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Biological docking and BSA binding studies of 1,4-disubstituted piperdine containing 1,2,4-triazoles: Comparative synthesis leveraging microwave assisted and conventional protocols

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Abstract: A biologically effective study regarding the synthesis of a library of hybrids based on 1,2,4-triazole, propanamides and piperidine was performed. The targeted hybrids, **9a-9l**, were synthesized through multistep protocol followed by a comparison of conventional and microwave assisted methodologies. Initially the carboxylate **3** was synthesized by the room temperature stirring of 4-methoxybenzenesulfonyl chloride (**1**) and ethyl isonipecotate (**2**). Carboxylate was converted into carbohydrazide **4**, which was refluxed with phenyl isothiocyanate and KOH to synthesize 1,2,4-triazole (**5**). A series of propanamides **8a-8l** were stirred at room temperature with compound **5** to avail the targeted library of hybrids **9a-9l**. All the designed hybrids were screened for antioxidant, urease, acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) inhibition potential. All the compounds were found active with variable potential. The best antioxidant agent was compound **9c** with IC_{50} value 45.2±0.15, AChE inhibitor **9e** (63.27±1.21), anti-urease agents were **9g** (20.2±0.21) and **9k (**19.2±0.09) and BChE inhibitors were **9d** (15.5±0.39) and **9e (**15.9±0.67). The computational and BSA binding studies of selected synthesized compounds against urease, BChE, AChE enzymes were carried out to elaborate the strong and weak enzymes inhibition potential through binding forces of synthesized compounds with the active sites of enzymes.

Keywords: 1,2,4-Triazole; Piperidine; Enzyme inhibition; Molecular docking; BSA binding

1. Introduction

Microwave assisted synthesis of heterocyclic compounds has become very effective. It has been frequently utilized by synthetic chemists around the world because of its efficiency in term of large scale reactions in minimum time, environment friendly behavior and high yield [1]. During the last few years, microwave assisted synthesis has been utilized at great extent on the basis of its improved yield, region-selectivity and chemo-selectivity [2,3].

Nitrogenous heterocyclic systems based on privileged patterns drawn from azinane and 1,2,4-triazole possessed extreme level of biological applications like natural products. These molecules could be the step toward the drug discovery route [4]. Triazoles and their derived compounds become very attractive and attained the attention of the world because their important usage in biology, agriculture, material science and in

pharmaceuticals [5]. The application of triazole based compounds in the different fields could be justified by the high thermal stability, heteroatomic character, hydrogen bonding and dipole moment properties [6,7].

Azole based compounds especially triazoles are the part of broad range of drugs utilized to cure different diseases. Triazoles are the part of Anastrozole, Letrozole and Vorozole as anticancer agents; Voriconazole as an antifungal agent; and Fluconazole and Itraconazole as antimycotic agents [8,9]. Another part of the main core, the piperidine ring of the designed drugs possesses a wide range of biological applications like anticancer, antimicrobial, antimycobacterial and antihypertensive ones [10,11].

Prompted by our previous designed projects, observations and literature survey, we have designed an array of compound having 1,2,4-triazole, piperidine and various substituted aromatic functionalities submerged in a single unit to enhance their biological potential. Likewise the microwave assisted versus conventional technique was employed to synthesize the designed products. It was observed that microwave assisted method is better and efficient comparative to the conventional one with respect to time and yield of the synthesized compounds.

2. Experimental

The services of local supplier were availed to access all the chemicals utilized during the current research. Chromatographic techniques were employed to judge the progress of reaction while the spectroscopic techniques were utilized to evaluate and confirm the structures of all the synthesized compounds.

2.1. Synthesis of ethyl 1-[(4-methoxyphenyl)sulfonyl]-4-piperidincarboxylate (3)

Compound **3** was synthesized by stirring 4-methoxybenzenesulfonyl chloride (**1**; 0.04 mol) with ethyl isonipecotate (**2**; 0.04 mol) for 14 hours. During the progress of reaction 10 % Na_2CO_3 solution was added in interval in-order to maintain the pH at 10. Reaction completion was confirmed by TLC and observed under UV lamp. By the addition of chilled distilled water, the precipitates of target compound **3** were availed.

2.2. Synthesis of 1-(4-methoxyphenylsulfonyl)piperidin-4-carbohydrazide (4)

Corresponding carbohydrazide **4** of compound **3** was synthesized by reflux reaction with hydrazine hydrate for 5 hours. Thin layer chromatography was used to judge the progress of reaction. At completion of the reaction, excess solvent was evaporated and crystals of the pure target compound **4** were obtained.

2.3. Synthesis of 5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazole-3-thiol (5)

The equimolar quantities (0.04 mol) of compound **4** and phenyl isothiocyanate were mixed in the presence of ethanol used as solvent and refluxed for 1 hour. The uncyclized product was obtained that was cyclized by reflux reaction in the presence of equimolar KOH. The reaction completion was checked by TLC. On the completion of reaction the dil. HCl was added to adjust the pH at 4-5 with continuous stirring. The obtained product was washed and dried at room temperature.

2.4. Synthesis of N-(substituted)-2-bromopropanamides (8a-8l)

Aralkyl/aryl amines (**7a-7l**; 0.02 mol) were reacted with equimolar quantity of 2-bromopropionyl bromide (**6**) in the presence of distilled water. By the addition of 10 % Na_2CO_3 solution, the pH was maintained at 9-10. Propanamides were obtained in the precipitate form and after washing were available for further utilization.

2.5. General procedure for the synthesis of N-(substituted)-2-[(5-{1-[(4-methoxyphenyl) sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl] propanamide (9a-9l)

Conventional synthesis: Compound **5** (0.0005 mol) was stirred in the presence of LiH for 15 minutes. Equimolar quantities of array of synthesized propanamides (**8a-8l**) were added in the presence of DMF and stirred at room temperature for 8-16 hours. The reaction progress was monitored through TLC. On completion of reaction, desired compounds were precipitated by adding chilled water, filtered, washed, and dried for analysis and further applications.

Microwave assisted synthesis: Compound **5** (0.0005 mol) was stirred in the presence of LiH for 15 minutes. Equimolar quantities of array of propanamides (**8a-8l**) were added in the presence of DMF and preceded in microwave for 30-60 seconds. The reaction progress was monitored through TLC. On completion of reaction, desired compounds were precipitated by adding chilled water, filtered, washed, and dried for analysis and further applications.

2.5.1. N-(2,5-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9a)

Light pink amorphous solid; yield: 88 %; M.P: 203.5 °C; Molecular formula: $C_{31}H_{35}N_5O_4S_2$; Molecular mass: 605.77 g/mol; IR (KBr, wave number, cm^{-1}) 2800 (Ar C-H), 1680 (C=O), 1600 (C=N), 1500 (Ar C=C), 1350 (CH_3), 1300 (S=O), 1240 (C-O-C), 710 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.51 (s, 1H, NH), 7.88 (s, 1H, H-6''''), 7.69 (d, *J* = 8.2 Hz, 2H, H-2', H-6'), 7.58-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.21 (d, *J* = 6.9 Hz, 2H, H-2'', H-6''), 7.06 (d, *J* = 7.7 Hz, 1H, H-3''''), 6.99 (d, *J* = 8.1 Hz, 2H, H-3', H-5'), 6.88 (d, J = 7.3 Hz, 1H, H-4'''') 4.66 (q, *J* = 7.3 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.76-3.65 (m, 2H, H_e-2, H_e-6), 2.53-2.51 (m, 1H, H-4), 2.40-2.36 (m, 2H, H_a-2, H_a-6), 2.32 (s, 3H, H-4'''), 2.26 (s, 3H, H-5'''), 2.07-1.81 (m, 4H, H_e-3, H_e-5, H_a-3, H_a-5), 1.61 (d, *J* = 7.8 Hz, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 168.00 (C-4'), 162.98 (C-5''''), 157.74 (C-3''''), 153.12 (C-6''''), 136.18 (C-1''''), 132.42 (C-1''), 130.68 (C-6''''), 130.38 (C-3'', C-5''), 131.20 (C-2''''), 126.74 (C-2' , C-6'), 129.01 (C-1'), 127.76 (C-5''''), 126.99 (C-2'', C-6''), 125.73 (C-4''), 125.28 (C-3''''), 122.63 (C-4''''), 114.20 (C-3',C-5'), 55.59 (C-1'''), 45.30 (C-2, C-6), 42.85 (C-2'''), 31.75 (C-4), 29.46 (C-3, C-5), 21.16 (C-4'''), 17.74 (C-5'''), 16.31 (C-3''').

2.5.2. N-(2,6-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9b)

Off white amorphous solid; yield: 87 %; M.P: 176.5 °C; Molecular formula: $C_{31}H_{35}N_5O_4S_2$; Molecular mass: 605.77 g/mol; IR (KBr, wave number, cm^{-1}) 2830 (Ar C-H), 1700 (C=O), 1620 (C=N), 1500 (Ar C=C), 1350 (CH_3), 1280 (S=O), 1140 (C-O-C), 730 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.31 (s, 1H, NH), 7.68 (d, *J* = 7.8 Hz, 2H, H-2', H-6'), 7.58-7.56 (m, 3H, H-3'', H-4'', H-5''), 7.20-7.19 (m, 2H, H-2'', H-6''), 7.10-7.04 (m, 3H, H-3'''', H-4'''', H-5''''), 6.98 (d, *J* = 7.8 Hz, 2H, H-3', H-5'), 4.68 (q, *J* = 7.3 Hz, 1H, H- 2'''), 3.86 (s, 3H, H-1'''), 3.74-3.68 (m, 2H, H_e-2, H_e-6), 2.56-2.52 (m, 1H, H-4), 2.41-2.36 (m, 2H, H_a-2, H_a-6), 2.173 (s, 6H, H-4''', H-5'''), 2.04-1.95 (m, 2H, H_e-3, H_e-5), 1.90-1.83 (m, 2H, H_a-3, H_a-5), 1.61 (d, *J* = 8.5 Hz, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 169.55 (C-4'), 162.99 (C-5''''), 157.67 (C-3''''), 152.72 (C-6''''), 135.29 (C-1''''), 133.95 (C-1''), 132.48 (C-1'), 130.74 (C-2'''', C-6''''), 130.74 (C-3'', C-5''), 129.75 (C-2', C-6'), 128.05 (C-3'''', C-5''''), 127.80 (C-4''), 127.07 (C-4''''), 126.97 (C-2'', C-6''), 114.21 (C-3', C-5'), 55.59 (C-1'''), 45.37 (C-2, C-6), 42.49 (C-2'''), 31.83 (C-4), 29.37 (C-3, C-5), 18.11 (C-3''', C-4'''), 16.54 (C-3''').

2.5.3. N-(3,5-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9c)

White amorphous solid; yield: 85 %; M.P: 98.0 °C; Molecular formula: $C_{31}H_{35}N_5O_4S_2$; Molecular mass: 605.77 g/mol; IR (KBr, wave number, cm^{-1}) 2840 (Ar C-H), 1700 (C=O), 1550 (C=N), 1500 (Ar C=C), 1320 (CH_3), 1250 (S=O), 1150 (C-O-C), 720 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.93 (s, 1H, NH), 7.69 (d, *J* = 7.6 Hz, 2H, H-2', H-6'), 7.57-7.54 (m, 5H, H-3'', H-4'', H-5'', H-2'''', H-6''''), 7.21 (d, *J* = 7.1 Hz, 2H, H-2'', H-6''), 6.99 (d, *J* = 7.9

Hz, 2H, H-3', H-5'), 6.75 (s, 1H, H-4''''), 4.51 (q, *J* = 7.0 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.80-3.75 (m, 2H, H_e-2, H_e-6), 2.52-2.48 (m, 1H, H-4), 2.31 (s, 6H, H-4''', H-5'''), 2.29-2.27 (m, 2H, H_a-2, H_a-6), 2.08-1.99 (m, 2H, H_e-3, H_e-5), 1.91-1.82 (m, 2H, H_a-3, H_a-5), 1.57 (d, *J* = 7.2 Hz, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm): 169.09 (C-4'), 162.99 (C-5''''), 157.64 (C-3''''), 152.84 (C-6''''), 138.60 (C-1''''), 138.19 (C-1''), 132.46 (C-1'), 130.69 (C-2'''', C-6''''), 130.37 (C-3'', C-5''), 129.77 (C-2', C-6'), 127.80 (C-3'''', C-5''''), 127.00 (C-4''), 125.77 (C-4''''), 117.40 (C-2'', C-6''), 114.20 (C-3', C-5'), 55.59 (C-1'''), 45.55 (C-2, C-6), 43.32 (C-2'''), 32.10 (C-4), 29.50 (C-3, C-5), 21.36 (C-4''', C-5'''), 16.24 (C-3''').

2.5.4. 2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-phenylpropanamide (9d)

White amorphous solid; yield: 89 %; M.P: 123.0 °C; Molecular formula: $C_{29}H_{21}N_5O_4S_2$; Molecular mass: 577.71 g/mol; IR (KBr, wave number, cm^{-1}) 2800 (Ar C-H), 1700 (C=O), 1630 (C=N), 1500 (Ar C=C), 1310 (CH_3), 1250 (S=O), 1130 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.19 (s, 1H, NH), 7.69 (d, *J* = 7.3 Hz, 2H, H-2', H-6'), 7.65 (d, *J* = 7.8 Hz, 2H, H-2'''', H-6''''), 7.58-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.33 (t, *J* = 7.2 Hz, 2H, H-3'''', H-5''''), 7.21 (d, *J* = 7.1 Hz, 2H, H-2'', H-6''), 7.11 (t, *J* = 7.4 Hz, H-4''''), 6.99 (d, *J* = 7.3 Hz, 2H, H-3', H-5'), 4.51 (q, *J* = 7.2 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.79-3.73 (m, 2H, H_e-2, H_e-6), 2.53-2.49 (m, 1H, H-4), 2.35-2.29 (m, 2H, H_a-2, H_a-6), 2.08-1.97 (m, 2H, H_e-3, H_e-5), 1.91-1.82 (m, 2H, H_a-3, H_a-5), 1.58 (d, *J* = 7.1 Hz, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 169.13 (C-4'), 163.00 (C-5''''), 157.68 (C-3''''), 152.85 (C-6''''), 138.43 (C-1''''), 132.43 (C-1''), 130.72 (C-1'), 130.38 (C-3'', C-5''), 129.78 (C-2', C-6'), 128.88 (C-2'''' ,C-6''''), 127.80 (C-4''), 127.00 (C-4''''), 123.98 (C-3'''', C-5''''), 119.65 (C-2'', C-6''), 114.21 (C-3', C-5'), 55.60 (C-1'''), 45.51 (C-2, C-6), 43.32 (C-2'''), 32.02 (C-4), 29.45 (C-3, C-5), 16.21 (C-3''').

2.5.5. 2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(2-methylphenyl)propanamide (9e)

Off white amorphous solid; yield: 85 %; M.P: 172.8 °C; Molecular formula: $C_{30}H_{33}N_5O_4S_2$; Molecular mass: 591.74 g/mol; IR (KBr, wave number, cm^{-1}) 2830 (Ar C-H), 1700 (C=O), 1630 (C=N), 1500 (Ar C=C), 1325 (CH_3), 1220 (S=O), 1110 (C-O-C), 740 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.59 (s, 1H, NH), 8.04 (d, *J* = 7.9 Hz, 2H, H-6''''), 7.69 (d, *J* = 7.4 Hz, 2H, H-2', H-6'), 7.59-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.21-7.18 (m, 4H, H-2'', H-6'', H-3'''', H-5''''), 6.99 (d, *J* = 7.4 Hz, 2H, H-3', H-5'), 4.66 (q, *J* = 7.2 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.76-3.65 (m, 2H, H_e-2, H_e-6), 2.55-2.51 (m, 1H, H-4), 2.41-2.34 (m, 2H, H_a-2, H_a-6), 2.31 (s, 3H, H-4'''), 2.08-2.04 (m, 2H, H_e-3, H_e-5), 1.95-1.88 (m, 2H, H_a-3, H_a-5), 1.57 (d, *J* = 7.1 Hz, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 169.46 (C-4'), 162.98 (C-5''''), 157.77 (C-3''''), 152.72 (C-6''''), 136.45 (C-1''''), 132.41 (C-1''), 130.70 (C-1'), 130.43 (C-6''''), 130.39 (C-3'', C-5''), 126.77 (C-2', C-6'), 128.87 (C-2''''), 128.80 (C-4''), 126.98 (C-5''''), 126.46 (C-3''''), 124.53 (C-4''''), 122.02 (C-2'', C-6''), 114.20 (C-3', C-5'), 55.59 (C-1'''), 45.41 (C-2, C-6), 42.88 (C-2'''), 31.76 (C-4), 29.46 (C-3, C-5), 18.22 ((C-4'''), 21.47 (C-3''').

2.5.6. 2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(3-methylphenyl)propanamide (9f)

Off white amorphous solid; yield: 85 %; M.P: 97.5 °C; Molecular formula: $C_{30}H_{33}N_5O_4S_2$; Molecular mass: 591.74 g/mol; IR (KBr, wave number, cm^{-1}) 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1320 (CH_3), 1250 (S=O), 1100 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.06 (s, 1H, NH), 7.69 (d, *J* = 7.2 Hz, 2H, H-2', H-6'), 7.58-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.50 (s, 1H, H-2''''), 7.43 (d, *J* = 8.0 Hz, H-6''''), 7.22-7.20 (m, 3H, H-2'', H-6'', H-5''''), 6.99 (d, *J* = 7.4 Hz, 2H, H-3', H-5'), 6.93 (d, *J* = 7.4 Hz, 1H, H-4''''), 4.51 (q, *J* = 7.2 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.79-3.74 (m, 2H, H_e-2, H_e- 6), 2.52-2.48 (m, 1H, H-4), 2.36 (s, 3H, H-4'''), 2.35-2.28 (m, 2H, H_a-2, H_a-6), 2.08-2.01 (m, 2H, H_e-3, H_e-5), 1.91-1.82 (m, 2H, H_a-3, H_a-5), 1.57 (d, *J* = 7.2 Hz, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 169.11 (C-4'), 162.99 (C-5''''), 157.66 (C-3''''), 152.84 (C-6''''), 138.81 (C-1''''),

138.31 (C-1''), 132.45 (C-1'), 130.70 (C-6''''), 130.37 (C-3'', C-5''), 129.77 (C-2', C-6'), 129.44 (C-2''''), 128.71 (C-2'', C-6''), 127.80 (C-3''''), 126.99 (C-4''), 124.81 (C-5''''), 116.77 (C-4''''), 114.20 (C-3', C-5'), 55.60 (C-1'''), 45.54 (C-2, C-6), 43.32 (C-2'''), 32.06 (C-4), 29.47 (C-3, C-5), 21.48 (C-4'''), 16.22 (C-3''').

2.5.7. N-(2-Ethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9g)

White amorphous solid; yield: 89 %; M.P: 175.6 °C; Molecular formula: $C_{31}H_{37}N_5O_4S_2$; Molecular mass: 607.78 g/mol; IR (KBr, wave number, cm^{-1}) 2830 (Ar C-H), 1690 (C=O), 1610 (C=N), 1550 (Ar C=C), 1325 (CH_3), 1250 (S=O), 1140 (C-O-C), 700 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.53 (s, 1H, NH), 7.93 (d, J = 7.9 Hz, 1H, H-6''''), 7.68 (d, J = 7.2 Hz, 2H, H-2', H-6'), 7.58-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.21-7.19 (m, 4H, H-2'', H-6'', H-3'''', H-5''''), 7.11 (t, J = 7.4 Hz, 1H, H-4''''), 6.98 (d, J = 7.3 Hz, 2H, H-3', H-5'), 4.66 (q, J = 7.2 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.76-3.66 (m, 2H, H_e-2, H_e-6), 2.65-2.60 (m, 2H, H-4'''), 2.56-2.52 (m, 1H, H-4), 2.40-2.35 (m, 2H, H_a-2, H_a-6), 2.08-2.04 (m, 2H, H_e-3, H_e-5), 1.96-1.89 (m, 2H, H_a-3, H_a-5), 1.56 (d, J = 7.7 Hz, 3H, H-3'''), 1.14 (t, J = 7.5 Hz, 3H, H-5''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.66 (C-4'), 162.99 (C-5''''), 157.76 (C-3''''), 152.80 (C-6''''), 135.51 (C-1''''), 135.22 (C-1''), 132.44 (C-1'), 130.71 (C-6''''), 130.41 (C-3'', C-5''), 129.76 (C-2', C-6'), 128.68 (C-4''), 127.81 (C-2''''), 126.95 (C-2'', C-6''), 126.35 (C-5''''), 125.07 (C-3''''), 123.18 (C-4''''), 114.21 (C-3', C-5'), 55.59 (C-1'''), 45.40 (C-2, C-6), 42.78 (C-2'''), 31.76 (C-4), 29.48 (C-3, C-5), 24.55 (C-3'''), 16.41 (C-5'''), 14.20 (C-4''').

2.5.8. N-(4-Ethoxyphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9h)

Light brown amorphous solid; yield: 87 %; M.P: 166.0 °C; Molecular formula: $C_{31}H_{35}N_5O_5S_2$; Molecular mass: 621.77 g/mol; IR (KBr, wave number, cm^{-1}) 2830 (Ar C-H), 1700 (C=O), 1610 (C=N), 1500 (Ar C=C), 1310 (CH_3), 1230 (S=O), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.99 (s, 1H, NH), 7.69 (d, J = 7.5 Hz, 2H, H-2', H-6'), 7.59-7.53 (m, 5H, H-3'', H-4'', H-5'', H-2'''', H-6''''), 7.21 (d, J = 7.8 Hz, 2H, H-2'', H-6''), 6.99 (d, J = 7.6 Hz, 2H, H-3', H-5'), 6.86 (d, J = 7.6 Hz, 2H, H-3'''', H-5''''), 4.49 (q, J = 7.2 Hz, 1H, H-2'''), 4.03 (q, J = 6.9 Hz, 2H, H-4'''), 3.88 (s, 3H, H-1'''), 3.78-3.73 (m, 2H, H_e -2, H_e-6), 2.52-2.48 (m, 1H, H-4), 2.35-2.28 (m, 2H, H_a-2, H_a-6), 2.07-1.98 (m, 2H, H_e-3, H_e-5), 1.90-1.82 (m, 2H, H_a-3, H_a- 5), 1.57 (d, J = 7.2 Hz, 3H, H-3'''), 1.41 (t, J = 6.9 Hz, 3H, H-5'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 168.78 (C-4'), 162.99 (C-5''''), 157.64 (C-3''''), 155.49 (C-4''''), 152.85 (C-6''''), 132.46 (C-1''''), 131.57 (C-1''), 130.69 (C-1'), 130.36 (C-3'', C-5''), 129.77 (C-2', C-6'), 127.79 (C-4''), 127.00 (C-2'''', C-6''''), 121.12 (C-2'', C-6''), 114.70 (C-3'''', C-5''''), 114.21 (C-3', C-5'), 63.71 (C-4'''), 55.60 (C-1'''), 45.52 (C-2, C-6), 43.33 (C-2'''), 32.02 (C-4), 29.45 (C-3, C-5), 16.31 (C-3'''), 14.85 (C-5''').

2.5.9. N-(2-Ethyl-6-methylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9i)

White amorphous solid; yield: 90 %; M.P: 162.0 °C; Molecular formula: $C_{32}H_{37}N_5O_4S_2$; Molecular mass: 619.79 g/mol; IR (KBr, wave number, cm^{-1}) 2840 (Ar C-H), 1670 (C=O), 1610 (C=N), 1500 (Ar C=C), 1340 (CH_3), 1250 (S=O), 1140 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.14 (s, 1H, NH), 7.68 (d, J = 7.6 Hz, 2H, H-2', H-6'), 7.59-7.57 (m, 3H, H-3'', H-4'', H-5''), 7.21 (d, J = 6.0 Hz, 2H, H-2'', H-6''), 7.14 (t, J = 7.6 Hz,1H, H-4''''), 7.08-7.07 (m, 2H, H-3'''', H-6''''), 6.98 (d, J = 7.6 Hz, 2H, H-3', H-5'), 4.69 (q, J = 7.2 Hz, 1H, H-2'''), 3.86 (s, 3H, H-1'''), 3.74-3.68 (m, 2H, H_e-2, H_e-6), 2.57-2.47 (m, 3H, H-4, H_a-2, H_a-6), 2.38 (q, J = 10.9 Hz, 2H, H-4'''), 2.14 (s, 3H, H-6'''), 2.04-1.82 (m, 4H, H_e-3, H_e-5, H_a-3, H_a-5),1.62 (3H, H-3 merged with HDO'''), 1.08 (t, J = 7.2 Hz, 3H, H-5'''); ^{13}C-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 169.80 (C-4'), 162.99 (C-5''''), 157.64 (C-3''''), 152.75 (C-6''''), 141.10 (C-1''''), 135.79 (C-1''), 133.30 (C-1'), 132.49 (C-6''''), 130.74 (C-4''), 130.46 (C-3'', C-5''), 129.75 (C-2', C-6'), 128.11 (C-5''''), 127.81 (C-2''''), 127.41 (C-3''''), 126.93 (C-2'', C-6''), 126.30 (C-4''''),

114.21 (C-3', C-5'), 55.58 (C-1'''), 45.36 (C-2, C-6), 42.34 (C-2'''), 31.82 (C-4), 29.37 (C-3, C-5), 24.82 (C-4'''), 18.18 (C-6'''), 16.54 (C-3'''), 14.64 (C-5''').

2.5.10. N-Cyclohexyl-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9j)

Off white amorphous solid; yield: 85 %; M.P: 135.0 °C; Molecular formula: $C_{29}H_{37}N_5O_4S_2$; Molecular mass: 583.76 g/mol; IR (KBr, wave number, cm^{-1}) 2820 (Ar C-H), 1700 (C=O), 1620 (C=N), 1500 (Ar C=C), 1310 (CH_3), 1250 (S=O), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 7.69 (d, *J* = 7.7 Hz, 2H, H-2', H-6'), 7.56-7.53 (m, 3H, H-3'', H-4'', H-5''), 7.19 (d, *J* = 7.1 Hz, 2H, H-2'', H-6''), 6.99 (d, *J* = 7.6 Hz, 2H, H-3', H-5'), 4.30 (q, *J* = 7.2 Hz, 1H, H-2'''), 3.88 (s, 3H, H-1'''), 3.77-3.71 (m, 2H, H_e-2, H_e-6), 2.55-2.47 (m, 1H, H-4) 2.37-2.30 (m, 2H, H_a-2, H_a-6), 1.89-1.84 (m, 4H, H_e-3, H_e-5, H_a-3, H_a-5),1.72-1.70 (m,1H, H-1''''), 1.49 (d, *J* = 7.2 Hz, 3H, H-3'''), 1.36-1.17 (m, 10H, H-2'''', H-3'''', H-4'''', H-5'''', H-6''''); ^{13}C-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 170.12 (C-4'), 163.00 (C-5''''), 157.46 (C-3''''), 152.16 (C-6''''), 133.51 (C-1''), 130.54 (C-1'), 130.28 (C-3'', C-5''), 129.76 (C-2', C-6'), 127.08 (C-4''), 124.98 (C-2'', C-6''), 114.20 (C-3', C-5'), 55.59 (C-1'''), 48.00 (C-1''''), 45.49 (C-2, C-6), 43.34 (C-2'''), 32.60 (C-4), 31.99 (C-2'''', C-6''''), 29.52 (C-3, C-5), 25.53 (C-3'''', C-5''''), 24.45 (C-4''''), 16.67 (C-3''').

2.5.11. Methyl 2-({2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanoyl}amino)benzoate (9k)

Off white amorphous solid; yield: 85 %; M.P: 130.0 °C; Molecular formula: $C_{32}H_{35}N_5O_6S_2$; Molecular mass: 649.78 g/mol; IR (KBr, wave number, cm^{-1}) 2810 (Ar C-H), 1680 (C=O), 1600 (C=N), 1510 (Ar C=C), 1300 (CH_3), 1230 (S=O), 1140 (C-O-C), 710 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 7.66 (d, *J* = 8.2 Hz, 2H, H-2', H-6'), 7.52-7.46 (m, 3H, H-3'', H-4'', H-5''), 7.26-7.25 (m, 2H, H-2'', H-6''), 7.14-6.98 (m, 5H, H-2'''', H-3'''', H-4'''', H-5'''', H-6''''), 6.95 (d, *J* = 7.9 Hz, 2H, H-3', H-5'), 4.68 (q, *J* = 7.0 Hz, 2H, H-2'''), 3.88 (s, 3H, H-1'''), 3.73-3.66 (m, 2H, H_e-2, H_e-6), 2.45-2.43 (m, 5H, H-5''', H_a-2, H_a-6), 2.43-2.41 (m, 1H, H-4), 1.99-1.82 (m, 4H, H_e-3, H_e-5, H_a-3, H_a-5), 1.79-1.78 (m, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 168.22 (C-2''''), 166.01 (C-4'), 162.91 (C-5''''), 161.01 (C-7''''), 154.93 (C-3''''), 152.38 (C-6''''), 132.74 (C-1''''), 131.56 (C-1''), 131.11 (C-6''''), 130.70 (C-3'', C-5''), 130.21 (C-1'), 129.75 (C-2', C-6'), 129.25 (C-4''), 128.61 (C-3''''), 128.01 (C-5''''), 127.60 (C-4''''), 127.47 (C-2'', C-6''), 114.20 (C-3',C-5'), 55.60 (C-4'''), 55.57 (C-1'''), 47.71 (C-2'''), 45.33 (C-2, C-6), 31.72 (C-4), 29.20 (C-3, C-5), 14.01 (C-5'''), 16.12 (C-3''').

2.5.12. N-(3,4-dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide (9l)

Light pink amorphous solid; M.P: 109.5 °C; Molecular formula: $C_{31}H_{35}N_5O_4S_2$; Molecular mass: 605.77 g/mol; **IR** (KBr, wave number, cm^{-1}) 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1375 (CH_3), 1250 (S=O), 1150 (C-O-C), 750 (C-H); **^{1}H-NMR** ($CDCl_3$, 600 MHz, δ (ppm)): 9.64 (s, 1H, NH), 7.69 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.64 (d, *J* = 7.9 Hz, 1H, H-6''''), 7.60-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.23 (dd, *J* = 7.7 Hz, 2H, H-2'', H-6''), 7.12 (t, *J* = 7.7 Hz, 1H, H-5''''),7.00 (d, *J* = 7.5 Hz, 1H, H-4''''), 6.99 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 4.60 (q, *J* = 7.0 Hz, 2H, H-2'''), 3.87 (s, 3H, H-1'''), 3.76-3.74 (m, 2H, H_e-2, H_e-6), 2.53-2.49 (m, 1H, H-4), 2.34-2.30 (m, 5H, H_a-2, H_a-6, H-4'''), 2.20 (s, 3H, H-5'''), 2.03-2.01 (m, 2H, H_e-3, H_e-5), 1.87-1.87 (m, 2H, H_a-3, H_a-5), 1.59 (d, *J* = 6.1 Hz, 3H, H-3'''); **^{13}C-NMR** ($CDCl_3$, 150 MHz, δ(ppm)): 167.01 (C-5''''), 163.00 (C-4'), 158.07 (C-3''''), 152.69 (C-6''''), 137.31(C-1''''),135.78 (C-1''), 132.43 (C-2''''), 130.75 (C-6''''), 130.43 (C-3'', C-5''), 129.75 (C-2'', C-6''), 128.98 (C-4''), 128.00 (C-3''''), 127.02 (C-5''''), 126.96 (C-2', C-6'), 125.69 (C-1'), 121.27 (C-4''''), 114.20 (C-3', C-5'), 55.57 (C-1'''), 45.43 (C-2, C-6), 42.67 (C-2'''), 31.96 (C-4), 29.44 (C-3, C-5), 20.60 (C-4'''), 13.83 (C-5'''), 16.65 (C-3''').

2.6. Antioxidant activity by DPPH method

The antioxidant activity of all the synthesized compounds was evaluated by reported method [12] with few modifications. 90 µL of DPPH with various concentrations of synthesized compounds (10 µL) were mixed and incubated at 35 °C for half an hour. Synergy HT BioTek® USA microplate reader was utilized to readout the absorbance at 517 nm. The decrease in the absorbance showed the DPPH scavenging activity. The given formula was utilized to calculate the % scavenging activity and Amherst USA software was used to calculate the IC_{50} values.

Percent scavenging activity = 100 - (Abs of test compound/Abs of control) x 100 Equ 1

2.7. Butyryl cholinesterase inhibition assay

BChE inhibition activity was studied by reported method [13,14] with a little modification. BChE is responsible for neuromuscular junction and brain synapses. A mixture of Na_2HPO_4, butyryl cholinesterase enzyme and synthesized compounds was prepared and absorbance was noted at 405 nm before and after incubation. EZ - Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA) was employed to calculate the IC_{50} values.

2.8. Urease inhibition assay

The urease inhibition potential of all the synthesized compounds was calculated by the reported method with a few variations in protocol [15]. A mixture of phosphate buffer, sample solution and enzyme solution was prepared and the absorbance was noted at 625 nm before and after incubation. EZ - Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA) was employed to calculate the IC_{50} values.

2.9. Statistical analysis

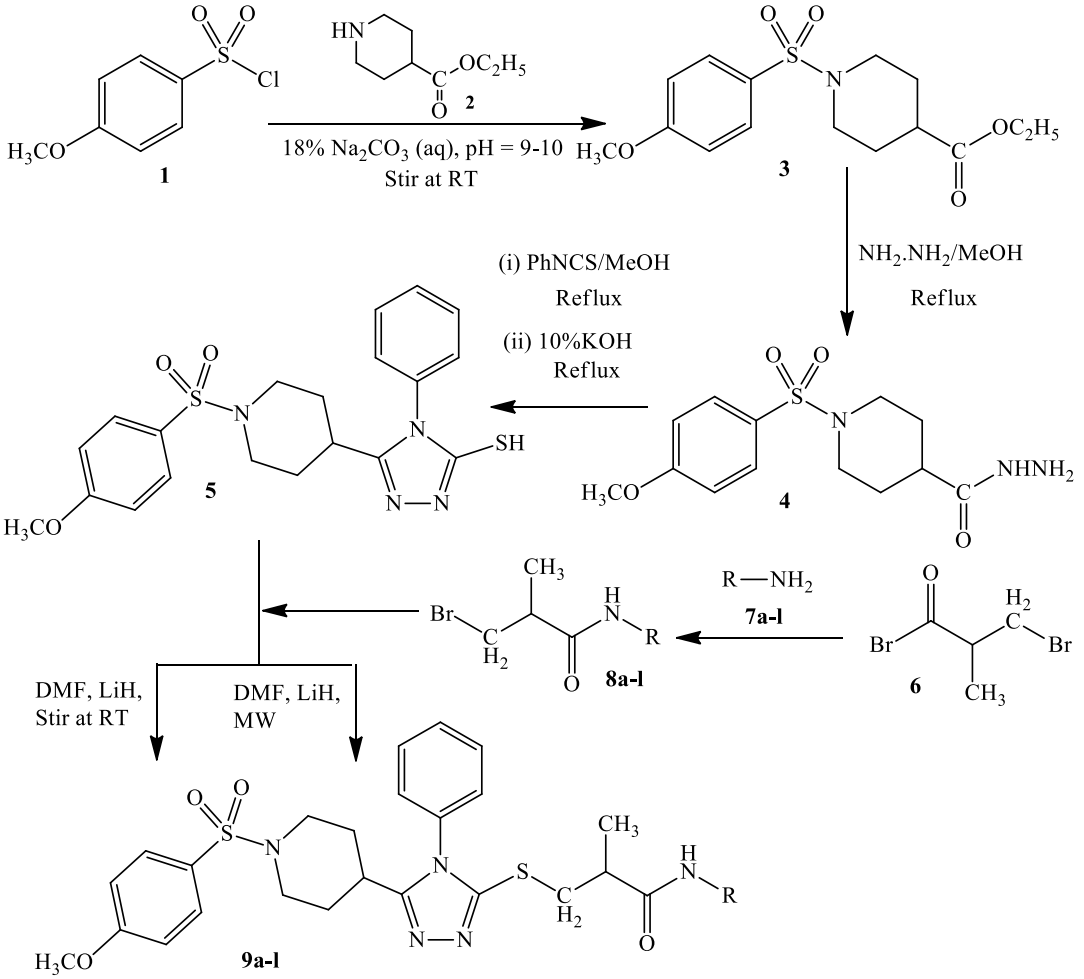
All the calculations were made in triplicate along with the statistical data and statistical analysis was performed by Microsoft Excel 2010. Results were presented in mean ± SEM. The IC_{50} values were calculated by using EZ-Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

2.10. *Molecular docking studies*

Molecular docking studies of synthesized compounds were carried out to investigate the putative binding interaction with urease and BChE. Protein structure of Urease and BChE were downloaded from protein data bank using PDB ID: 4h9m and 4BDS with resolution of 1.5 Å and 2.1 Å respectively [16,17]. ChemDraw Professional 15.0 was utilized to generate the structure of synthesized compound [18]. Then converted into minimized 3D structures by Ligand Preparation tool implemented in Discovery Studio 4.0. Prior to molecular docking studies Discovery Studio Client software was used for preparation of target protein structure as well as ligand structures [19]. For docking calculations FRED version 3.2.0 was used [20]. FRED requires a set of input conformers for each compound. The conformers of each ligand were generated by OMEGA 3.0.0 [21]. We used default settings of OMEGA for generation of conformers. The active site of urease was defined in such a manner that it contained all residues staying within 10 Å of nickel atoms and the non-standard residues (KCX and CME) in the X-ray structures of urease while in case of BChE active site was selected around the co-crystal ligand, tacrine within the active site of BChE. FRED default parameters were used setting the high dock resolution. Docking protocol was optimized using the re-docking of co-crystal ligand with in the active site. FRED generated ten poses for each ligand and pose with lowest chemguass4 was selected for further analysis. Binding interactions of best docked poses were visualized using Discovery Studio client v16.1.0 [19].

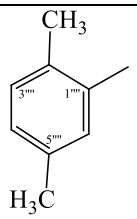
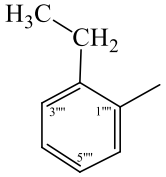
3. Results and discussion

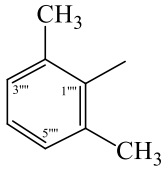
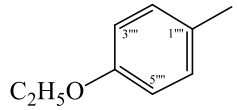
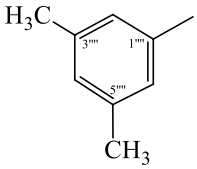
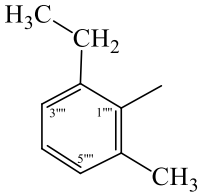
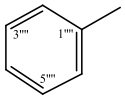
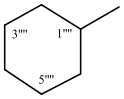
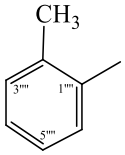
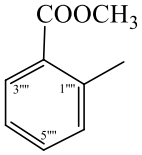
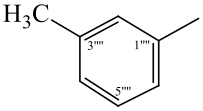
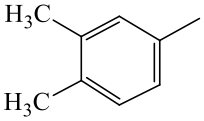
Comparative synthetic study through conventional and microwave assisted methods was the main objective of the current studies. Through comparative synthetic strategies, we have found that the microwave assisted technique is more suitable and efficient with respect to time and yield Table 2. The protocol for the synthesized hybrids is given in scheme 1 and the varying groups are listed in Table 1. All the synthesized hybrids of 1,2,4-triazole were characterized through spectroscopic techniques to justify their structures after their successful synthesis followed by different analytical techniques TLC, filtration, extraction, crystallization and re-crystallization. The whole library of synthesized hybrids was subjected to biological screening against different enzyme and securitization of most active synthesized members. The factors responsible for better biological activity were analyzed through docking studies.



Scheme 1. General scheme for the synthesis library of hybrids of 1,2,4-triazole having piperidine and propanamides.

Table 1. Different *N*-substituted aryl/phenyl/alkyl groups.

Compound	R	Compound	R
9a		**9g**	

9b		**9h**	
9c		**9i**	
9d		**9j**	
9e		**9k**	
9f		**9l**	

3.1. Chemistry

For the detailed structural discussion, compound **9a** was selected. It was obtained as light pink amorphous solid. The protons of aromatic ring having sulfonyl group was justified by two doublet peaks appearing at 7.69 (d, *J* = 8.2 Hz, 2H, H-2', H-6') and 6.99 (d, *J* = 8.1 Hz, 2H, H-3', H-5') respectively. The aromatic ring attached with nitrogen of triazole ring have been justified by the following two signals appeared at 7.58-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.21 (d, *J* = 6.9 Hz, 2H, H-2'', H-6''). Similarly the third aromatic ring of amide was justified by one singlet and two doublet peaks as 7.88 (s, 1H, H-6''''), 7.06 (d, *J* = 7.7 Hz, 1H, H-3'''') and 6.88 (d, *J* = 7.3 Hz, 1H, H-4'''') respectively. The nine protons of piperidine ring were justified by the following signals 3.76-3.65 (m, 2H, H_e-2, H_e-6), 2.53-2.51 (m, 1H, H-4), 2.40-2.36 (m, 2H, H_a-2, H_a-6), 2.07-1.81 (m, 4H, H_e-3, H_e-5, H_a-3, H_a-5). Three protons of methoxy group attached with aromatic ring of sulfonyl moiety was justified by a singlet signal appearing at δ 3.88 while the –CH group attached with heteroatom was justified by quartet signal as 4.66 (q, *J* = 7.3 Hz, 1H, H-2'''). Two substituted methyl groups attached at position 2 & 5 were justified by two singlet peaks appearing at 2.32 (s, 3H, H-4''') and 2.26 (s, 3H, H-5''') respectively. The carbon skeleton of the concerned compound was explained on the basis of ^{13}C-NMR spectral data. All the quaternary carbons of aromatic rings and two quaternary carbons of triazole ring were justified by the following peaks appearing at 168.00 (C-4'), 129.01 (C-1'), 132.42 (C-1''), 136.18 (C-1''''), 131.20 (C-2''''), 127.76 (C-5''''), 162.98 (C-5'''') and 157.74 (C-3''''). The carbons of aromatic ring having sulfonyl moiety were justified by two peaks at 126.74 (C-2', C-6') and 114.20 (C-3',C-5'). The aromatic carbons of the ring attached to the triazole ring were justified by 130.38 (C-3'', C-5'') and 126.99 (C-2'', C-6'') respectively. The carbon of methoxy group was justified by the peak 55.59 (C-1''') while the carbons at *S*-substitution and methyl groups attached to amidic aromatic ring were justified by the following peaks as 42.85 (C-2'''), 16.31 (C-3'''), 21.16 (C-4''') and 17.74 (C-5'''). The detailed spectroscopic studies made us able to define the structure of concerned compound **9a** with name of *N*-(2,5-dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]propanamide.

Table 2. Comparison of conventional and microwave assisted methods.

Compounds	Reaction Time		Reaction Yield (%)	
	Conventional (hours)	Microwave (sec)	Conventional	Microwave
9a	10	32	62	94
9b	13	36	58	92
9c	14	43	66	93
9d	11	33	61	88
9e	8	59	70	86
9f	11	39	67	89
9g	12	40	72	67
9h	14	48	54	92
9i	16	47	68	90
9j	13	52	76	88
9k	9	38	49	85
9l	8	49	58	82

3.2. Acetyl cholinesterase inhibition potential

The given drugs' ability to inhibit the AChE enzyme was assessed (Table 3). All of the compounds **9a-l** have been found to have various levels of activity. With IC_{50} values of 63.45±1.21 in comparison to the standard used, compound 9g exhibited the most potential among the synthesized compounds. Even though synthetic compounds have slightly lower potential than expected, they nevertheless have the strongest potential to block AChE's activity. The modification at the aromatic ring connected to the amidic functionality may be a root cause of the synthesized compounds' varied potential.

3.3. Antioxidant activity studies

The whole array of designed hybrids was screened for antioxidant potential (Table 3). Every member of the current synthesis was found very active for their antioxidant potential with variable range. The following members, **9b-d** and **9k** and **9l** were found the most active among the synthesized compounds. The compound **9c** showed highest potential among the synthesized compounds and also comparable to that of BHA used as reference standard. The highest antioxidant potential possessed by **9c** might be due to the presence of methyl group at *meta* position of phenyl ring attached to nitrogen of amide functionality.

3.4. Urease inhibition studies

Urease inhibition potential of every compound was tested and very outstanding results were availed as shown in Table 3. The list of compounds showed variable potential. The active compounds possessed least, comparable and better potential than the standard thiourea. Compounds **9b, 9c** and **9g-9i** have little low activity compared to the thiourea, while compound **9a** presented potential with IC_{50} value of 21.1±0.11 as compared to that of thiourea with IC_{50} value of 21.3±0.24. The urease inhibition potential of compounds **9g** and **9k** was even better than the standard utilized here with IC_{50} values of 20.2±0.21 and 19.2±0.09 respectively. The better potential showed by these two compounds might due the variable substitution at aromatic ring of amidic group.

3.5. Butyryl cholinesterase (BChE) studies

The inhibition potential of the presented compounds **9a-9l** against BChE enzyme was evaluated (Table 3). All the compounds were found active with variable range. Two compounds **9d** and **9e** showed highest potential among the synthesized compounds with IC_{50} values of 15.5±0.39 and 15.9±0.67 respectively as compared to the standard utilized. Although the potential of synthesized compounds is little less than the standard yet they could perform at highest rank to inhibit the action of BChE. The variable potential of the synthesized compounds might be due to the variation at the aromatic ring attached at amidic ring.

Table 3. Anti-oxidant and enzyme inhibition studies.

Compounds	IC_{50} Values			
	AChE	Antioxidant	Urease inhibition	BChE
9a	270.83±1.21	65.2 ± 0.15	21.1 ± 0.11	66.1 ± 0.62
9b	269.25±1.13	52.1 ± 0.26	36.2 ± 0.21	79.2 ± 0.22
9c	134.63±1.12	45.2 ± 0.15	39.4 ± 0.45	77.7 ± 0.36
9d	215.91±1.13	49.5 ± 0.21	> 500	15.5 ± 0.39
9e	63.27±1.21	72.1 ± 0.69	> 500	15.9 ± 0.67
9f	169.83±1.12	77.7 ± 0.29	> 500	19.2 ± 0.67
9g	63.45±1.21	75.5 ± 0.32	20.2 ± 0.21	29.2 ± 0.26
9h	87.32±1.18	85.5 ± 0.55	45.5 ± 0.52	26.1 ± 0.14
9i	182.73±1.15	82.2 ± 0.56	49.2 ± 0.26	39.5 ± 0.21
9j	-	55.5 ± 0.20	55.2 ± 0.11	79.8 ± 0.29
9k	133.91±1.12	52.1 ± 0.09	19.2 ± 0.09	72.1 ± 0.35
9l	402.83±1.12	47.7±0.29	-	29.2±0.37
BHA		**44.2 ± 0.41**	-	-
Thiourea		-	**21.3 ± 0.24**	-
Eserine	**0.19±0.05**	-	-	**7.8 ± 0.05**

3.6. Docking studies

3.6.1. Docking studies against against urease and BChE enzymes

Now a day's molecular docking calculations are broadly used for investigating the binding affinities of ligands with target structures. We also performed molecular docking studies of all compounds **9a-9l**, Table 4, to investigate the putative binding orientation of these compounds within the active site of urease and BChE. Binding affinity of synthesized compound against urease and BChE were evaluated based on chemguass4 score implemented in FRD docking software are shown in Table 4. FRED uses multi-conformer docking procedure which separately creates a set of low-energy conformers, and then does rigid docking for each conformer.

Best docked pose based on lowest chemguass4 was selected for further deciphering the biding interactions. Detail binding interaction of most potent compound **9k** and compound **9d** against urease and BChE, respectively are shown in Figure 1 and Figure 2. Compound **9k** was forming a great network of different hydrogen bonding, hydrophobic and electrostatic interactions with various amino acid residues within the active site of urease. Two hydrogen bonds were formed by amino acid residue His593 and CME592 as shown in green dotted line in Figure 1. Two pi-anion electrostatic interactions were formed with Asp494 and Glu525 while only one pi-pi T-shaped was formed with triazole moiety of compound **9k** and amino acid residue His593 as shown by pink dotted line. Additionally, Met637, His593, Leu523 and Arg439 presented hydrophobic interactions with side chains of compound **9k**.

Compound **9d** was most potent compound against urease, so it was selected to investigate the detail binding interaction with the target structure. Figure 2 shows the binding interactions of compound **9d** with different amino acid residue of urease. One of oxygen atom attached to sulfur was making hydrogen bond with amino acid residue of Asn289 as shown in green dotted line. Ala277 and Ser287 along with Gly283 were also forming carbon hydrogen bond as shown in light green color in Figure 2. Additional to hydrogen bonding network and hydrophobic interactions were formed by Phe329, Trp332, Ala277 and Ala328.

Table 4. Chemgauss4 scores of all synthesized compounds against Urease and BChE.

Compound	ChemGauss4 Score Against Urease	ChemGauss4 Score Against BChE
9a	-6.57	-9.80
9b	-6.06	-9.92

9c	-7.49	-8.98
9d	-6.23	-8.82
9e	-6.56	-9.47
9f	-4.66	-8.94
9g	-4.17	-8.95
9h	-5.37	-8.21
9i	-6.17	-9.32
9j	-4.27	-9.02
9k	-6.75	-8.68
9l	-4.21	-9.12

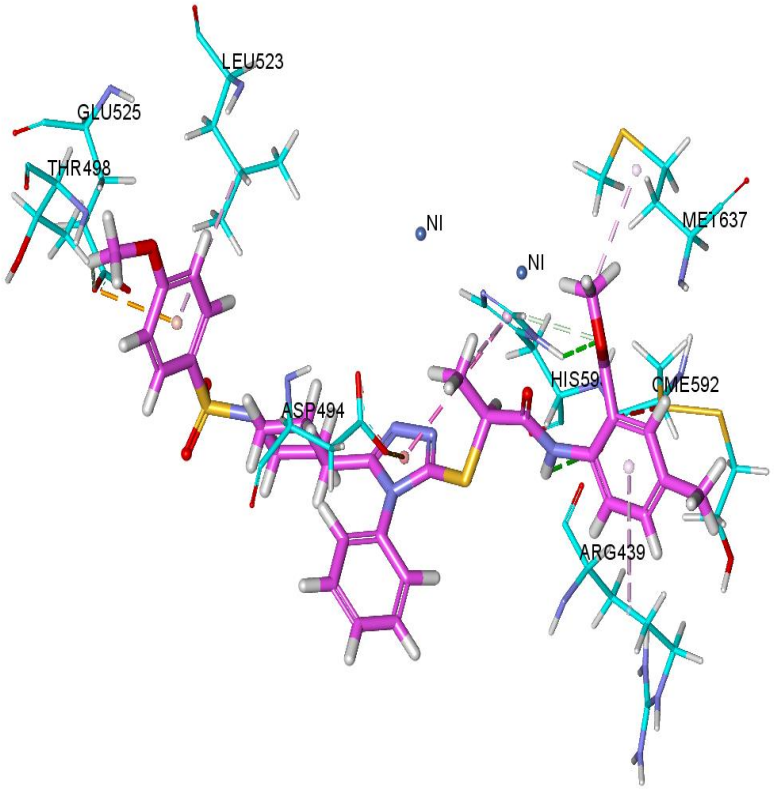


Figure 1. Binding orientation of most potent Compound **9k** within the active site if Urease (4H9M). Hydrogen bonding are shown in green line while other Hydrophobic are shown in pink dotted line.

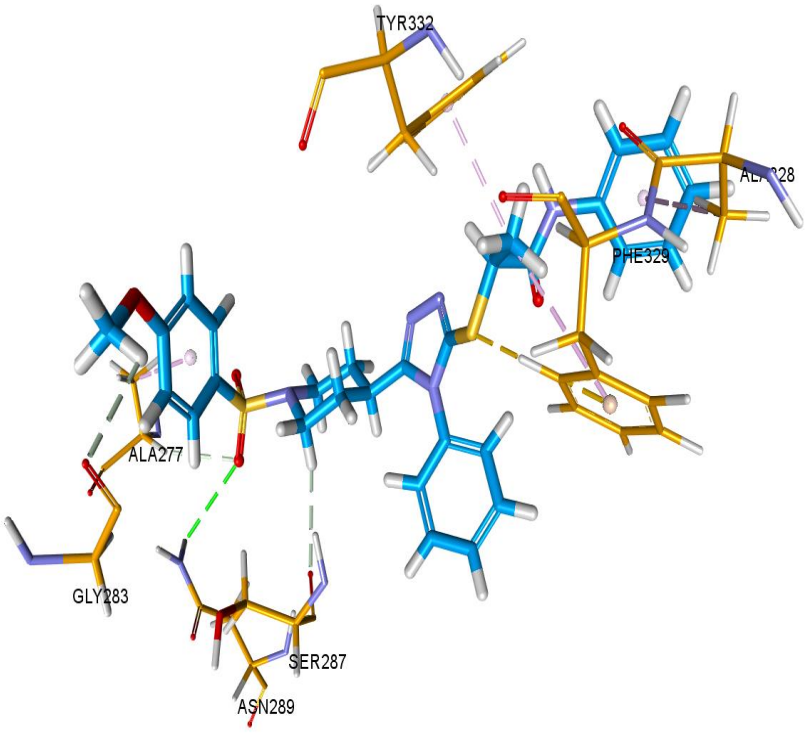


Figure 2. Binding orientation of most potent Compound **9d** within the active site if Urease (4BDS). Hydrogen bonding is shown in green line while other Hydrophobic are shown in pink dotted line.

3.6.2. Docking studies against AChE enzyme

To determine the binding orientation of compound **9l** with the protein constituting the active site of the AChE enzyme, a molecular docking analysis of the relevant a compound was conducted. Out of all the synthesized compounds in the corresponding series, the ligand **9l** had the greatest ability to block the AChE enzyme. In order to dock either ligand **9l** or the active site of the AChE enzyme, SybylX-1.3 (module Surflex-Dock) was utilized (Table 5, Figure 3). By re-docking the docked compounds into the active cite of AChE, the legitimacy of the chemical was verified. In order to verify the docking methodology, donepezil was removed from the co-crystal complex 4EY7In and then docked again in the same binding pocket. Experimentally, the docking capabilities and donepezil's AChE binding behavior were compared. This proves the accuracy of our docking method. Additionally, it was demonstrated that the inhibitor exhibits a similar binding mechanism to that of the experimentally discovered 4EY7 complex. According to the visual analysis of docked ligands, compound **9l**, an inhibitor, deeply enters the CAS and PAS of the AChE enzyme. The ligand has created an H-bonding interaction with the backbone of Glu292, Ser203, Phe295, Arg296, and Phe338 in the active site of AChE. It is possible that the methyl groups substituted at the aromatic ring linked with the amidic group created the hydrophobic contact necessary for its optimal activity in the CAS area. C scorea, PMF scoree, and Polar scorec are experimental findings that are 6.84, 15.098, and 0.09, respectively. This proves the accuracy of our docking method. Numerous amino acids, including Glu 292, Ser 203, Phe 295, Arg 296 and Phe 338, interacted with inhibitor drugs to increase their effectiveness. The variation in ligand acceptor interactions could be the cause of compound **9l's** possible inhibitory behavior.

Table 5. Surflex score of docked ligands compound **9l** for AChE enzyme.

Docking complex = AChE- 9l							
C score[a]	**Crash score**[b]	**Polar score**[c]	**D score**[d]	**PMF score**[e]	**G score**[f]	**Chem score**[g]	**Amino acid interaction**
6.84	-3.89	0.09	-215.074	15.098	-345.68	-32.964	Glu292, Ser203, Phe295, Arg296, Phe338

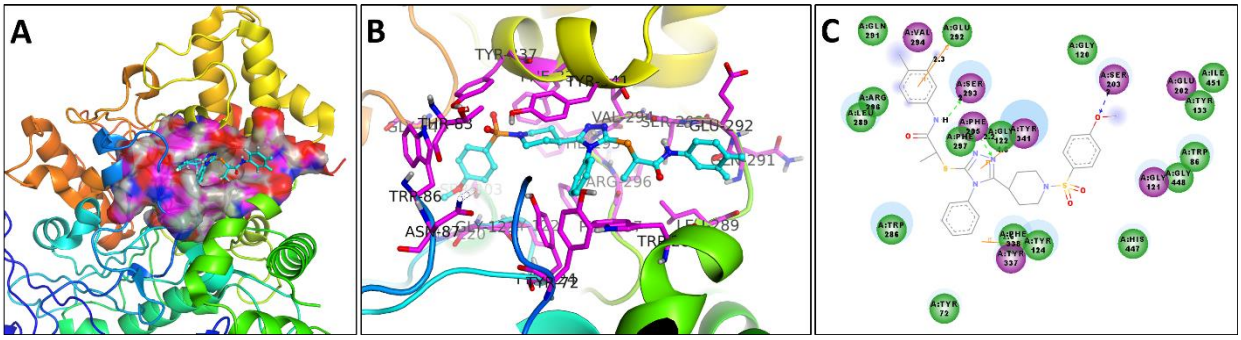


Figure 3. Molecular docking generated poses for selected AChE inhibitor (**9l**) (A) Compound **9l** bonded with in the active site of AChE enzyme (B) binding mode of **9l** in AChE ligand binding site (C) 2D-ligand-protein interaction diagram was generated for the best poses obtained with compound **9l** against AChE enzyme.

3.7. BSA binding studies

The quenching of BSA with the derivatives appears to follow a static process rather than a dynamic one, as the predicted kq was greater than the maximum scattering collision quenching rate constant (2×10^{10} M^{-1} s^{-1}) in dynamic quenching. The apparent constant (Ka) and the total number of binding sites (n) can be determined when tiny molecules bind individually to a set of analogous sites on a macromolecule using the double reciprocal plot. The derivatives' double reciprocal charts are displayed. The intercept and slope of the linear plot, respectively, are used to determine the values of Ka and n. Among the studied compounds **9a** showed least bonding constant values while 9f showed the highest bonding constant value with BSA. So this binding pattern will justify that strongest bonding will lead does not radially release the drug and least bonding will not carry the drug in proper way towards the target as **9a** and **9f** respectively. The compounds **9c** showing intermediate bonding capabilities with BSA perform better to biologically.

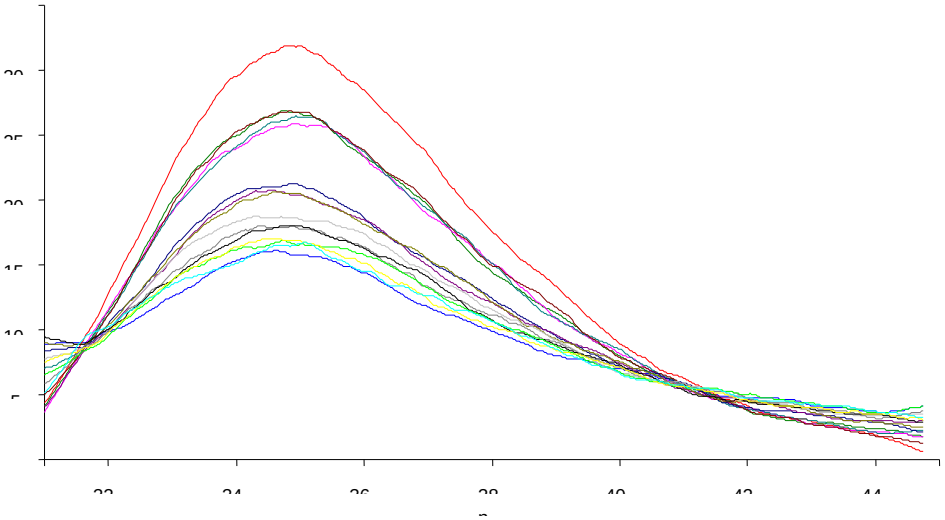


Figure 4. Fluorescence graph of BSA in the presence of **9a.**

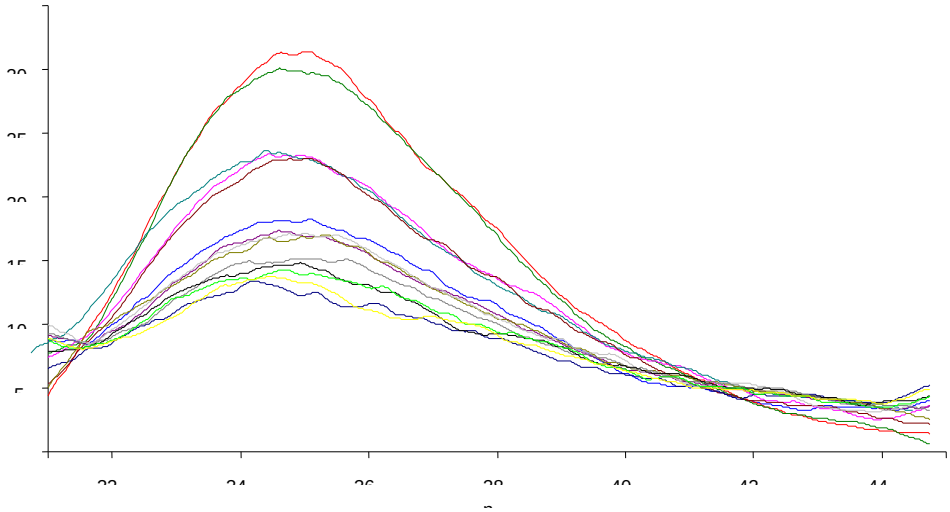


Fig. 5. Fluorescence graph of BSA in the presence of **9c**

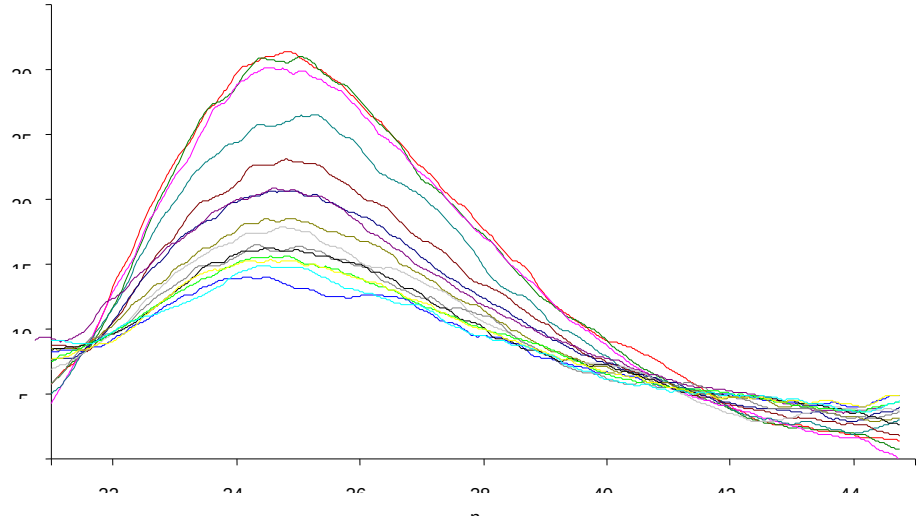


Fig. 6. Fluorescence graph of BSA in the presence of **9e**

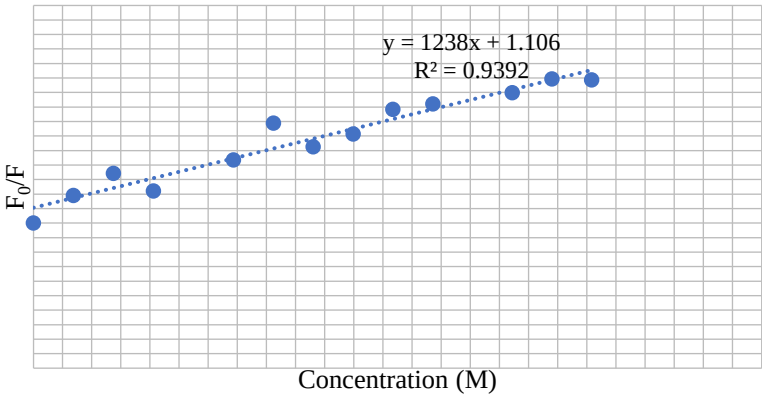


Fig. 7. Stern-Volmer plots of **9a**

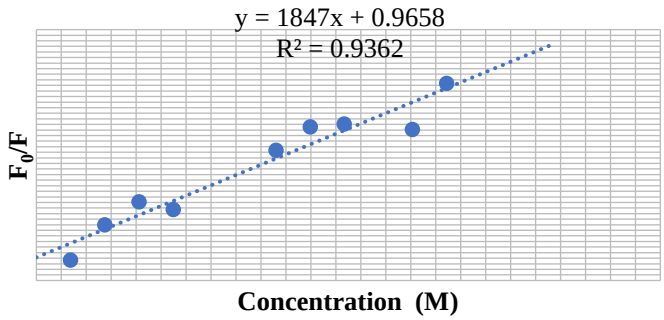


Fig. 8. Stern-Volmer plots of **9c**

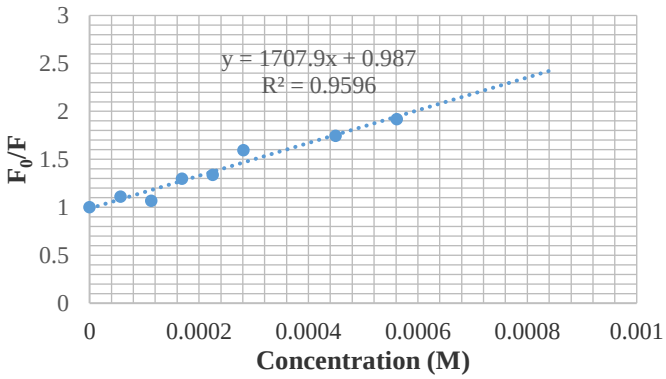


Fig. 9. Stern-Volmer plots of **9f**

Table 6. Stern–Volmer quenching constants, binding constant and number of binding site for compounds.

Compounds	$K_{SV} \times 10^1$ (M^{-1})	$k_q \times 10^{11}$ (M^{-1} s^{-1})	K_a (M^{-1})	n
9a	1.238	1.238	61.2	0.58
9c	1.847	1.847	2.33×10^3	1.03
9f	1.708	1.708	4.11×10^3	1.11

4. Conclusion

Attempts were made to synthesize a library of hybrids of 1,2,4-triazole having azinane and propanamides as key components responsible for versatile biological potential. Synthesis was in comparison fashion through conventional and microwave assisted methods. The highest yield with high purity in minimum time was obtained through microwave assisted technique. The whole library of designed hybrids was characterized by spectroscopic techniques. Docking, BSA binding as well as biological studies were carried out to know the biological potential of synthesized compounds against antioxidant activity, urease, AChE and BChE inhibition potential. All the hybrids were found active in variable potential against mentioned activities. Compounds **9g** and **9k** were the most active member of the series and proved best against urease enzyme. After further biological exploration these compounds might be the best anti-urease drugs in the market in future to serve the humanity.

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References

1. H. S. Jhuang, Y. W. Liu, D. M. Reddy, Y. Z. Tzeng, W. Y. Lin, C. F.Lee, Microwave assisted synthesis of thioesters from aldehydes and thiols in water, J. Chin. Chem. Soc. 65 (2018) 24-27.
2. P. Lidström, J. Tierney, B. Watheyb, J. Westmana, Microwave assisted organic synthesisÐa review, Tetrahedron. 57 (2001) 9225-9283.
3. J. Lee, M. Hong, Y. Jung, E. J. Cho, H. Rhee, Synthesis of 1,3,5-trisubstituted-1,2,4-triazoles by microwave-assisted *N*-acylation of amide derivatives and the consecutive reaction with hydrazine hydrochlorides, Tetrahedron. 68 (2012) 2045-2051.
4. [4] A. A. H. Sebeka, A. M. A. Osman, I. E. T. El Sayed, M. El Bahanasawy, M. A. Tantawy, Synthesis and antiproliferative activity of novel neocryptolepine-hydrazides hybrids, J. Appl. Pharm. Sci. 7 (2017) 9-15.
5. [5] T. M. Potewar, K. T. Petrova, M. T. Barros, Efficient microwave assisted synthesis of novel 1,2,3-triazole–sucrose derivatives by cycloaddition reaction of sucrose azides and terminal alkynes, Carbohydr. Res. 379 (2013) 60-67.
6. [6] J. N. Sangshetti, A. R. Chabukswar, D. B. Shinde, Microwave assisted one pot synthesis of some novel 2,5-disubstituted 1,3,4-oxadiazoles as antifungal agents, Bioorg. Med. Chem. Lett. 21 (2011) 444-448.
7. [7] A. S. Medvedeva, M. M. Demina, T. V. Kon'kova, T. L. H. Nguyen, A. V. Afonin, I. A. Ushakov, Microwave assisted solvent- and catalyst-free three-component synthesis of *NH*-1,2,3-triazoloimines, Tetrahedron. 73 (2017) 3979-3985.
8. [8] R. Kharb, P. C. Sharma, M. S. Yar, Pharmacological significance of triazole scaffold, J. Enzyme Inhib. Med. Chem. 26 (2011) 1-21.
9. [9] P. Mishra, N. Upmanyu, A. Mehta, In vitro anti-microbial activity of some 5-aryl-4-(substituted amino)-3-mercapto-1,2,4-triazoles, Indian J Microbiol. 46 (2006) 401.
10. [10] S. K. Sahu, B. K. Dubey, A. C Tripathi, M. Koshy, S. K. Saraf, Piperidin-4-one: the potential pharmacophore, Mini-Rev. Med. 13 (2013) 565-583.
11. [11] M. Gopalakrishnan, J. Thanusu, V. Kanagarajan, R. Govindaraju, Unexpected formation of 3-chloro-1-hydroxy-2,6-diarylpiperidin-4-ones: synthesis, antibacterial and antifungal activities, J. Enzyme Inhib. Med. Chem. 24 (2009) 52-58.
12. [12] I. I. Koleva, T. A. Van Beek, J. P. H. Linssen, A. D. Groot, L. N. Evstatieva, Screening of plant extracts for antioxidant activity: a comparative study on three testing methods, Phytochem. Anal. 13 (2002) 8-17.
13. [13] M. Cygler, J. D. Schrag, J. L. Sussman, M. Harel, I. Silman, M. K. Gentry, B. P. Doctor, Relationship between sequence conservation and three-dimensional structure in a large family of esterases, lipases, and related proteins, Protein Sci. 2 (1993) 366-382.
14. [14] V. Tougu, Acetylcholinesterase: mechanism of catalysis and inhibition, Curr. Med. Chem. 1 (2001) 155-170.
15. [15] H. L. Mobley, M. J. Cortesia, L. E. Rosenthal, B. D. Jones, Characterization of urease from Campylobacter pylori, J. Clin. Microbiol. 26 (1988) 831-836.
16. [16] A. Balasubramanian, K. Ponnuraj, Crystal structure of the first plant urease from jack bean: 83 years of journey from its first crystal to molecular structure, J. Mol. Biol. 400 (2010) 274-283.
17. [17] F. Nachon, E. Carletti, C. Ronco, M. Trovaslet, Y. Nicolet, L. Jean, P. Y. Renard, Crystal structures of human cholinesterases in complex with huprine W and tacrine: elements of specificity for anti-Alzheimer's drugs targeting acetyl-and butyryl-cholinesterase, Biochem. J. 453 (2013) 393-399.
18. [18] Elmer, P. ChemBioDraw Professional, version (15.0.0.106); Cambridge Soft Waltham: MA, USA, 2017.
19. [19] BIOVIA Discovery Studio Client, v16. 1.0. 15350, Dassault Systemes: San Diego, 2015
20. [20] FRED, version 3.0.0; OpenEye Scientific Software: Santa Fe, NM, USA, 2013. Available from: www.eyesopen.com.
21. [21] OMEGA, version 2.4.6; OpenEye Scientific Software: Santa Fe, NM, USA, 2013. Available from: www.eyesopen.com.