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Authors: Sania Pervaiz, Sadaf Mutahir, Islam Ullah Khan, Muhammad Ashraf, Xiao Liu, Sidrah Tariq, Bao-jing Zhou, and Muhammad Asim Khan

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Organocatalyzed solvent free and efficient synthesis of 2,4,5-trisubstituted imidazoles as potential acetylcholinesterase inhibitors for Alzheimer's disease

Sania Pervaiz [a], Sadaf Mutahir [b*], Islam Ullah Khan [a, c *], Muhammad Ashraf [d], Xiao Liu [b], Sidrah Tariq [a], Bao-jing Zhou [b], Muhammad Asim Khan [b*].

[a] Department of Chemistry, Government College, University, Lahore 54000, Pakistan

[b]School of Chemical Engineering, Nanjing University of Science & Technology, Nanjing, 210094, China.

[c]Department of Chemistry, Faculty of Sciences, University of Sialkot- 51040, Sialkot, Pakistan

[d]Department of Chemistry, and Department of Biochemistry & Biotechnology, The Islamia University of Bahawalpur, Bahawalpur 63100, Pakistan

Corresponding authors,

khanabdali@njust.edu.cn (M. A. Khan), sadafmutahir@njust.edu.cn (S. Mutahir) and iukhan@gcu.edu.pk (I. U. Khan).

Abstract

The catalytic potential of pyridine-2-carboxlic acid has been evaluated for efficient, green and solvent free synthesis of 2,4,5-trisubstituted imidazole derivatives. The compounds were synthesized by condensation reaction of substituted aromatic aldehydes, benzil and ammonium acetate (**3a-3m**) in one pot in a good to excellent yield (74-96 %). These compounds were evaluated against acetylcholinesterase (AChE) enzyme activity to explore their potential against Alzheimer's disease. Among this series of compounds **3m** bearing one ethoxy and a hydroxyl group on the phenyl ring on 2,4,5-trisubstituted imidazoles proved potent AChE inhibitor (102.56±0.14). Structure–activity relationship (SAR) of these compounds was developed. Molecular dockings were carried out for the inhibitors **3m**, **3e**, **3k**, **3c**, **3a**, **3d**, **3j**, and **3f** in order to further investigate the binding mechanism. The inhibitor molecule was molecularly docked with acetylcholinesterase to further study its binding mechanism. The amino group of the

inhibitor **3m** forms an H bond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 3.051 Å.

Keywords: 2,4,5-trisubstituted imidazoles, Organocatalyst, Acetylcholinesterase, Alzheimer's disease, Molecular docking

Introduction

Organocatalyst is an organic molecule with low molecular weight, capable of catalyzing a wide variety of chemical reactions and has many advantages over traditional metal catalysts. It is greener, has synthetic range, mild reaction conditions, economic and less toxic, hence highly appreciated by pharmaceutical industry because it reduces the risk of metal contamination. Organocatalyst having acidic hydrogen has ability to catalyze wide variety of reactions which possibily interact with functional groups of substance, hence they play a valuable role in organic synthesis. Organocatalysts transfer proton to the substrate in transition state and stabilizes the intermediate products by hydrogen bonding.[1-4] Organocatalysis now-a-days has become the hot topic in synthetic chemistry. Our research group is actively engaged in the synthesis of potent organic motifs by organo-catalysis and so far we have explored the catalytic potential of N-acetyl glycine in the synthesis of bis-biphenyl substituted thiazolidinones,[5] and pyrimidines,[6] a step towards the green and one pot synthesis of organic compounds avoiding the risk of metal contamination and obtaining good yield.

Alzheimer's disease (AD) is a neurodegenerative brain turmoil, which is linked with the low level of acetylcholine neurotransmitter. This disorder is associated by slow but sure loss of memory and cognitive impairments. About 4-8 % of the elderly population of the world are affected by this disorder.[7-10] In Alzheimer's disease, cognitive impairment is due to the reduction in levels of acetylcholine in synaptic cleft which occurs by the loss of basal forebrain cholinergic cells. Acetylcholinesterase is a hydrolase enzyme that hydrolyzes acetylcholine, which is the major constituent of central cholinergic pathways; hence impart an important role in the nervous system.

Restraining the acetylcholinesterase (AChE) enzyme is one of the approaches for the treatment of Alzheimer's disease which increases the synaptic levels of acetylcholine. For clinical treatment of AD galanthamine, donepezil, rivastigmine and tacrine are mainly used as AChE inhibitors (Fig. 1). [11-13].

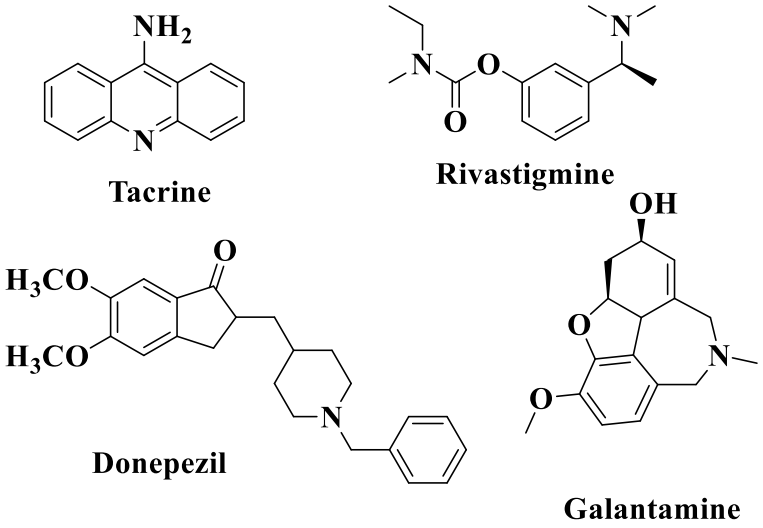

Figure 1: Structures of AChE inhibitors

The increasing mortality rate, complexities in disease and the limited option of drugs for the curing of AD, demands new and more effective pharmacological products. Keeping these factors in view researchers is aiming to develop new potential drugs. [14-16]

Substituted imidazole nucleus is well recognized for a wide range of therapeutic effects such as it is used as farnesyltransferase inhibitors [17], potent BRAF kinase inhibitor,[18] orally active 5-lipoxygenase (5-LO) inhibitors,[19] CSBP kinase inhibitors,[20], cytokine biosyhthesis inhibitor,[21] transforming growth factor b1 (TGF-b1) type 1 activating receptor-like kinase (ALK5).[22]

Synthesis of 2,4,5-trisubstituted imidazoles is usually carried out by cyclocondensation of three components i.e. an aldehyde and ammonium acetate with a 1,2-diketone, α-hydroxyketone or α-ketomonoxime. To obtain good yield reaction is generally catalyzed by the selection of different compounds such as silica gel, BF_3. SiO_2, molecular iodine, zirconium (IV) acetylacetonate $Zr(acac)_4$, silica sulfuric acid, scolecite, Ammonium Chloride, nanocrystalline magnesium chloride, $TiCl_4.SiO_2$, tetrabutylammonium hexatungstate $[TBA]_2[W_6O_{19}]$, ZnO nanorods, $Eu(OTf)_3$, silica chloride, silica bonded propyl-N-sulfamic acid nanocatalyst ($NHSO_3H$-KIT-5), $Ce(SO_4)_2$. $4H_2O$, keggin-structured phosphotungstic acid (HPW).[23-38]

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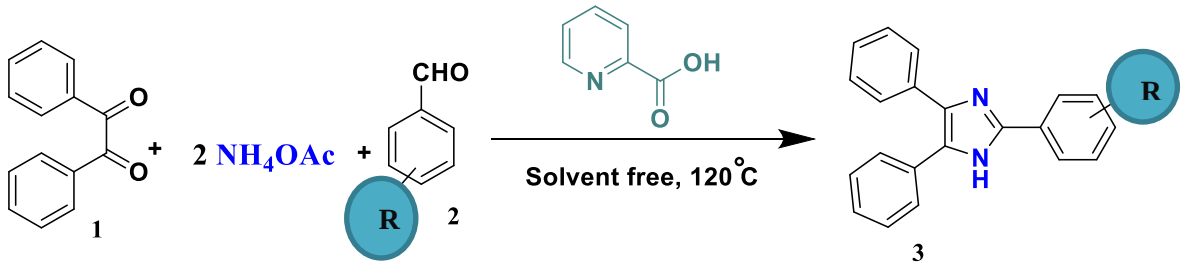


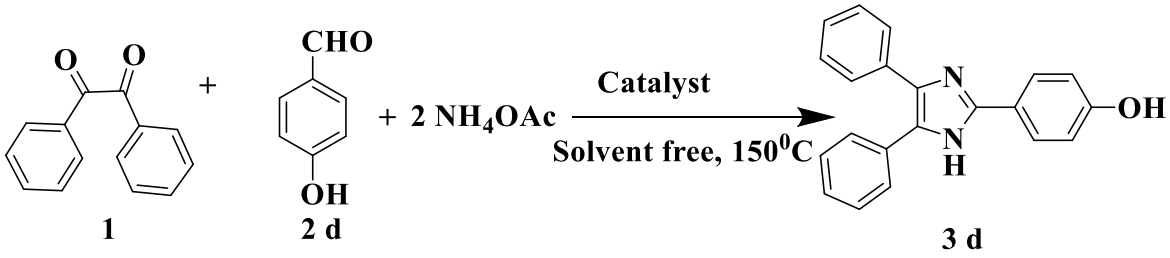
Scheme 1: Synthesis of 2,4,5-trisubstituted imidazoles.

All these new methodologies have their own advantages but most of these are associated with drawbacks like use of toxic solvents, prolong time, complicated work-up, purification difficulties, poor yield, expensive reagents, hence there is a dire need to develop such synthetic routes that are environmental friendly and economically. Various organocatalysts are being used now-a-days to accelerate chemical reaction with easy handling. [39] We herein report the organocatalyzed synthesis of 2,4,5-trisubstituted imidazole in solvent free system.

Result and discussion

2.1. Chemistry

For investigating the catalytic potential of various catalysts and for the choice of best catalyst model reactions were carried out. We have selected the reaction of Benzil **(1)** with 4-hydroxybenzaldehyde **(2 d)** and ammonium acetate for optimization of reactions. (Scheme 2).



Scheme 2: Model reaction for the optimization

Different catalysts were used to get comparative yields for model reaction and the obtained results are summarized in **table 1**.

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Table 1: Comparative study for the optimization of best catalyst (Scheme 2)

Entry	**Catalyst**	**Yield**	**Time of reaction** (hours)
1	Nicotinic acid	72%	2-3
2	Pyridine-2-carboxylic acid	78%	2-3
3	Pyridine-2,3-dicarboxylic acid	54%	4-5
4	Pyridine-2,4,6-tricarboxylic acid	43%	Overnight
5	Benzoic acid	67%	3-4
6	No catalyst	-	-

Among all tested catalysts for the synthesis of 4-(4,5-diphenyl-1H-imidazol-2-yl)phenol, pyridine-2-carboxylic acid has shown better catalytic ability resulting 78% yield in 2-3 hours (Table 1, entry 2) so, it is selected for carrying out a whole series of compounds. Further optimization of catalyst for minimum concentration and temperature was determined; results are shown in Table 2.

Table 2: Optimization of the reaction conditions with the selected catalyst (pyridine-2-carboxylic acid) (Scheme 2)

Entry	Catalyst (eq)	Time (hours)	Temperature (°C)	% yield
1	1.5	2-3	120	69
2	1.0	2-3	120	72
3	0.5	2-3	120	78
4	0.5	2-3	90	75
5	0.5	2-3	150	78

2,4,5-trisubstituted imidazoles **3a–3m** were synthesized by condensation of the aromatic aldehydes **,** benzil, and ammonium acetate in good to excellent yields (74 -96 %) as depicted in scheme 1. The condensation reaction was catalyzed by pyridine -2- carboxylic acid. Physical analysis and spectroscopic studies were applied for the conformation of synthesized compounds.

The plausible mechanism for the synthesis of 2,4,5-trisubstitued imidazoles is shown in Figure 2. Organo- catalyst pyridine-2-carboxylic acid's proton attacked on the carbonyl group of aldehyde and benzyl. Nitrogen of ammonia attach as nucleophile on the protonated carbonyl group of

aldehydes, diamine intermediate formed. Benzil and diamine intermediate condensed to cyclic intermediate state which rearranged by a [1, 5]-H shift to the trisubstituted imidazoles. [40-42]

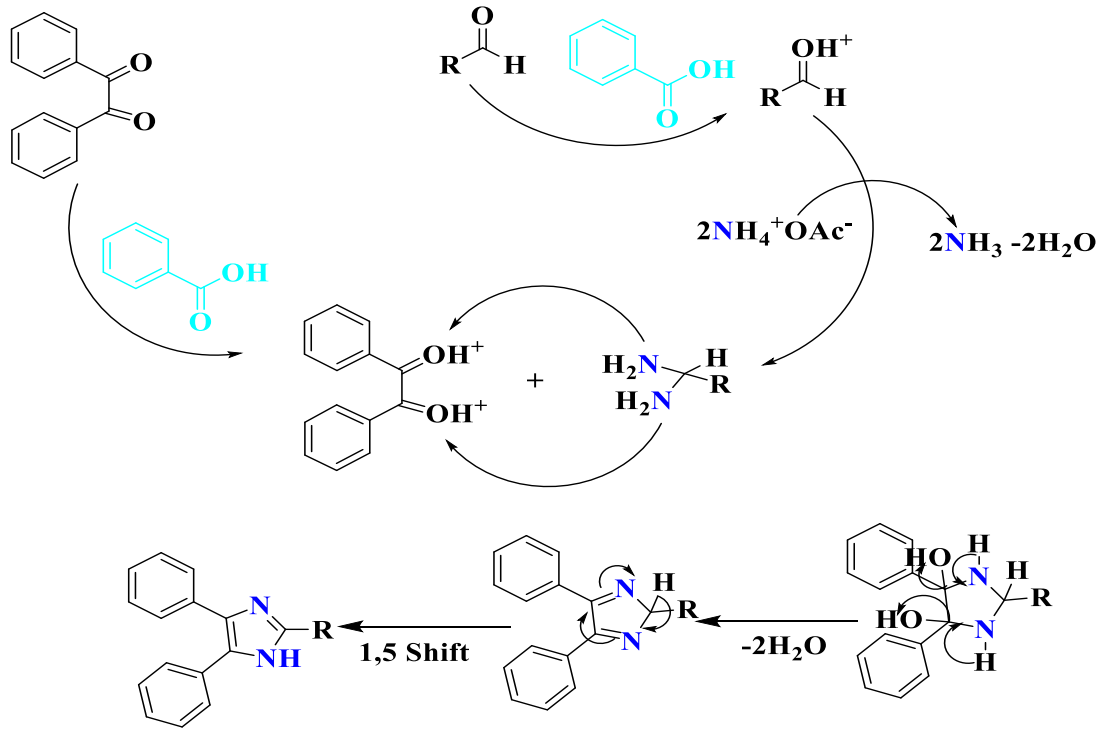

Figure 2: Proposed mechanism for the synthesis of 2,4,5-trisubstituted imidazoles by catalytic condensation using pyridine-2-carboxlic acid.

2.2. AChE inhibition activity

In vitro inhibition against AChE was performed for all synthesized 2,4,5-trisubstituted imidazoles **3a–3m.** Eserine was used as control in this assay. [43-45] The results were summarized in table 3 which illustrates that **3m**, **3k**, and **3e** are, the more potent AChE inhibitor as compared to the rest of synthesized compounds that showed moderate activity. Compound **3m** bearing one ethoxy and one hydroxyl group on phenyl moiety at position 2 of imidazole nucleus proved most active compound with IC_{50} value of 102.18±0.12 μM. When we replaced the group from ethoxy to methoxy on the phenyl moiety in compound **3k** there is a slight decrease in activity with IC_{50} value of 125.86±0.13 μM. There is slightly diminishing effect in activity when the phenyl moiety have only methoxy group in **3c** (128.22±0.17 μM). In compound **3e** hydroxyl group is at C-2 of phenyl moiety having IC_{50} value of 112.40±0.16 μM when hydroxyl group is at C-3 of compound **3a** it has the inhibitory value IC_{50} value of 158.14±0.19 while in compound **3d**

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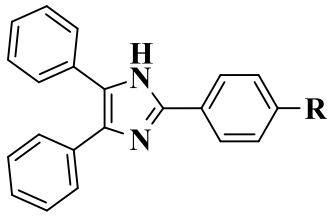
hydroxyl group is at C-4 of phenyl moiety having IC_{50} value of 172.16±0.14 μM. Change in the position of –OH group from carbon 2 to 4 of phenyl ring there is decreased in the AChE inhibitory activity. It can be depicted from the activity pattern that phenyl group bearing hydroxyl group along with ethoxy and methoxy groups are more effective AChE inhibitor as compared to alone hydroxyl group (compound **3m** vs. **3e)**. The unsubstituted phenyl ring **2i** proved inactive but when electron withdrawing group are attached either it act as moderate AChE inhibitor (**3j)** or proved in active (**3b**, **3g**, & **3l)**.

The overall order of reactivity is **3m**>**3e**>**3k**>**3c**>**3a**>**3d**>**3j**>**3f**

Table 3

Yield (%) and in vitro AChE inhibitory activity of 2,4,5-trisubstituted imidazoles **3a–3m** [(inhibition percentage and IC_{50} values are means given with SEM, (ND, not determined)].



(3a–3m)

Entry	compound	R	Yield (%)	% Inhibition	IC_{50} (μM)
1	**3a**	3-OH–C_6H_4	83	87.46±0.21	158.14±0.19
2	**3b**	2-Cl–C_6H_4	76	23.75±0.16	ND
3	**3c**	4-OCH_3–C_6H_4	74	86.25±0.23	128.22±0.17
4	**3d**	4-OH–C_6H_4	78	42.53±0.25	172.16±0.14
5	**3e**	2-OH–C_6H_4	79	87.15±0.28	112.40±0.16
6	**3f**	C_6H_4N	77	77.58±0.22	332.636±0.16
7	**3g**	4-Cl–C_6H_4	79	34.75±0.24	ND
8	**3h**	C_6H_5	81	23.24±0.23	ND
9	**3i**	4-$(CH_3)_2N$–C_6H_4	78	37.68±.025	ND
10	**3j**	4-Br–C_6H_4	96	81.27±0.19	243.55±0.16
11	**3k**	3-OCH_3-4-OH–C_6H_4	81	87.51±0.18	125.86±0.13
12	**3l**	4-F–C_6H_4	89	14.52±0.12	ND
13	**3m**	3-OC_2H_5-4-OH–C_6H_4	78	76.54±0.22	102.56±0.14
14	**Eserine**		-	91.27±1.17	0.04±0.0001

Molecular Docking Studies

Molecular dockings were carried out for the inhibitors **3m**, **3e**, **3k**, **3c**, **3a**, **3d**, **3j**, and **3f** in order to further investigate the binding mechanism (Fig. 3). The inhibitor molecule was molecularly docked with acetylcholinesterase to further study its binding mechanism. Obviously, the different groups on the inhibitor molecule are the main reasons leading to different docking results. Generally, the inhibitor molecule will be inserted into a residue pocket formed by THR121, PHE290, PHE331, PHE330, TYP84, TYR334, ASP72, TYR70, TRP270. The amino group of the inhibitor **3m** forms an H bond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 3.051 Å. The amino group of the inhibitor **3e** forms Hbond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 2.685 Å. The amino group of the inhibitor **3k** forms an H bond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 3.025 Å. The docking results of the inhibitor **3c** and acetylcholinesterase have two main conformations. One is that the inhibitor N atom forms a H bond with the oxygen atom of the residue (i.e., THR121), and the bond length is 2.970 Å. Second, the inhibitor N atom forms a H bond with the oxygen atom of the residue (ie, THR121), and the bond length is 3.197 Å. The amino group of the inhibitor **3a** forms H bond with the oxygen atom of the residue (ie SER122) with a bond length of 2.603Å; the hydroxyl group and the N atom of the residue ASP72, respectively. It forms a bond with the O atom of the residue ASN85, and the bond lengths are 2.479 Å and 1.718 Å, respectively. The amino group of the inhibitor **3d** forms a H bond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 2.896 Å. The amino group of the inhibitor **3j** forms a H bond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 3.783 Å. The amino group of the inhibitor **3f** forms a H bond with the oxygen atom of the residue (i.e., SER122) with a bond length of 2.450Å. The interaction between the inhibitor and the enzyme is enhanced by the interaction of the H bond, resulting in high binding affinity.

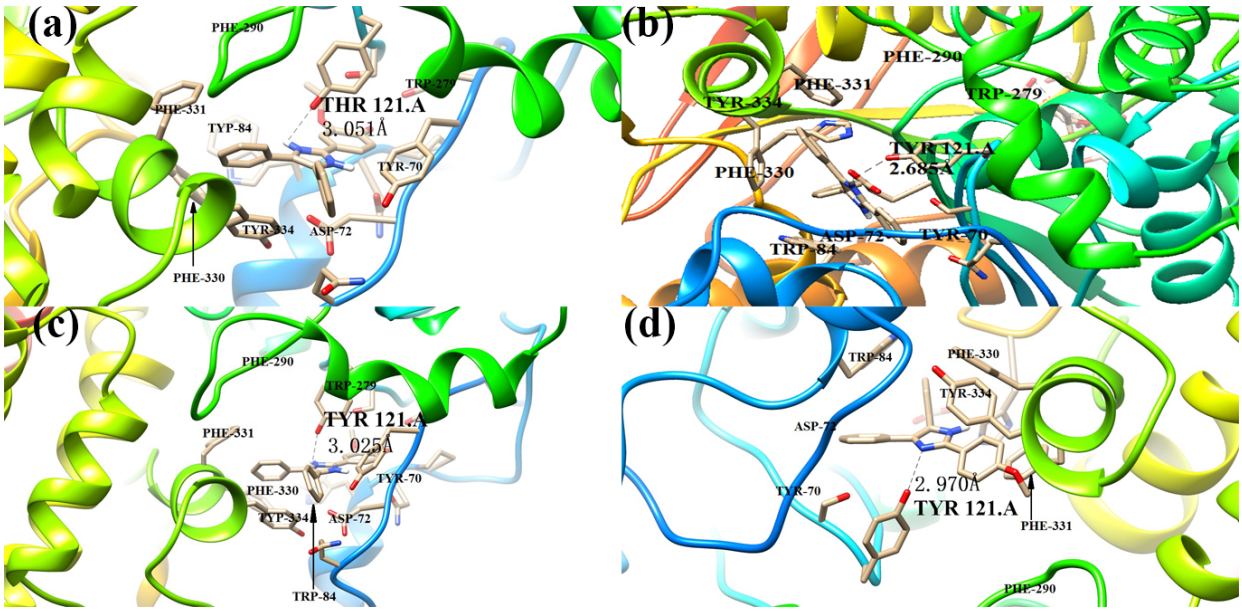


Figure 3: Illustrations of close contacts between the residues and inhibitors: (a) **3m**, (b) **3e**, (c) **3k**, (d) **3c**, surface representations are employed for the enzyme.

Conclusions

In summary, We report the new methodology for the synthesis of 2,4,5-trisubstituted imidazoles by utilizing the pyridine-2-carboxlic acid as an organocatalyst. The remarkable catalytic activity of pyridine-2-carboxlic acid exhibited one pot synthesis, short time of reaction, amount of catalyst, easy work up and pure products were obtained by simple crystallization. Products (**3a-3m**) were evaluated for in vitro AChE inhibitory activity. The substituents on triphenyl imidazole had a profound effect on the acetylcholinesterase inhibitory activity. Compound **3m** proves the most active compound with IC_{50} value of 102.18±0.12 μM and this could be a good drug candidate for the treatment of AD. Molecular dockings were carried out for the inhibitors **3m**, **3e**, **3k**, **3c**, **3a**, **3d**, **3j**, and **3f** in order to further investigate the binding mechanism. The inhibitor molecule was molecularly docked with acetylcholinesterase to further study its binding mechanism. The amino group of the inhibitor **3m** forms an H bond with the oxygen atom of the residue (i.e., THR121), and has a bond length of 3.051 Å.

Experimental

General methods

All chemicals and solvents used were of analytical grade and were purchased from Sigma, Aldrich and Merck Chemical Company and used without further purification. IR spectra in KBr

pellets were recorded on FT-IR Perkin Elmer spectrum BX spectrophotometer at the frequency range of 4000–400 cm^{-1}. Melting points were taken in open capillary tubes and are uncorrected. TLC was performed on silica coated aluminum sheets (silica gel, 0.2 mm, E. Merk, Germany) and visualized in low UV light. Elemental analysis was carried by CHNS analyzer (vail micro cube, Elementary Germony). ^{1}H NMR spectra were recorded in solvents such as DMSO-d6, $CDCl_3$ and MeOD. Splitting patterns were as follows s (singlet), d (doublet), dd (double doublets), t (triplet), and m (multiplet). Chemical shifts values are reported in δ-units and coupling constants are given in Hz.

General procedure for synthesis of 2,4,5-trisubstituted imidazoles (3a-3m)

For the synthesis of 2,4,5-trisubstituted imidazoles **(3a-3m)** benzil (1) (1eq), ammonium acetate (2 eq), corresponding aldehydes **(2a-2m)** (1 eq) and pyridine 2 carboxylic acid (0.5eq) were grounded in a mortar and pestle. The finely ground mixture was transferred into a round bottom flask and stirred for two to three hours at 120 °C. The reaction was observed by TLC. After the completion of reaction 5 % $NaHCO_3$ was added. Through solvent extraction, product was extracted with ethyl acetate which was dried over $MgSO_4$ and evaporated. Products were purified by recrystallization from methanol

3-(4,5-diphenyl-1*H*-imidazol-2-yl)phenol [46] (3a)

Shiny brown solid, yield: 83%; m.p: 249-251 °C; FT-IR (υmax, cm^{-1}): 3048 (N-H), 3088 (Ar CH), 2900 (Alkyl C-H), 1684 (C=N), 1652 (C=C); ^{1}H-NMR (300 MHz, DMSO-d6): δ 12.65 (s, NH ,1H), 9.56 (s, O-H, 1H), 9.05 (s, Ar-H, H-5, 1H), 8.75 (s, Ar-H, 1H), 8.2 (s, Ar-H, 1H), 7.7-7.2 (m, Ar-H, 10H), 6.7 (d, J=5.96, Ar-H, 1H). Anal. Calcd for $C_{21}H_{16}N_2O$: C, 80.75; H, 5.17; N, 8.97; O, 2.12. Found: C, 80.68; H, 5.62; N, 8.83.

2-(2-chlorophenyl)-4,5-diphenyl-1*H*-imidazole [47] (3b)

White solid, yield: 69%; m.p: 242-244 °C; FT-IR (υmax, cm^{-1}): 3057 (N-H), 3092 (Ar CH), 2951 (Alkyl C-H), 1640 (C=N), 1600 (C=C); ^{1}H-NMR (300 MHz, DMSO-*d6*): δ 9.05 (s, O-H, 1H), 8.75 (d, J=6.98, Ar-H, 1H), 8.25 (t, J=8.93, Ar-H, 1H), 7.7 (t, J=5.69, Ar-H, 1H) 7.6-7.0 (m, Ar-H, 10H). Anal. Calcd for $C_{21}H_{15}ClN_2$: C, 76.24; H, 4.57; N, 8.47; Cl, 10.72. Found: C, 76.53; H, 4.82; N, 8.75.

2-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazole [41] (3c)

White solid, yield: 74%; m.p: 230-232 °C; FT-IR (υmax, cm^{-1}): 3034 (N-H), 3066 (Ar CH), 2837 (Alkyl C-H), 1657(C=N), 1635 (C=C); ^{1}H-NMR (DMSO-d6, 300MHz): 8.0 (d, J= 4.9, Ar-H, 2H) 7.5-6.9 (m, Ar-H, 10H), 7.0 (d, J= 5.31, Ar-H, 2H) 3.75 (s, CH_3, 3H). Anal. Calcd for $C_{22}H_{18}N_2O$: C, 80.96; H, 5.56; N, 8.58; O, 4.90. Found: C, 80.82; H, 5.76; N, 8.69.

4-(4,5-diphenyl-1*H*-imidazol-2-yl)phenol [48] (3d)

White solid, yield: 78%; m.p: 239-241 °C; FT-IR (υmax, cm^{-1}): 3046 (N-H), 3071 (Ar CH), 2870 (Alkyl C-H), 1683 (C=N), 1625 (C=C); ^{1}H-NMR (300 MHz, DMSO-*d6*): δ 12.41 (s, N-H, 1H), 9.15 (s, O-H, 1H), 8.2 (s, H-1, 1H), 7.9 (s, Ar-H, 1H), 7.6-7.1 (m, Ar-H, 10H), 6.8 (s, Ar-H, 2H). Anal. Calcd for $C_{21}H_{16}N_2O$: C, 80.75; H, 5.17; N, 8.97; O, 2.12. Found: C, 80.93; H, 5.47; N, 8.76.

2-(4,5-diphenyl-1*H*-imidazol-2-yl)phenol [48] (3e)

White solid, yield: 71%; m.p: 205-207 °C; FT-IR (υmax, cm^{-1}): 3044 (N-H), 3120 (Ar CH), 2836 (Alkyl C-H), 1683(C=N), 1614 (C=C); ^{1}H-NMR (300 MHz, DMSO-*d6*): δ 12.5 (s, N-H, 1H), 8.0 (d, J = 6.21, Ar-H, 2H), 7.50-7.1 (m, Ar-H, 12H). Anal. Calcd for $C_{21}H_{16}N_2O$: C, 80.75; H, 5.17; N, 8.97; O, 2.12. Found: C, 80.58; H, 5.45; N, 8.78.

3-(4,5-diphenyl-1*H*-imidazol-2-yl)pyridine [49] (3f)

White solid, yield: 77%; m.p: 234-236 °C; FT-IR (υmax, cm^{-1}): 3245 (N-H), 3080 (Ar CH), 2910 (Alkyl C-H), 1698 (C=N), 1599 (C=C); 1H-NMR (300 MHz, DMSO-d6): δ 12.92 (s, N-H, 1H), 9.3 (s, Ar-H, H-1, 1H), 8.5(d, J = 8.74, Ar-H, 1H), 8.4 (d, J = 3.29, Ar-H, 1H), 7.6-7.2 (m, Ar-H, 11H). Anal. Calcd for $C_{20}H_{15}N_3$: C, 80.78; H, 5.08; N, 14.13. Found: C, 80.83; H, 5.25; N, 8.45.

2-(4-chlorophenyl)-4,5-diphenyl-1*H*-imidazole [41] (3g)

White solid, yield: 78%; m.p: 259-261 °C; FT-IR (υmax, cm^{-1}): 3038 (N-H), 3065 (Ar CH), 2825 (Alkyl C-H), 1640 (C=N), 1600 (C=C); ^{1}H-NMR (300 MHz, $CDCl_3$): δ 13.45 (s, N-H, 1H), 9.3 (s, Ar-H, 2H), 8.6 (s, Ar-H, 2H), 8.2-7.7 (m, Ar-H, 10H). Anal. Calcd for $C_{21}H_{15}ClN_2$: C, 76.24; H, 4.57; N, 8.47; Cl, 10.72. Found: C, 76.68; H, 4.78; N, 8.64.

2,4,5-triphenyl-1*H*-imidazole [41] (3h)

White solid, yield: 81%; m.p: 271-273 °C; FT-IR (υmax, cm^{-1}): 3036 (N-H), 3055 (Ar CH), 2850 (Alkyl C-H), 1680 (C=N), 1602 (C=C); ^{1}H-NMR (300 MHz, DMSO-*d6*): δ 12.6 (s, N-H, 1H), 8.1 (d, J = 4.75, Ar-H, 2H), 7.6-7.2 (m, Ar-H, 13H). Anal. Calcd for $C_{21}H_{16}N_2$: C, 85.11; H, 5.44; N, 9.45. Found: C, 85.38; H, 5.85; N, 9.78.

4-(4,5-diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline [41] (3i)

White solid, yield: 78%; m.p: 256-258 °C; FT-IR (υmax, cm^{-1}): 3048 (N-H), 3090 (Ar CH), 2938 (Alkyl C-H), 1653 (C=N), 1633 (C=C); ^{1}H-NMR ($CDCl_3$): δ 12.4 (s, N-H, 1H), 8.0 (d J = 5.31, Ar-H, 2H), 7.5-7.2 (m, Ar-H, 10H), 7.0 (d, J = 4.94, Ar-H, 2H), 3.8 (s, H-$(CH_3)_2$, 6H). Anal. Calcd for $C_{23}H_{21}N_3$: C, 81.38; H, 6.24; N, 12.38. Found: C, 81.45; H, 6.12; N, 12.43.

2-(4-bromophenyl)-4,5-diphenyl-1*H*-imidazole [41] (3j)

White solid, yield: 93%; m.p: 247-248 °C FT-IR (υmax, cm^{-1}): 3030 (N-H), 3078 (Ar CH), 2956 (Alkyl C-H), 1665 (C=N), 1620 (C=C); ^{1}H-NMR (300 MHz, DMSO-*d6*): δ 8.0 (d, J = 5.31, Ar-H, 2H), 7.6 (d, J = 5.13, Ar-H, 2H), 7.5-7.2 (m, Ar-H, 10H). Anal. Calcd for $C_{21}H_{15}BrN_2$: C, 67.28; H, 4.03; N, 7.47; Br, 21.29. Found: C, 67.31; H, 4.15; N, 7.38.

4-(4,5-diphenyl-1*H*-imidazol-2-yl)-2-methoxyphenol [33] (3k)

FT-IR (υmax, cm-1): 3031 (N-H), 3073 (Ar CH), 2895 (Alkyl C-H), 1698 (C=N), 1600 (C=C); ^{1}H-NMR (500 MHz, MeOD): δ 7.6 (d, J = 2.0, Ar-H, 1H), 7.4-7.2 (m, Ar-H, 11H), 6.8 (d, J = 8, Ar-H, 1H) 3.9 (s, CH_3, 3H). Anal. Calcd for $C_{22}H_{18}N_2O_2$: C, 77.17; H, 5.30; N, 8.81; O, 9.35. Found: C, 77.24; H, 5.38; N, 8.78.

2-(4-fluorophenyl)-4,5-diphenyl-1*H*-imidazole [50] (3l)

Pale white solid, yield: 89%; m.p: 191-193 °C FT-IR (υmax, cm^{-1}): 3029 (N-H), 3069 (Ar CH), 2908 (Alkyl C-H), 1683 (C=N), 1607 (C=C); ^{1}H-NMR (300 MHz, DMSO-*d6*): δ 12.6 (s, N-H, 1H), 8.1 (t, J = 8.6, Ar-H, 2H), 7.5-7.2 (m, Ar-H, 12H). Anal. Calcd for $C_{21}H_{15}FN_2$: C, 80.24; H, 4.81; N, 8.91; F, 6.04. Found: C, 80.35; H, 4.84; N, 8.84.

4-(4,5-diphenyl-1*H*-imidazol-2-yl)-2-ethoxyphenol [49] (3m)

White solid, yield: 78%; m.p: 255-257°C. FT-IR (υmax, cm-1): 3031 (N-H), 3061 (Ar CH), 2890 (Alkyl C-H), 1698 (C=N), 1600 (C=C); 1H-NMR(600 MHz DMSO): δ 12.5 (s, N-H,1H), 9.1 (s, O-H, 1H) , 7.1-7.6 (m, Ar-H, 12H), 6.9 (d, J = 7.9, Ar-H, 1H) 4.1 (q, J = 6.7, H-CH_2,

2H), 1.3 (t, J = 6.4, H-CH_3 3H,). Anal. Calcd for $C_{23}H_{20}N_2O_2$: C, 77.51; H, 5.66; N, 7.86; O, 8.98. Found: C, 77.42; H, 5.58; N, 8.93.

Acetylcholinesterase assay

Ellman's method with minor modifications was used for the determination of AChE inhibition activity.[51] Total volume of the reaction mixture was 100 µl, 60µl was buffer solution (Na_2HPO_4, 50 mM) for maintaining pH at 7.7. Test compound (10 µl, 0.5 mM well) was added, followed by the addition of 10 µl (0.005 unit well) enzyme. The reactants were mixed and pre-read at 405 nm. At 37 °C contents were pre-incubated for 10 min. The reaction was initiated by the addition of 10 µl of substrate (acetylthiocholine iodide, 0.5 mM), then 10 µl DTNB (0.5 mM). Absorbance was measured at 405 nm after 30 minutes of incubation at 37 °C. In all experiments synergy HT (BioTek, USA) 96-well plate reader was used. As a positive control eserine (0.5 mM) was used. Following equation was used for the calculation of percent AChE inhibition activity.

$$\text{Inhibition (\%)} = \frac{\text{(absorbance of control- absorbance of Test)}}{\text{absorbance of Control} * 100}$$

EZ–Fit Enzyme kinetics software (Perella Scientific Inc. Amherst, USA) was used for estimation of IC_{50} values of synthesized compounds.

Molecula Docking

The structure of acetylcholinesterase was obtained from the protein data bank (PDB code: 1EVE). The structures of inhibitors were optimized at B3LYP/6-31G level using Gaussian 09. Docking studies were performed for four inhibitors using Autodock package. We used Chimera software to determine the hydrogen bonds. The angstroms and degrees were set to 1.0 Å and 90 Å, respectively, except for the seventh (the angstroms and degrees were set to 2.0 Å and 90 Å), in order to identify the H bonds.

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Supplementary Data

Supplementary data related to this article can be found in supporting file.

Author Contribution Statement

Sania Pervaiz, Islam Ullah Khan and Sadaf Mutahir designed and planned the experiments. Sania Pervaiz and Sidrah Tariq synthesized the compounds. Muhammad Ashraf evaluated all synthesized compounds for AChE inhibition. Sania Pervaiz wrote the manuscript. Bao-jing Zhou and Xiao Liu performed molecular docking. Sadaf Mutahir and Muhammad Asim Khan wrote molecular docking report, and provided guidance in writing manuscript. Islam Ullah Khan supervised the whole project.

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