |
| 4f |  | 4m |  |
| 4g |  | 4n |  |
| | | 4o |  |

**Scheme: 2** Synthetic pathway for the synthesis of tetrazole derivatives **4** (**a-o**)

Characteristic peaks for the different substituted functional moieties such as methoxy, nitro and carboxylic group's etc., have been discussed in the experimental section. In $^{1}H$ NMR spectral analysis, each synthesized compound displayed a sharp singlet resonating at around δ 8.07-8.95, attributed to the H-5 (-CH=N) proton of the tetrazole nucleus, this

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prominent downfield shift displayed by H-5 proton of the tetrazole nucleus is probably due to its acidic nature imposed by adjacent nitrogen atoms of the tetrazole ring (See supplementary information). In $^{13}C$ NMR spectral study, the absorption band resonating at around δ 135-148 has been assigned to the $sp^2$ hybridized carbon of the tetrazole ring (See supplementary information). The detailed spectral assignment has been discussed in experimental section. The mass spectral analysis of all the synthesized tetrazole derivatives has been found to be in good conformity with the proposed structures.

In our present study, a series of 1-substituted-1*H*-tetrazole **4** (**a-o**) were synthesized from different heterocyclic/aromatic amino compounds with $NaN_3$ and triethyl *ortho*-formate at reflux temperature in acetic acid in the absence of catalyst. It was observed that the reaction took prolonged time (4-6 hrs) for completion with a moderate yield (61-72 %) of the products (Table 1).

In order to develop efficient, eco-friendly and environmentally benevolent approach for the synthesis of 1-substituted-1*H*-tetrazole compounds, we explored the efficacy of silica-supported boric acid ($SiO_2$-$H_3BO_3$) catalyst by carrying out the solvent free reaction of variety of substituted amino compounds with sodium azide and triethyl *ortho*-formate at 90 $^oC$. The reaction progressed smoothly and resulted in the formation of corresponding products **4**(**a-o**) in excellent yields (90-97 %) within (30-55 mins) as monitored by TLC (Table 1). The present protocol is applicable to a broader substrate scope (electron-rich and electron-deficient). The plausible mechanistic pathway for the synthesis of target compounds **4** (**a-o**) is shown in Scheme 3. In the present study, we probed the optimized reaction conditions including loading of catalyst, effect of temperature and solvents in terms of time and yield on a model reaction using 5-methylisoxazol-3-amine (**1a**) with sodium azide (**2**) and triethyl *ortho*-formate (**3**) to establish the best possible reaction conditions for the synthesis of tetrazole derivatives.

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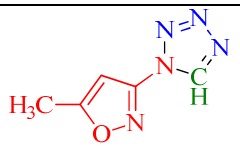
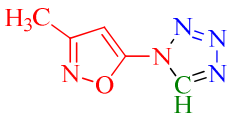
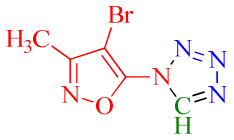
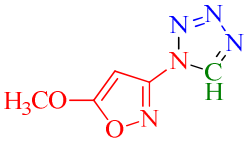
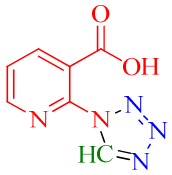
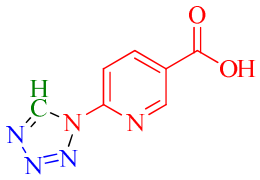
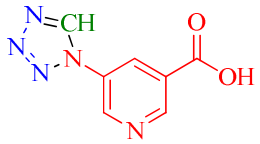
**Table 1** Silica-supported boric acid ($SiO_2$-$H_3BO_3$) catalyzed synthesis of 1-substituted-1*H*-tetrazole derivatives **4** (**a-o**)

| Products | Structure | Reaction in presence of acetic acid | | Reaction in presence of catalyst | | M.P (°C) |
|---|---|---|---|---|---|---|
| | | Time (hrs) | Yield (%) | Time (min) | Yield (%) | |
| **4a** |  | 5 | 69 | 30 | 95 | 160 |
| **4b** |  | 4.5 | 72 | 45 | 93 | 189-191 |
| **4c** |  | 4 | 65 | 35 | 96 | 174 |
| **4d** |  | 5 | 68 | 40 | 91 | 196 |
| **4e** |  | 4.7 | 63 | 50 | 97 | 231-233 |
| **4f** |  | 4.2 | 67 | 42 | 95 | 211 |
| **4g** |  | 5.5 | 70 | 46 | 90 | 203 |

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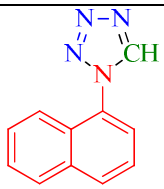
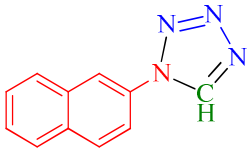
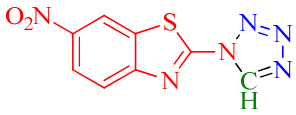
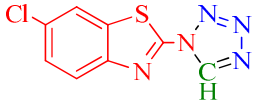
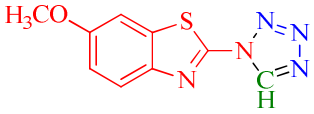
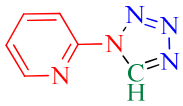
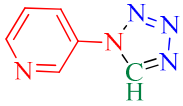
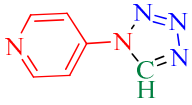
| | | | | | | |
|---|---|---|---|---|---|---|
| **4h** |  | 6 | 61 | 35 | 96 | 98 (95-96)[31b] |
| **4i** |  | 5.5 | 64 | 40 | 92 | 116 (114-115)[44] |
| **4j** |  | 5.0 | 67 | 55 | 91 | 263 |
| **4k** |  | 4.8 | 71 | 48 | 97 | 174 |
| **4l** |  | 4.2 | 66 | 43 | 93 | 181-183 |
| **4m** |  | 5 | 72 | 35 | 95 | 127 (128)[45] |
| **4n** |  | 5.5 | 70 | 40 | 94 | 79 (77)[32c,45] |
| **4o** |  | 5.3 | 68 | 42 | 96 | 171 |

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To achieve the optimum concentration of catalyst, the model reaction was investigated for different concentrations 0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 mol% (entries 2-7, Table 2) of catalyst ($SiO_2$-$H_3BO_3$) at 90 $^oC$ under solvent free condition. The model reaction was primarily tested in the absence of catalyst, it was found that reaction took elongated time (8 hrs) for completion with poor yield (42 %) of product obtained (entry 1, Table 2). The model reaction was then tested for 0.5 mol% of catalyst followed by 1.0, 1.5, 2.0, 2.5 and 3.0 mol% and the results were noted in terms of reaction time and yield. The results revealed that at every subsequent increase in concentration of catalyst by 0.5 %, there has been noteworthy increase in the yield from 64-95 %, with drop in reaction time from 8-0.5 hrs. It can be inferred from (entry 6, Table 2) that 2.5 mol% of the catalyst is satisfactory to gain the optimum yield in the shortest reaction time under neat conditions at 90 $^oC$. Using less than 2.5 mol% of catalyst, moderate yield of the product (64-83 %) was obtained with higher reaction time. The use of excess mole% of catalyst (>2.5 mol%) does not further enhance the yield, possibly due to the saturation of the catalyst surface.

**Table 2** Effect of catalyst loading on the yield and reaction time of model reaction (**4a**)[a]

| Entry | Catalyst (mol%) | Time (hrs)[b] | Yield (%)[c] |
|---|---|---|---|
| **1** | No catalyst | 8.0 | 42 |
| **2** | 0.5 | 4.5 | 64 |
| **3** | 1.0 | 4.0 | 71 |
| **4** | 1.5 | 2.0 | 75 |
| **5** | 2.0 | 1.0 | 83 |
| **6** | 2.5 | 0.5 | 95 |
| **7** | 3.0 | 0.5 | 95 |

[a]*Reaction condition*: 5-methylisoxazol-3-amine (**1a**, 2 mmol), $NaN_3$ (**2**, 2 mmol), triethyl *ortho*-formate (**3**, 2.4 mmol), solvent free, 90 $^oC$; [b]Reaction Progress monitored by TLC.
[c]Isolated yield of products.

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To optimize the reaction temperature, we conducted the model reaction at different temperatures under solvent free condition. It was observed that the increase in temperature from 25 $^{o}$C to 90 $^{o}$C, has a prominent effect on the reaction in terms of yield and reaction time. Initially model reaction was allowed to stir at room temperature (25 $^{o}$C), the results obtained were not satisfactory with prolonged reaction time of 3 hrs and moderate yield of 54 %. The increase in temperature coupled with stirring produced a profound effect on the yield of the product from 54-95 % during the course of reaction (entries 1-5, Table 3). However, further increase in the temperature up to 110 $^{o}$C did not show any further increase in the yield of the product. Thus, keeping in view the above optimized reaction conditions, 90 $^{o}$C was chosen as the optimal temperature for all the reactions.

**Table 3** Effect of temperature on model reaction[a]

| Entry | Temperature ($^{o}$C) | Time (hrs)[b] | Yield (%)[c] |
|---|---|---|---|
| **1** | 25 | 3.0 | 54 |
| **2** | 50 | 2.5 | 77 |
| **3** | 70 | 2.0 | 82 |
| **4** | 80 | 1.0 | 86 |
| **5** | 90 | 30[d] | 95 |
| **6** | 110 | 30[d] | 95 |

[a]*Reaction condition*: 5-methylisoxazol-3-amine (**1a**, 2 mmol), $NaN_3$ (**2**, 2 mmol), triethyl *ortho*-formate (**3**, 2.4 mmol), catalyst (2.5 mol%), solvent free, refluxing at different temperatures.
[b]Reaction Progress monitored by TLC (entry 1-4, hrs).
[c]Isolated yield of products.
[d]Reaction Progress monitored by TLC (entry 5-6, min).

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To evaluate the efficiency of the solvent free reaction conditions in comparison with solvent conditions, the model reaction was examined in different solvent systems in the presence of 2.5 mol% of $SiO_2$-$H_3BO_3$ catalyst. The model reaction was initially performed in common organic solvents like MeOH and EtOH, a moderate yield of the product (**4a**) was obtained after a stretched time period (entries 2-3, Table 4). This moderate yield can be attributed to the nucleophilic nature of these solvents, due to presence of lone pair of electrons on oxygen atom. Thus nucleophilic competition between these solvents (MeOH, EtOH) and primary amine group of the substrate for electrophilic carbon of triethyl *ortho*-formate will eventually resulted in lower yield of desired product. Moreover, this low yield in these coordinating solvents (MeOH, EtOH) can be ascribed due to the accessibility of the acidic Lewis sites of $B(OH)_2$ in these solvents. Whereas in acetic acid, the yield of the desired product increased significantly with reduced reaction time (entry 4, Table 4). This improvement in yield in $CH_3COOH$ solvent is probably due to its ability to facilitate the elimination of ethoxy group (OEt) from triethyl *ortho*-formate, thus enhances the electrophilicity of carbon, rendering it more feasible for nucleophilic attack by the amines. However, when the reaction was carried out in non-coordinating solvents like, THF and DMF, no further significant increase in the yield of the product was observed in comparison to $CH_3COOH$, after a stretched reaction time (entries 5-6, Table 4). Moreover, when the reaction was explored under solvent-free condition, it was noted that the yield of product enhanced sharply with significant drop in reaction time. Thus, nature (nucleophlicity/protocity) of the solvent has a considerable effect on the yield and reaction time. These results suggest that, solvent free reaction condition coupled with 2.5 mol% of $SiO_2$-$H_3BO_3$ at 90 $^oC$ is the best synthetic approach for the synthesis of 1-substituted tetrazole derivatives in terms of reduced reaction times and excellent yields of the products.

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**Table 4** Effect of various solvents on model reaction[a]

| Entry | Solvent | Time (hrs)[b] | Yield (%)[c] |
|---|---|---|---|
| **1** | Solvent free | 30 min | 95 |
| **2** | MeOH | 7 | 59 |
| **3** | EtOH | 8 | 54 |
| **4** | $CH_3COOH$ | 5 | 79 |
| **5** | THF | 9 | 73 |
| **6** | DMF | 8 | 76 |

[a]*Reaction condition*: 5-methylisoxazol-3-amine (**1a**, 2 mmol), $NaN_3$ (**2**, 2 mmol), triethyl *ortho*-formate (**3**, 2.4 mmol), catalyst (2.5 mol%), different solvents, refluxing at 90 $^oC$.
[b]Reaction Progress monitored by TLC.
[c]Isolated yield of products.

A comparative study of a variety of heterogeneous catalysts was conducted to confirm the superiority of silica-boric acid over other heterogeneous acid catalysts. When the model reaction was investigated with silica-supported catalysts such as $HClO_4$-$SiO_2$, $NaHSO_4$-$SiO_2$, $NH_4OAc$-$SiO_2$ and $P_2O_5$-$SiO_2$ the reaction took prolonged time period, and the yields were not satisfactory (entries 2-5, Table 5). However, relatively good yield of the product **4a** (77-81 %) was obtained by using $Yb(OTf)_3$ and $In(OTf)_3$ as heterogeneous catalysts but again reaction took protracted time period for completion (entries 6-7, Table 5). When the model reaction was coupled with silica-boric acid catalyst, the yield of the product **4a** (95 %) increased exponentially with prominent drop in reaction time. These results suggest that silica-boric acid is the catalyst of choice for the present synthetic protocol.

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**Table 5** Comparison of catalytic activity of different catalysts on the model reaction[a]

| Entry | Catalyst | Time (hrs)[b] | Yield (%)[c] |
|---|---|---|---|
| **1** | $SiO_2$-$H_3BO_3$ | 30 min | 95 |
| **2** | $SiO_2$-$HClO_4$ | 5 | 50 |
| **3** | $SiO_2$- $NaHSO_4$ | 7 | 55 |
| **4** | $SiO_2$- $NH_4OAc$ | 6 | 59 |
| **5** | $SiO_2$- $P_2O_5$ | 6.5 | 62 |
| **6** | $Yb(OTf)_3$ | 5 | 77 |
| **7** | $In(OTf)_3$ | 3 | 81 |

[a]*Reaction condition*: 5-methylisoxazol-3-amine (**1a**, 2 mmol), $NaN_3$ (**2**, 2 mmol), triethyl *ortho*-formate (**3**, 2.4 mmol), different catalyst (2.5 mol%), solvent free, refluxing at 90 $^oC$.
[b]Reaction Progress monitored by TLC.
[c]Isolated yield of products.

The reusability of the silica-boric acid catalyst was also examined on model reaction in order to satisfy the green chemistry criteria. The recycling of catalyst is an important step as it reduces the cost of the process. The catalyst was reused five times and the results demonstrate that catalyst can be reused as such without a significant loss in yield (Fig. 7). After completion of the reaction, the catalyst was removed by simple filtration. The recovered catalyst was dried under vacuum at 100 $^oC$ for 6 hrs and tested up to five more reaction cycles. Recycling and reuse of the catalyst showed minimal decreases in yields. The product was obtained in 93%, 91%, 88%, 85%, 80% yields after successive cycles. (Fig. 7), thus proving the catalyst's reusability. To ascertain the variation in morphological features of the recovered catalyst, we carry out its SEM-EDX analysis. It was observed that the composition of the catalytic system was almost consistent with the fresh catalyst (Fig. 8) and also there was no significant change in the morphology of the catalyst (Fig. 9) as compared to the fresh catalyst.

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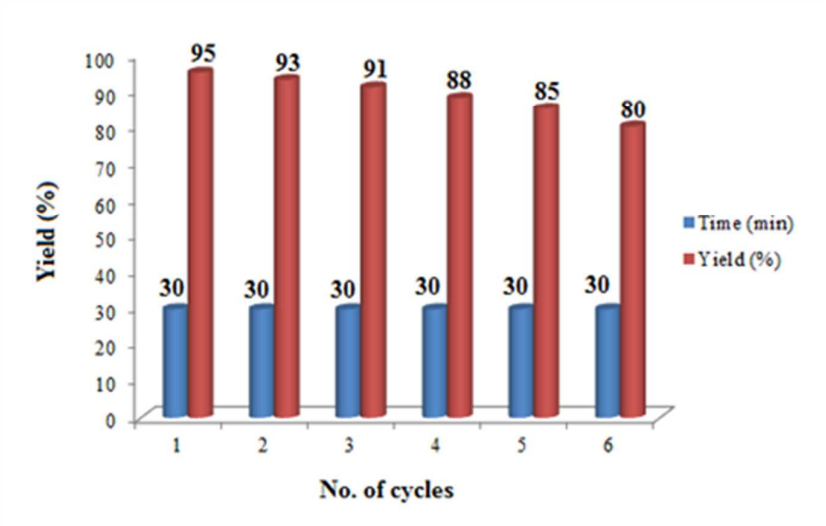


**Fig. 7** Recycling data of the catalyst ($SiO_2$-$H_3BO_3$) for the model reaction

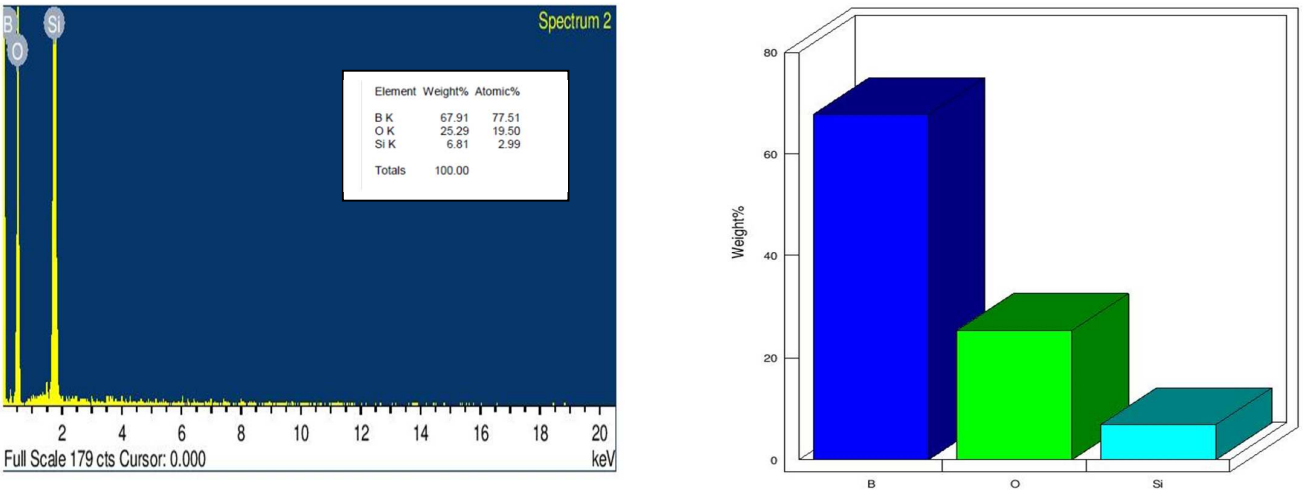


**Fig. 8** Energy dispersive X-ray (EDX) analysis of recovered catalyst after five runs

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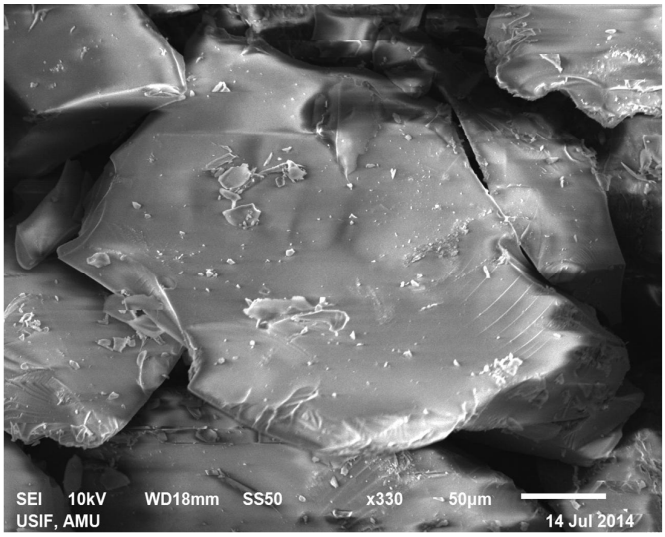


**Fig. 9** SEM image of recovered catalyst after five runs

Under these optimized reaction conditions discussed above, the scope and generality of the present protocol was further demonstrated by carrying out the reaction of different heterocyclic/aromatic amines with sodium azide and triethyl *ortho*-formate under solvent free condition. All the reactions proceeded smoothly to afford the desired products **4(a-o)** in excellent yields (90-97 %) within 30-55 min (Table 1).

The probable silica-boric acid catalyzed mechanistic pathway for the synthesis of tetrazole derivatives is shown in Scheme 3, in which the Lewis acidity of the catalyst probably has an important role in the promotion of the cyclization process. It is believed that silica-boric acid activates the ethoxy group of triethyl *ortho*-formate and facilitates its elimination by cleaving the C-O bond of triethyl *ortho*-formate to generate carbenium ion (**I**), which is resonance stabilized by oxygen atom of another ethoxy group followed by nucleophilic attack of amine. The next step involves the expulsion of second ethoxy group generating carbenium ion (**II**), which can be stabilized by the neighbouring O or N atom to form either oxonium (**A**) or iminium (**B**) cation, respectively. It is believed that the formation of iminium cation is preferential over oxonium cation due to the greater basicity of nitrogen with respect to oxygen. The next step involves the sequential attack of azide anion leading to

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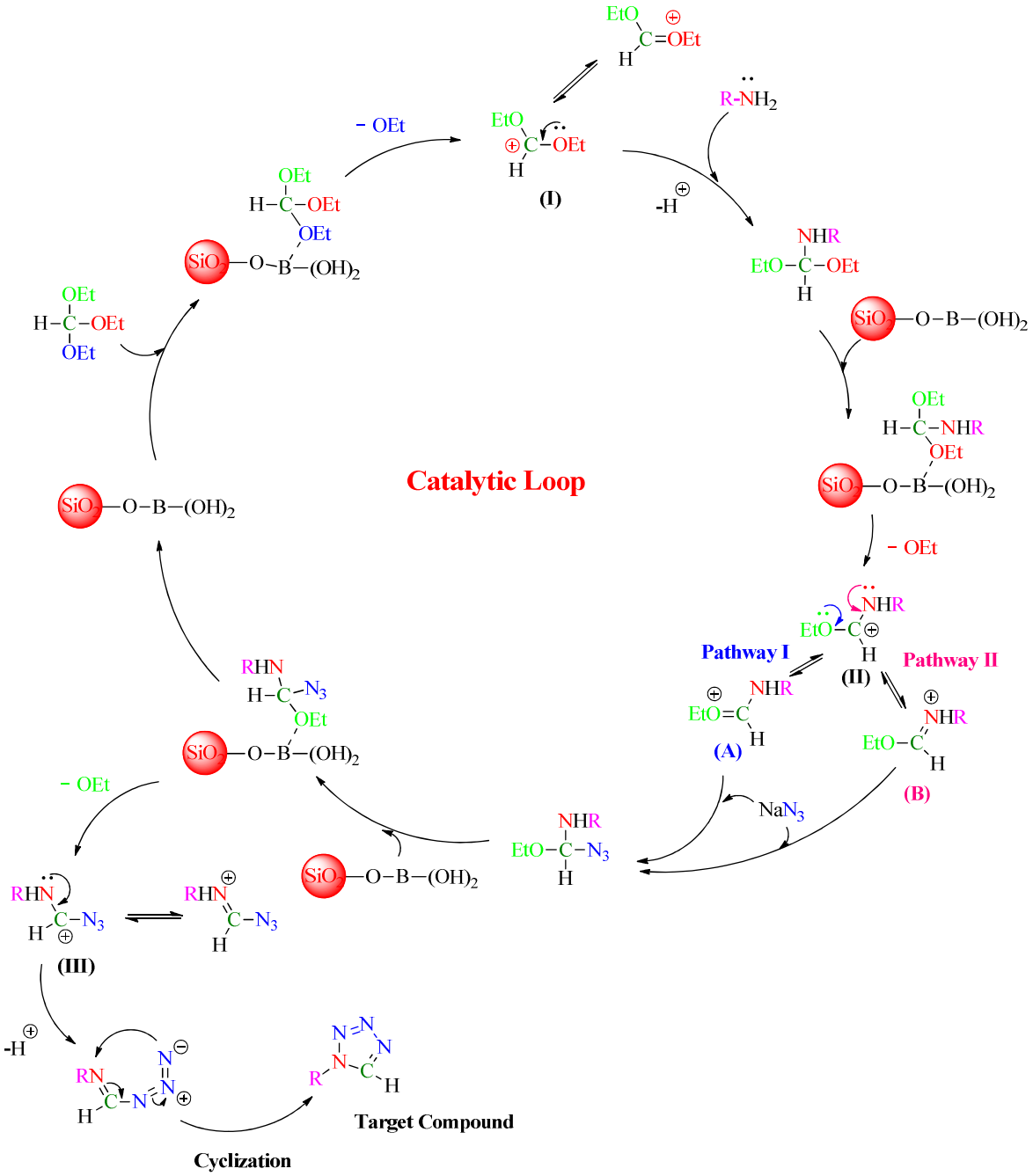
the formation of proposed imidoylazide intermediate (**III**).[41,42] As the three nitrogens bend away from the linear arrangement and the fourth nitrogen atom comes within the bonding distance, eventually leads to the formation of cyclized tetrazoles **4** (**a-o**).




**Scheme: 3** Plausible mechanistic catalytic loop for the synthesis of compounds **4** (**a-o**)

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### 2.3. AChE and BuChE inhibition results

All the synthesized tetrazole derivatives **4** (**a-o**) were analyzed for acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibition studies. The $IC_{50}$ values for AChE and BuChE inhibition are summarized in **Table 6**. It can be inferred from the data reported in **Table 6**, that compounds bearing nicotinic acid nucleus i.e., **4e**, **4f** and **4g** exhibited strongest inhibition to AChE with an $IC_{50}$ values of 0.43±0.02 μM, 0.21±0.02 μM and 0.26±0.01 μM, respectively, followed by compound **4c** ($IC_{50}$ = 1.03±0.01 μM), **4d** ($IC_{50}$ = 1.25±0.01 μM), **4b** ($IC_{50}$ = 1.46±0.01 μM), **4a** ($IC_{50}$ = 1.52±0.02 μM), **4m** ($IC_{50}$ = 2.08±0.02 μM), **4n** ($IC_{50}$ = 2.13±0.02 μM), **4o** ($IC_{50}$ = 2.17±0.01 μM), **4l** ($IC_{50}$ = 2.71±0.01 μM), **4k** ($IC_{50}$ = 2.89±0.01 μM), **4j** ($IC_{50}$ = 3.01±0.02 μM), **4h** ($IC_{50}$ = 3.29±0.02 μM) and **4i** ($IC_{50}$ = 3.32±0.02 μM). Of these nicotinic acid analogues **4f** was found to be most potent AChE inhibitor. Among the isoxazole nucleated tetrazole analogues **4** (**a-d**), compound **4c** ($IC_{50}$ = 1.03±0.01 μM) with methyl and bromine substituted isoxazole ring displayed encouraging activity against AChE, methoxy substituted isoxazole ring (compound **4d)** showed lower activity than **4c** by a value of 0.22 but higher than methyl substituted isoxazole analogues (compound **4a** and **4b**) by a value of 0.27 and 0.21, respectively. These results suggest that the nature of substituent inflict a prominent effect on AChE activity. Compound **4m**, **4n** and **4o** comprising pyridine nucleus exhibited nearly similar results with $IC_{50}$ values of 2.08±0.02 μM, 2.13±0.02 μM and 2.17±0.01 μM, respectively. Among benzothiazole nucleated tetrazole derivatives, compound **4l** ($IC_{50}$ = 2.71±0.01 μM) with methoxy substituent attached to the benzothiazole nucleus at 6 position exhibited better AChE inhibition. It was noticed that when the methoxy group at the 6 position in compound **4l** is replaced by chlorine (compound **4k**), the activity decreased significantly by 0.18. However, when the same methoxy group was replaced by nitro group in compound **4j**, there was further dip in AChE inhibition by value of 0.30. Thus the nature of substituent at 6 position in benzothiazole based tetrazole derivatives plays an essential role in

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AChE inhibition activity. It can be seen that compound **4h** and **4i** exhibited moderate activity among all the synthesized tetrazole derivatives with $IC_{50}$ values of 3.29±0.02 μM and 3.32±0.02 μM, respectively. In view of the above results it can be concluded that in general electron pumping groups enhance AChE activity while as electron withdrawing groups reduce it. The level of *in vitro* BuChE inhibition was also examined using the same method. Most of these compounds exhibited moderate inhibitory potency against BuChE, but there were no apparent patterns in the levels of inhibition.

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**Table 6** *In vitro* inhibition $IC_{50}$ (μM) and selectivity of tetrazole derivatives and tacrine for *h*AChE and *h*BuChE

| Entry | Product | $IC_{50}$ (μM)[a] | | Selectivity for *h*AChE[d] |
|---|---|---|---|---|
| | | ***h*AChE[b] ± SEM** | ***h*BuChE[c] ± SEM** | |
| **1** | **4a** | 1.52±0.02 | 1.05±0.03 | 0.69 |
| **2** | **4b** | 1.46±0.01 | 1.71±0.01 | 1.17 |
| **3** | **4c** | 1.03±0.01 | 2.29±0.02 | 2.23 |
| **4** | **4d** | 1.25±0.01 | 4.58±0.02 | 3.67 |
| **5** | **4e** | 0.43±0.02 | 2.43±0.01 | 5.64 |
| **6** | **4f** | 0.21±0.02 | 1.53±0.01 | 7.31 |
| **7** | **4g** | 0.26±0.01 | 0.85±0.03 | 3.27 |
| **8** | **4h** | 3.29±0.02 | 2.39±0.02 | 0.72 |
| **9** | **4i** | 3.32±0.02 | 1.69±0.01 | 0.51 |
| **10** | **4j** | 3.01±0.02 | 13.64±0.02 | 4.53 |
| **11** | **4k** | 2.89±0.01 | 19.57±0.02 | 6.77 |
| **12** | **4l** | 2.71±0.01 | 27.30±0.01 | 10.07 |
| **13** | **4m** | 2.08±0.02 | 0.39±0.03 | 0.19 |
| **14** | **4n** | 2.13±0.02 | 0.70±0.01 | 0.33 |
| **15** | **4o** | 2.17±0.01 | 0.54±0.02 | 0.25 |
| **16** | **Tacrine (Standard)** | 0.09±0.02 | 0.14±0.01 | 0.16 |

[a]**$IC_{50}$** values represent the concentration of inhibitor required to decrease the enzyme activity by 50%; **SEM** = Standard error of the mean
[b]***h*AChE** = Acetylcholinesterase from human serum
[c]***h*BuChE** = Butyrylcholinesterase from human serum
[d]Selectivity for *h*AChE = $IC_{50}$ (*h*BuChE)/$IC_{50}$ (*h*AChE).

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## 3. Conclusions

The present protocol reports the convenient, eco-friendly and scalable protocol approach for the synthesis of tetrazole derivatives **4** (**a-o**) in excellent yields (90-97 %) by employing silica-supported boric acid as a catalyst. This solvent-free, green synthetic procedure eliminates the use of toxic solvents and thus makes it attractive one in organic synthesis. The noteworthy features of this protocol are shorter reaction time, high purity, mild reaction conditions, operational simplicity, cleaner reaction profile, enhanced reaction rates and easy workup. Moreover, $SiO_2$-$H_3BO_3$ exhibited stability towards air and water and is easy to prepare from cheap materials. The present protocol demonstrates that, optimization conditions i.e., 2.5 mol% of catalyst under solvent-free condition at 90 $^oC$ are the best parameters for the synthesis of tetrazole analogues in excellent yields for scalable purposes. All the compounds displayed moderate to good AChE inhibition potency. The results validate that the nature of substituents attached to the heterocyclic moieties is crucial for biological activity. We believe that this synthetic approach provides a better scope for the synthesis of tetrazole analogues and will be a more practical alternative to the other existing methods.

## 4. Experimental

### 4.1. Materials and general methods

All reagents were purchased from Sigma-Aldrich (India), Alfa Aesar and Merck as 'synthesis grade' and used without further purification. The purity of all the compounds was checked by thin layer chromatography (TLC) on glass plates coated with silica gel G254 (E-Merck). Human AChE (EC: 3.1.1.7) and BuChE (EC: 3.1.1.8) lyophilized powder were purchased from Sigma-Aldrich. Melting points of all synthesized compounds were determined on a Kofler apparatus and are uncorrected. Fourier transform-infrared (FT-IR) spectra were recorded in the 400-4000 $cm^{-1}$ wave-number range with a Perkin-Elmer (2000 FTIR)

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Spectrometer by KBr pellet method. $^{1}H$ NMR and $^{13}C$ NMR spectra were recorded on a Bruker Avance-II 400 MHz instrument in pure deuterated DMSO-$d_6$ solvent. The chemical shifts (δ) are reported in ppm relative to the TMS as an internal standard and J values are reported in Hertz. Mass spectra were recorded on a JEOL D-300 mass spectrometer. Elemental analysis (C, H, N) was conducted using Thermo Scientific (FLASH 2000) CHN Elemental Analyser. X-ray diffractograms (XRD) of the catalyst was recorded in the 2θ range of 20-80° with scan rate of 4°/ min on a Shimadzu-6100 X-ray diffractometer with Ni-filtered Cu K$\alpha$ radiation at a wavelength of 1.54060 A$^{o}$. The scanning electron microscope (SEM-EDX) analysis was obtained using a JEOL (JSM-6510) equipped with an energy dispersive X-ray spectrometer at different magnification and transmission electron microscope (TEM) results were recorded using JEOL (JEM-2100F) model.

### 4.2. Synthesis of silica-supported boric acid ($SiO_2$-$H_3BO_3$) catalyst

Boric acid (3.0 g) was taken in a 250 ml round bottom flask with 60 mL water and heated to 60-80 $^{o}$C. Silica gel (60-120 mesh, 27.0 g) was added gradually with constant stirring and refluxed for 5 hrs. Water was evaporated under reduced pressure and the residue was stirred at 100 $^{o}$C for 6-7 hrs under vacuum to give free flowing white powder of silica-boric acid (Scheme 1). It was kept under vacuum at room temperature.[36] The various catalysts i.e. $SiO_2$-$HClO_4$,[43a] $SiO_2$-$NaHSO_4$,[43b] $SiO_2$-$NH_4OAc$[43c] and $SiO_2$-$P_2O_5$[43d] used for the comparative study with respect to silica-boric acid have been synthesized according to the previously published standard procedures.

### 4.3. General procedure for the synthesis of 1-substituted-1*H*-tetrazoles

To a mixture of amine **1** (2.0 mmol), $NaN_3$ **2** (2.0 mmol) and triethyl *ortho*-formate **3** (2.4 mmol), 2.5 mol% of silica-supported boric acid was added and the reaction mixture was

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allowed to stirr at 90 $^{o}$C for 30-55 min. After completion of the reaction (as monitored by TLC), the reaction mixture was allowed to cool at room temperature and diluted with cold water (5 mL). The catalyst was separated by filtration and the resulting solution was extracted with ethyl acetate (3×10 mL). The combined organic layers were washed with brine, dried over anhydrous $Na_2SO_4$ and evaporated under reduced pressure. The crude product obtained was crystallized with chloroform-methanol to afford the pure products. The recovered catalyst was reused for subsequent cycles without a significant loss in yield.

### 4.4. Spectral characterization

#### 4.4.1. 5-methyl-3-(1*H*-tetrazol-1-yl) isoxazole (4a)

Compound **4a** crystallized from $CHCl_3$-MeOH as yellow crystalline solid. Yield: 95%, m.p. 160 °C; Anal. calc. for $C_5H_5N_5O$: C, 39.74; H, 3.33; N, 46.34; found: C, 39.75; H, 3.34; N, 46.36. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1065 (C-O), 1128 (C-N), 1508 (N=N), 1584 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 2.24 (s, 3H, $CH_3$), 6.35 (s, 1H,*isoxazole ring*), 8.07 (s, 1H, -CH=N*tetrazole ring*). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 15.25 ($CH_3$), 102.44 (C-4), 135.05 (-CH=N*tetrazole ring*), 148.54 (C-3), 155.03 (C-5). MS (ESI) m/z: 151.05 $[M+H]^{+\bullet}$.

#### 4.4.2. 3-methyl-5-(1*H*-tetrazol-1-yl) isoxazole (4b)

Compound **4b** crystallized from $CHCl_3$-MeOH as brownish solid. Yield: 94%, m.p. 189-191 °C; Anal. calc. for $C_5H_5N_5O$: C, 39.74; H, 3.33; N, 46.34; found: C, 39.76; H, 3.32; N, 46.37. IR $\nu_{max}^{KBr}$ cm$^{-1}$: 1058 (C-O), 1135 (C-N), 1511 (N=N), 1578 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 2.15 (s, 3H, $CH_3$), 6.38 (s, 1H,*isoxazole ring*), 8.15 (s, 1H, -CH=N*tetrazole ring*). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 17.24 ($CH_3$), 104.20 (C-4), 137.18 (-CH=N*tetrazole ring*), 143.25 (C-5), 152.31 (C-3). MS (ESI) m/z: 151.05 $[M+H]^{+\bullet}$.

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#### 4.4.3. 4-bromo-3-methyl-5-(1*H*-tetrazol-1-yl) isoxazole (4c)

Compound **4c** crystallized from $CHCl_3$-MeOH as white solid. Yield: 96%, m.p. 174 °C; Anal. calc. for $C_5H_4BrN_5O$: C, 26.11; H, 1.75; N, 30.45; found: C, 26.13; H, 1.77; N, 30.43. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 574 (C-Br), 1082 (C-O), 1142 (C-N), 1509 (N=N), 1587 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 2.17 (s, 3H, $CH_3$), 8.26 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 16.32 ($CH_3$), 102.38 (C-4), 140.25 (-CH=$N_{tetrazole\ ring}$), 147.28 (C-5), 156.24 (C-3). MS (ESI) m/z: 228.96 $[M+H]^{+\bullet}$.

#### 4.4.4. 5-methoxy-3-(1*H*-tetrazol-1-yl) isoxazole (4d)

Compound **4d** crystallized from $CHCl_3$-MeOH as yellowish crystals. Yield: 92%, m.p. 196 °C; Anal. calc. for $C_5H_5N_5O_2$: C, 35.93; H, 3.02; N, 41.90; found: C, 35.94; H, 3.01; N, 41.93. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1078 (C-O), 1140 (C-N), 1512 (N=N), 1582 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 2.94 (s, 3H, $OCH_3$), 5.78 (s, 1H,$_{isoxazole\ ring}$), 8.23 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 53.65 ($OCH_3$), 107.28 (C-4), 142.20 (-CH=$N_{tetrazole\ ring}$), 145.27 (C-3), 157.80 (C-5). MS (ESI) m/z: 167.04 $[M+H]^{+\bullet}$.

#### 4.4.5. 2-(1*H*-tetrazol-1-yl) nicotinic acid (4e)

Compound **4e** crystallized from $CHCl_3$-MeOH as white crystalline solid. Yield: 97%, m.p. 231-233 °C; Anal. calc. for $C_7H_5N_5O_2$: C, 43.98; H, 2.64; N, 36.64; found: C, 43.97; H, 2.66; N, 36.65. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1132 (C-N), 1510 (N=N), 1592 (C=N), 1712 (C=O), 3212 (-OH). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.23 (t, 1H, H-5), 7.85 (d, 1H, H-6), 8.02 (d, 1H, H-4), 8.59 (s, 1H, -CH=$N_{tetrazole\ ring}$), 9.84 (brs, 1H, OH). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 123.59 (C-5), 129.25 (C-4), 132.18 (C-3), 142.24 (C-6), 144.21 (-CH=$N_{tetrazole\ ring}$), 148.13 (C-2), 171.35 (C=O). MS (ESI) m/z: 191.04 $[M+H]^{+\bullet}$.

#### 4.4.6. 6-(1*H*-tetrazol-1-yl) nicotinic acid (4f)

Compound **4f** crystallized from $CHCl_3$-MeOH as light yellow solid. Yield: 93%, m.p. 211 °C; Anal. calc. for $C_7H_5N_5O_2$: C, 43.98; H, 2.64; N, 36.64; found: C, 43.99; H, 2.63; N,

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36.67. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1135 (C-N), 1514 (N=N), 1602 (C=N), 1723 (C=O), 3225 (-OH). $^1H$ NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.62 (dd, 2H, H-4 and H-5), 7.84 (s, 1H, H-2), 8.58 (s, 1H, -CH=$N_{tetrazole\ ring}$), 9.79 (brs, 1H, OH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$, δ, ppm): 124.20 (C-5), 130.25 (C-4), 134.28 (C-3), 145.21 (-CH=$N_{tetrazole\ ring}$), 146.10 (C-6), 151.25 (C-2), 169.25 (C=O). MS (ESI) m/z: 191.04 $[M+H]^{+\bullet}$.

#### 4.4.7. 5-(1*H*-tetrazol-1-yl) nicotinic acid (4g)

Compound **4g** crystallized from $CHCl_3$-MeOH as cream colored solid. Yield: 94%, m.p. 203 °C; Anal. calc. for $C_7H_5N_5O_2$: C, 43.98; H, 2.64; N, 36.64; found: C, 43.96; H, 2.65; N, 36.66. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1141 (C-N), 1510 (N=N), 1594 (C=N), 1708 (C=O), 3221 (-OH). $^1H$ NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.72 (s, 1H, H-6), 7.80 (s, 1H, H-2), 7.92 (s, 1H, H-4), 8.48 (s, 1H, -CH=$N_{tetrazole\ ring}$), 9.73 (brs, 1H, OH). $^{13}C$ NMR (100 MHz, DMSO-$d_6$, δ, ppm): 128.51 (C-4), 131.42 (C-3), 138.82 (C-5), 140.20 (C-6), 142.12 (-CH=$N_{tetrazole\ ring}$), 150.24 (C-2), 168.25 (C=O). MS (ESI) m/z: 191.04 $[M+H]^{+\bullet}$.

#### 4.4.8. 1-(naphthalen-1-yl)-1*H*-tetrazole (4h)

Compound **4h** crystallized from $CHCl_3$-MeOH as violet crystals. Yield: 97%, m.p. 98 °C, reported 95-96 °C;[31b] Anal. calc. for $C_{11}H_8N_4$: C, 67.34; H, 4.11; N, 28.55; found: C, 67.36; H, 4.12; N, 28.57. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1141 (C-N), 1572, 1462 (C=$C_{aromatic}$), 1505 (N=N), 1597 (C=N). $^1H$ NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.53 (m, 2H, H-3 and H-6), 7.58 (m, 1H, H-7), 7.64 (m, 1H, H-2), 7.85 (m, 2H, H-4 and H-5), 7.88 (m, 1H, H-8), 8.78 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}C$ NMR (100 MHz, DMSO-$d_6$, δ, ppm): 125.13 (C-7), 126.20 (C-3 and C-6), 127.15 (C-4 and C-5), 127.28 (C-8), 128.05 (C-1), 130.25 (C-10), 132.33 (C-9), 132.82 (C-2), 148.25 (-CH=$N_{tetrazole\ ring}$). MS (ESI) m/z: 196.07 $[M+H]^{+\bullet}$.

#### 4.4.9. 1-(naphthalen-2-yl)-1*H*-tetrazole (4i)

Compound **4i** crystallized from $CHCl_3$-MeOH as pinkish solid. Yield: 95%, m.p. 116 °C, reported 114-115 °C.[44] Anal. calc. for $C_{11}H_8N_4$: C, 67.34; H, 4.11; N, 28.55; found: C, 67.33;

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H, 4.14; N, 28.56. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1146 (C-N), 1575, 1456 (C=$C_{aromatic}$), 1503 (N=N), 1593 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.45 (m, 2H, H-6 and H-7), 7.68 (m, 1H, H-3), 7.79 (m, 1H, H-5), 7.86 (d, 1H, H-4), 7.92 (m, 1H, H-8), 8.04 (d, 1H, H-1), 8.89 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 125.54 (C-3), 126.24 (C-6 and C-7), 127.38 (C-2), 128.05 (C-8), 128.21 (C-5), 129.33 (C-1), 130.79 (C-10), 132.43 (C-4), 133.25 (C-9), 144.25 (-CH=$N_{tetrazole\ ring}$). MS (ESI) m/z: 196.07 $[M+H]^{+\bullet}$.

**4.4.10. 6-nitro-2-(1*H*-tetrazol-1-yl)benzo[d]thiazole (4j)**

Compound **4j** crystallized from $CHCl_3$-MeOH as yellowish solid. Yield: 92%, m.p. 263 °C; Anal. calc. for $C_8H_4N_6O_2S$: C, 38.71; H, 1.62; N, 33.86; found: C, 38.73; H, 1.61; N, 33.89. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 625 (C-S), 1147 (C-N), 1507 (N=N), 1520, 1325 ($NO_2$), 1591 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.58 (d, 1H, H-4), 7.67 (d, 1H, H-5), 7.95 (s, 1H, H-7), 8.57 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 122.38 (C-5), 124.47 (C-4), 125.29 (C-7), 138.24 (C-8), 142.26 (C-6), 144.21 (-CH=$N_{tetrazole\ ring}$), 147.28 (C-9), 152.31 (C-2). MS (ESI) m/z: 248.01 $[M+H]^{+\bullet}$.

**4.4.11. 6-chloro-2-(1H-tetrazol-1-yl)benzo[d]thiazole (4k)**

Compound **4k** crystallized from $CHCl_3$-MeOH as white solid. Yield: 94%, m.p. 174 °C; Anal. calc. for $C_8H_4ClN_5S$: C, 40.43; H, 1.70; N, 29.47; found: C, 40.45; H, 1.68; N, 29.48. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 632 (C-S), 710 (C-Cl), 1137 (C-N), 1503 (N=N), 1596 (C=N).$^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.54 (d, 1H, H-4), 7.59 (d, 1H, H-5), 7.85 (s, 1H H-7), 8.47 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 120.12 (C-7), 123.21 (C-5), 125.34 (C-4), 130.51 (C-6), 134.27 (C-8), 141.24 (-CH=$N_{tetrazole\ ring}$), 144.20 (C-9), 155.18 (C-2). MS (ESI) m/z: 236.99 $[M+H]^{+\bullet}$.

**4.4.12. 6-methoxy-2-(1H-tetrazol-1-yl)benzo[d]thiazole (4l)**

Compound **4l** crystallized from $CHCl_3$-MeOH as white creamy solid. Yield: 96%, m.p. 181-183 °C; Anal. calc. for $C_9H_7N_5OS$: C, 46.34; H, 3.02; N, 30.03; found: C, 46.32; H, 3.03; N,

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30.05. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 638 (C-S), 1128 (C-O), 1143 (C-N), 1508 (N=N), 1603 (C=N).$^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 2.34 (s, 3H, $OCH_3$), 7.43 (d, 1H, H-5), 7.51 (d, 1H, H-4), 7.71 (s, 1H, H-7), 8.42 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 51.21 ($OCH_3$), 115.21 (C-7), 121.35 (C-5), 126.13 (C-4), 136.18 (C-8), 140.29 (-CH=$N_{tetrazole\ ring}$), 142.15 (C-9), 150.28 (C-6), 152.20 (C-2). MS (ESI) m/z: 233.04 $[M+H]^{+\bullet}$.

**4.4.13. 2-(1*H*-tetrazol-1-yl) pyridine (4m)**

Compound **4m** crystallized from $CHCl_3$-MeOH as colorless crystalline solid. Yield: 95%, m.p. 127 °C, reported 128 °C;[45] Anal. calc. for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60; found: C, 48.92; H, 3.38; N, 47.62. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1135 (C-N), 1512 (N=N), 1588 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.38 (m, 1H, H-5), 7.94 (m, 1H, H-3), 8.12 (m, 1H, H-4), 8.36 (m, 1H, H-6), 8.95 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 118.14 (C-5), 121.05 (C-3), 130.36 (C-4), 138.54 (C-2), 142.44 (C-6), 144.72 (-CH=$N_{tetrazole\ ring}$). MS (ESI) m/z: 147.05 $[M+H]^{+\bullet}$.

**4.4.14. 3-(1*H*-tetrazol-1-yl) pyridine (4n)**

Compound **4n** crystallized from $CHCl_3$-MeOH as white solid. Yield: 93%, m.p. 79 °C, reported 77 °C. [32c,45] Anal. calc. for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60; found: C, 49.08; H, 3.39; N, 47.56. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1138 (C-N), 1507 (N=N), 1593 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ, ppm): 7.14 (m, 1H, H-5), 7.42 (d, 1H, H-4), 7.96 (s, 1H, H-2), 8.11 (d, 1H, H-6), 8.85 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 119.05 (C-3), 122.81 (C-5), 132. 63 (C-4), 138.24 (C-6), 141.44 (-CH=$N_{tetrazole\ ring}$), 143.72 (C-2). MS (ESI) m/z: 147.05 $[M+H]^{+\bullet}$.

**4.4.15. 4-(1*H*-tetrazol-1-yl) pyridine (4o)**

Compound **4o** crystallized from $CHCl_3$-MeOH as colourless crystals. Yield: 96%, m.p. 171 °C; Anal. calc. for $C_6H_5N_5$: C, 48.98; H, 3.43; N, 47.60; found: C, 48.93; H, 3.41; N, 47.68. IR $\nu_{max}^{KBr}$ $cm^{-1}$: 1141 (C-N), 1503 (N=N), 1598 (C=N). $^1$H NMR (400 MHz, DMSO-$d_6$, δ,

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ppm): 7.28 (d, 2H, H-2 and H-6), 8.07 (d, 2H, H-3 and H-5), 8.78 (s, 1H, -CH=$N_{tetrazole\ ring}$). $^{13}$C NMR (100 MHz, DMSO-$d_6$, δ, ppm): 121.08 (C-3 and C-5), 139.41 (C-2 and C-6), 142.26 (-CH=$N_{tetrazole\ ring}$), 145.83 (C-4). MS (ESI) m/z: 147.05 $[M+H]^{+\bullet}$.

### 4.5. *In vitro* inhibition studies on AChE and BuChE

The ability of all the synthesized tetrazole derivatives **4** (**a-o**) to inhibit AChE and BuChE activity was assessed by Ellman's method.[46] AChE stock solution was prepared by dissolving human recombinant AChE (EC: 3.1.1.7) lyophilized powder (Sigma-Aldrich) in 0.1 M phosphate buffer (pH = 8.0) containing Triton X-100 (0.1%). Five increasing concentrations of test compounds were assayed to obtain % inhibition of the enzymatic activity in the range of 20-80. The assay solution consisted of a 0.1 M phosphate buffer pH 8.0, with the addition of 340 μM 5,5'-dithio-bis(2-nitrobenzoic acid), 0.02 unit/mL of human recombinant AChE from human serum and 550 μM of substrate (acetylthiocholine iodide, ATCh). Increasing concentration of tested inhibitor were added to the assay solution and pre-incubated for 5 min at 37 °C with the enzyme followed by the addition of substrate. Initial rate assays were performed at 37 °C with a Jasco V-530 double beam Spectrophotometer. Absorbance value at 412 nm was recorded for 5 min and enzyme activity was calculated from the slope of the obtained linear trend. Assays were carried out with a blank containing all components except AChE to account for the non-enzymatic reaction. The reaction rates were compared and the percent inhibition due to the presence of tested inhibitors was calculated. Each concentration was analyzed in triplicate, and $IC_{50}$ values were determined graphically from log concentration-inhibition curves (GraphPad Prism 4.03 software, GraphPad Software Inc.). Tacrine was used as a standard inhibitor. The *in vitro* BuChE inhibition assay was also determined with the similar method as described above.

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