Conventional versus microwave assisted synthesis, molecular docking and enzyme inhibitory activities of new 3,4,5-trisubstituted-1,2,4-triazole analogues

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Abstract: *N*-(Substituted)-5-(1-(4-methoxyphenylsulfonyl)piperidin-4-yl)-4*H*-1,2,4-triazol-3-ylthio) acetamide were synthesized by following conventional as well as microwave assisted protocol through five consecutive steps under the impact of various reaction conditions to control the reaction time and the yield of product. Starting from 4-methoxybenzenesulfonyl chloride and ethyl isonipecotate, product 3 was obtained which was converted into product 4 by treating with hydrazine hydrate. In step 3, the product 4 was refluxed with methyl isothiocyanate and KOH to yield compound 5 which was finally treated with variety of *N*-substituted acetamides to yield an array of different new compounds (8a-k). These synthesized compounds were evaluated for their inhibition potential against bovine carbonic anhydrase (*b*CA-II), acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) enzymes. Compound 8g demonstrated good activity against *b*CA-II, AChE and BChE with IC_{50} values of 8.69 ± 0.38 μM, 11.87±0.19 μM and 26.01±0.55 μM respectively. SAR studies assisted with molecular docking were carried out to explore the mode of binding of the compounds against the studied enzymes.

Keywords: 1,2,4-Triazoles, Acetamides, Microwave assisted synthesis, Piperidine, Enzyme inhibition.

INTRODUCTION

The synthesis of compounds relating to the azole family has attracted the attention of researcher working on heterocyclic compounds (Zbancioc and Mangalagiu, 2010; Zbancioc and Mangalagiu, 2006; Mitsumori *et al.*, 2003 and Cheng *et al.*, 1999). The literature has demonstrated that these compounds possess a wide range of biological activities (Zbancioc *et al.*, 2010). In the last few decades, a variety of heterocyclic compounds has been synthesized by following different varying procedures (Dumitrascu *et al.*, 2010 and Caira *et al.*, 2011). Triazoles and oxadiazoles represent biologically important class of compounds which have been synthesized with best yield and minimum cost (Chandgude and Dömling, 2016 and Myznikov *et al.*, 2007). Potentially 1,2,4-triazole based hybrids have high level of biological potential like antimicrobial, antiviral (Ye *et al.*, 2016), antifungal (Haddad *et al.*, 2015), antitumor and anti-tubercular (Jander *et al.*, 1989 and Moloney *et al.*, 1999).

Enzymes have five main classes which catalyze the different types of reactions. The process of irreversible and reversible dehydration of carbon dioxide occurred by the universal enzyme known as carbonic anhydrase (Supuran, 2011). This reaction is very crucial because it is the part of many physiological processes. Similarly the acetylcholinestrase enzyme is involved in mental disorder by the hydrolization process of acetylcholine to choline. The conversion of acetylcholine to choline results into the problem known as Alzheimer's disease (Ibrahim, 2009).

Azole based heterocyclic compounds possessed vide range of biological applications as examined by our reported synthesized compounds (Aziz-ur-Rehman *et al.*, 2018). The inauguration of new compounds bearing multiple functionalities is the running methodology in search of drug candidates. The heterocyclic compounds based on 1,2,4-triazole and piperidine nucleus have been synthesized through multicomponent reactions (Wu *et al.*, 2013). The conventional and microwave assisted methodologies were accompanied for the synthesis of all compounds (8a-k; table 1) mentioned in scheme-1. Among the different approaches microwave assisted synthesis was well established and used to synthesize various triazole nucleus (Kanagaraju and Thangamani, 2014). Three different functionalities including 4-methoxybenzene sulfonyl, piperidine and triazole were condensed into one unit. The current synthesized

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compound was further treated with a variety of *N*-substituted acetamides for the green synthesis of our target compounds. The heteroatoms present in triazole and piperidine ring system are of great interest from biological point of view (Elya *et al.*, 2012). They provide the hydrophilic interaction and hydrogen bond sites to block the negative effect of any enzyme or bacterial strain causing different range of diseases among the living organisms. So we envisioned to synthesize a library of compounds containing 1,2,4-triazole in order to evaluate their anti-enzymatic profile.

MATERIALS AND METHODS

General

The ^{1}H-NMR spectral information was registered on Bruker spectrometers operating at 600 MHz while ^{13}C-NMR spectral studies were registered on Bruker AM-400 spectrometer (150 MHz) in $CDCl_3$, using TMS as internal standard. The infra-red spectroscopic studies were made by using KBr pellet method on a Jasco-320-A spectrophotometer (wave number in cm^{-1}). Griffin and George apparatus was used to calculate the melting points of all the synthesized compounds. Reaction progress and purity was confirmed by thin layer chromatography (TLC) on a pre-coated silica gel plates system with *n*-hexane:ethyl acetate as an eluant and visualized under UV lamp or iodine vapors. The chemical utilized for the progress and synthesis of this series of compounds were of Alfa Aesar and Sigma Aldrich brands supplied by the local supplier.

Synthesis of ethyl 1-[(4-methyoxyphenyl)sulfonyl]-4-piperidinecarboxylate (3)

4-Methoxybenzensulfonyl chloride (1; 0.04 mol) and ethyl isonipecotate (2; 0.04 mol) were mixed in aqueous medium and 18 % sodium carbonate solution for the green synthesis of 1-[(4-methyoxyphenyl)sulfonyl]-4-piperidinecarboxylate (3). TLC was utilized to observe the reaction improvement. The product was obtained in the form of precipitates by addition of chilled distilled water and dil. HCl (to maintain pH at 4-5) which were filtered, washed and dried at room temperature.

Synthesis of 1-(4-methoxyphenylsulfonyl)piperidine-4-carbohydrazide (4)

A mixture of product 3 (0.05mol) and hydrazine hydrate was reacted in the presence of methanol as solvent for 5 hours to synthesize compound 4. Progress of reaction was monitored by thin layer chromatography. The compound 4 in the form of crystals was collected by evaporating the excess of methanol.

Synthesis of 5-(1-(4-methoxyphenylsulfonyl)piperidin-4-yl)-4-methyl-4H-1,2,4-triazole-3-thiol (5)

Methyl isothiocyanate (0.0304 mol) and compound 4 (0.0304 mol) were refluxed in ethanol (20mL) for 3 hours. As the reaction completed, checked by TLC, the un-cyclized compound in the form of precipitates was obtained which was again refluxed with equimolar aq. KOH (18%) for 2 hours. Reaction progress monitored thoroughly with TLC. Dil. HCl was added to adjust pH at 4-5 with continuous stirring until the precipitation of product 5. Precipitates were filtered, washed and dried at room temperature for further utilization.

General synthetic procedure for N-substituted-2-bromoacetamides (7a-k)

A mixture of 2-bromoacetyl bromide (0.02 mol), aryl/phenyl amines (6a-k; 0.02mol) and 18% Na_2CO_3 solution was vigorously shacked in the presence of aqueous medium. Reaction was supervised with TLC. The obtained precipitates were filtered, washed and dried at room temperature for next step utilization.

General synthesis of N-(substituted)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8a-k)

Method A: Product 5 (0.0005 mol) reacted in DMF with equimolar lithium hydride (LiH) for half an hour followed by the addition of *N*-substituted acetamides (7a-k, 0.0005 mol). The reaction mixture was kept on stirring until the reaction completed, confirmed by TLC. By the addition of chilled distilled water, the precipitates of compounds, 8a-k, were availed.

Method B: Product 5 (0.0005 mol) in DMF with equimolar lithium hydride (LiH) was reacted by stirring for half an hour followed by the addition of *N*-substituted acetamides (7a-k, 0.0005 mol). The reaction was proceeded in the microwave (Model: AM820 C×C(F)-PM, Frequency: 2450 MHz, Power Consumption: 1200-1270 W) for 30-70 sec (table 2). The best yield of the products, 8a-k, was achieved. At the completion of reaction, fine precipitates of the target compounds were attained by the addition of distilled water followed by the filtration, washing and drying at room temperature.

Carbonic anhydrase inhibition (in vitro) assay

A reported method (Qurrat-ul-Ain *et al.*, 2015) was employed to evaluate the synthesized compounds for their anti-carbonic anhydrase potential. A mixture of buffer solution, enzyme and test compounds was incubated for 30 minutes at 25˚C. Before incubation the absorbance was calculated at 400 nm. Then substrate was added and incubated for 30 minutes and absorbance noted. Acetazolamide utilized as standard. Results were tabulated (table 3) in the form of IC_{50} values.

% inhibition = 100 - [(absorbance of test compound ÷absorbance of control) × 100]

AChE & BChE inhibition (in vitro) assay

The pre-reported method was adopted to measure the inhibition of acetyl cholinesterase and butyryl cholinesterase enzyme (Ellman *et al.*, 1961). Test

compound (0.5 mM well^{-1}), a buffer solution of Na_2HPO_4 buffer (pH 7.7), acetyl cholinesterase and butyryl cholinesterase enzyme (0.005 mM well^{-1}) were mixed and absorption was measured at 405 nm. Then the whole mixture was incubated for 20min at 30ºC. As 10µL acetyl thiocholine iodide (for AChE) or butyryl thiocholine iodide (for BChE) and 10 µL DTNB were added to the reaction mixture, reaction was started. As the incubation done absorption was taken again at 405 nm by using 96-well plate reader Synergy HT, Biotek, USA. Galantamine was used as standard. The IC_{50} was calculated was calculated with the assistance of EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Docking studies

Docking experiments were performed *via* Molecular Operating Environment (MOE) docking program version 2016. Crystal structures of the enzymes were retrieved from protein data bank (PDB). For *b*CA-II PDB code 1V9E and for AChE PDB code 1EVE was selected for studies. Preparation of ligand structures, enzyme structures (protonate 3d and energy minimization), determination of active site and validation of docking protocol was performed according to our reported procedures (Rashid *et al.*, 2016). The view of the docking results and the analysis of their surface with graphical representations were performed using MOE and discovery studio visualization (Iftikhar *et al.*, 2017).

Spectral characterization

N-(2,4-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8a)

White solid; Yield: 89 %; M.P: 184.6ºC; Mol. formula: $C_{25}H_{31}N_5O_4S_2$; Mol. mass: 529.67 g/mol; IR (KBr, wave number, cm^{-1}) 2830 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1370 (CH_3), 1300 (S=O), 1200 (C-O-C), 730 (C-H)**;** ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.58 (s, 1H, NH), 7.74 (d, *J* = 8.2 Hz, 2H, H-2', H-6'), 7.71 (s, 1H, H-3"), 7.03 (d, *J* = 8.3 Hz, 2H, H-3', H-5'), 6.99-6.97 (m, 2H, H-5", H-6"), 3.99 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.79–3.77 (m, 2H, H_{eq}-2, H_{eq}-6), 3.47 (s, 3H, H-2'"), 2.69-2.66 (m, 1H, H-4) 2.64-2.61 (m, 2H, H_{ax}-2, H_{ax}-6), 2.29 (s, 3H, H-4'"), 2.18 (s, 3H, H-5'"), 2.08-2.01 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 167.79 (C-4'), 162.53 (C-5'"), 153.80 (C-3'"), 149.98 (C-6'"), 135.50 (C-1"), 133.63 (C-2"), 129.52 (C-5"), 129.45 (C-4"), 129.37 (C-6"), 128.23 (C-2', C-6'), 127.56 (C-3"), 126.70 (C-1'), 114.38 (C-3', C-5'), 55.56 (C-1'"), 44.94 (C-2, C-6), 34.28 (C-3'"), 31.15 (C-4), 30.07 (C-2'"), 28.11 (C-3, C-5), 22.54 (C-4'"), 14.51 (C-5'").

N-(2,3-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8b)

Light pink amorphous solid; Yield: 87%; M.P: 186.0ºC; Mol. formula: $C_{25}H_{31}N_5O_4S_2$; Mol. mass: 529.67 g/mol: IR (KBr, wave number, cm^{-1}) 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1372 (CH_3), 1275 (S=O), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.65 (s, 1H, NH), 7.73 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.75 (d, 1H, H-6"), 7.07 (t, *J* = 7.8 Hz, 1H, H-5"), 7.02 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 6.99 (d, *J* = 7.4, 1H, H-4"), 3.99 (s, 2H, H-3'"), 3.89 (s, 3H, H-1'"), 3.84-3.82 (m, 2H, H_{eq}-2, H_{eq}-6), 3.47 (s, 3H, H-2'") 2.66-2.64 (m, 1H, H-4), 2.58-2.54 (m, 2H, H_{ax}-2, H_{ax}-6), 2.29 (s, 3H, H-4'"), 2.14 (s, 3H, H-5'"), 2.14-2.00 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.98 (C-4'), 163.09 (C-5'"), 157.72 (C-3'"), 151.76 (C-6'"), 137.32 (C-1"), 135.63 (C-2"), 129.79 (C-2', C-6'), 129.30 (C-1'), 127.99 (C-3"), 127.14 (C-6"),125.63 (C-5"), 121.49 (C–4 "), 114.28 (C-3', C-5'), 55.62 (C-1'"), 45.49 (C-2, C-6), 36.08 (C-3'"), 32.04 (C-4), 30.08 (C-2'"), 29.12 (C-3, C-5), 20.56 (C-4'"), 13.83 (C-5'").

N-(3,4-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8c)

Off white amorphous solid; Yield: 95 %; M.P: 185 ºC; Mol. formula: $C_{25}H_{31}N_5O_4S_2$; Mol. mass: 529.67 g/mol; IR (KBr, wave number, cm^{-1}) 2835 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1375 (CH_3), 1310 (S=O), 1220 (C-O-C), 730 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.94 (s, 1H, NH), 7.73 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.39 (s, 1H, H-2"), 7.34-7.32 (m, 1H, H-6"), 7.04-7.02 (m, 3H, H-3', H-5', H-5"), 4.69 (dd, *J* = 6.9 Hz, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.84-3.81 (m, 2H, H_{eq}-2, H_{eq}-6), 3.48 (s, 3H, H-2'"), 2.67-2.63 (m, 1H, H-4), 2.54-2.52 (m, 2H, H_{ax}-2, H_{ax}-6), 2.22 (s, 3H, H-4'"), 2.21 (s, 3H, H-5'"), 2.08-1.96 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 168.54 (C-4'), 163.13 (C-5'"), 157.43 (C-3'"), 151.58 (C-6'"), 137.05 (C-1"),135.90 (C-3"), 132.52 (C-4"), 129.80 (C-2', C-6'), 127.69 (C-1'), 127.12 (C-6"), 120.96 (C-2"), 117.21 (C-5"), 114.32 (C-3', C-5'), 55.65 (C-1'"), 45.47 (C-2, C-6), 45.08 (C-3'"), 32.13 (C-4), 30.56 (C-2'"), 28.94 (C-3, C-5), 19.17 (C-4'"), 16.95 (C-5'").

N-(3,5-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8d)

Grey amorphous solid; Yield: 93 %; M.P: 176.6ºC; Mol. formula: $C_{25}H_{31}N_5O_4S_2$; Mol. mass: 529.67 g/mol; IR (KBr, wave number, cm^{-1}) 2825 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1372 (CH_3), 1250 (S=O), 1150 (C-O-C), 720 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.61 (s, 1H, NH), 7.75-7.73 (m, 3H, H-2', H-6'), 7.04-7.02 (m, 3H, H-3', H-5'), 6.87 (d, *J* = 2.62, 1H, H-4"), 6.63 (d, *J* = 2.62, 1H, H-2", H-6"), 4.00 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.79–3.77 (m, 2H, H_{eq}-2, H_{eq}- 6), 3.45 (s, 3H, H-2'"), 2.68-2.66 (m, 1H, H-4), 2.64–2.66 (m, 2H, H_{ax}-2, H_{ax}-6), 2.31 (s, 3H, H-4'"), 2.18 (s, 3H, H-5'"), 2.08-2.00 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.83 (C-4'), 163.07 (C-

5""), 157.71 (C-3""), 151.79 (C-6""), 136.13 (C-1"), 135.84 (C-2"), 130.22 (C-5"), 129.81 (C-2', C-6'), 127.79 (C-1'), 126.28 (C-6"), 125.72 (C-3"), 123.03 (C-4"), 114.28 (C-3', C-5'), 55.63 (C-1'"), 45.38 (C-2, C-6), 36.00 (C-3'"), 31.80 (C-4), 30.05 (C-2'"), 29.04 (C-3, C-5), 21.13 (C-4'"), 17.68 (C-5'").

N-(2,6-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamid (8e)

White amorphous solid; Yield: 95%; M.P: 178.3°C; Mol. formula: $C_{25}H_{31}N_5O_4S_2$; Mol. mass: 529.67 g/mol; IR (KBr, wave number, cm^{-1}) 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1350 (CH_3), 1280 (S=O), 1125 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.41 (s, 1H, NH), 7.74 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.09-7.04 (m, *J* = 7.44 Hz, 3H, H-3", H-4", H-5"), 7.02 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 4.00 (s, 2H, H-3'"), 3.89 (s, 3H, H-1'"), 3.81–3.79 (m, 2H, H_{eq}-2, H_{eq}-6), 3.50 (s, 3H, H- 2'"), 2.68-2.63 (m, 1H, H-4), 2.61– 2.59 (m, 2H, H_{ax}-2, H_{ax}-6), 2.14 (s, 6H, H-4'", H-5'"), 2.08-2.01 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 167.04 (C-4'), 163.08 (C-5""), 157.60 (C-3""), 152.00 (C-6""), 141.82 (C-1"), 135.28 (C-2", 6"), 133.82 (C-4"), 129.79 (C-3", 5"), 128.06 (C-2', C-6'), 127.22 (C-1'), 114.28 (C-3', C-5'), 55.61 (C-1'"), 45.38 (C-2, C-6), 35.59 (C-3'"), 31.95 (C-4), 30.15 (C-2'"), 29.07 (C-3, C-5), 18.19 (C-4'", C-5'").

N-(2,5-dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8f)

Grey white amorphous solid; Yield: 97 %; M.P: 180.0 °C; Mol. formula: $C_{25}H_{31}N_5O_4S_2$; Mol. mass: 529.67 g/mol; IR (KBr, wave number, cm^{-1}) 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1550 (Ar C=C), 1300 (CH_3), 1250 (S=O), 1150 (C-O-C), 725 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.59 (s, 1H, NH), 7.73 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.71 (d, *J* = 7.2 Hz, 1H, H-4") 7.04 (d, *J* = 7.5 Hz, 1H, H-3"), 7.02 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 6.87 (d, *J* = 7.4 Hz,1H, H-6"), 4.00 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.80–3.77 (m, 2H, H_{eq}-2, H_{eq}-6), 3.46 (s, 3H, H-2'"), 2.67-2.63 (m, 1H, H-4), 2.61–2.59 (m, 2H, H_{ax}-2, H_{ax}-6), 2.31 (s, 3H, H-4'"), 2.17 (s, 3H, H-5'"), 2.08-2.00 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.81 (C-4'), 163.08 (C-5""), 157.71 (C-3""), 151.73 (C-6""), 136.12 (C-1"), 130.22 (C-3", C-5"), 129.80 (C-2', C-6'), 127.91 (C-1'), 126.35 (C-2"), 125.76 (C-6"), 123.12 (C-4"), 114.28 (C-3', C-5'), 55.61 (C-1'"), 45.36 (C-2, C-6), 36.03 (C-3'"), 31.84 (C-4), 30.04 (C-2'"), 29.05 (C-3, C-5), 17.63 (C-4'", C-5'").

N-(4-ethoxyphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (8g)

Tea pink solid; Yield: 91 %; M.P: 145.8°C; Mol. formula: $C_{25}H_{31}N_5O_5S_2$; Mol. mass: 545.67 g/mol; IR (KBr, wave number, cm^{-1}) 2825 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1320 (CH_3), 1250 (S=O), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.05 (s, 1H, NH), 7.76 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.47 (d, *J* = 9.0 Hz, 2H, H-2", H-6"), 7.04 (d, *J* = 8.2 Hz, 2H, H-3', H-5'), 6.85 (d, *J* = 9.0 Hz, 2H, H-3", H-5"), 4.02 (q, *J* = 6.9 Hz, 2H, H-4'"), 3.91 (s, 3H, H-1'"), 3.90 (s, 3H, H-2'"), 3.88–3.84 (m, 2H, H_{eq}-2, H_{eq}-6), 3.46 (s, 2H, H-3'"), 2.68-2.64 (m, 1H, H-4), 2.60-2.56 (m, 2H, H_{ax}-2, H_{ax}-6), 2.14-2.02 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5), 1.41 (t, *J* = 6.9 Hz, 3H, H- 5'"), 1.27 (s, 3H, H-2'"); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.16 (C-4'), 163.09 (C-5""), 157.64 (C-3""), 155.68 (C-4"), 151.91 (C-6""), 131.24 (C-1"), 129.82 (C-2', C-6'),128.65 (C-1'), 121.36 (C-2", C-6"), 114.67 (C-3", C-5"), 114.28 (C-3', C-5'), 63.68 (C-1'"), 55.64 (C-2, C-6), 45.54 (C-4'"), 36.31 (C-3'"), 32.11 (C-4), 30.13 (C-2'"), 29.11 (C–3, C-5), 14.83 (C-5'").

2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-phenylacetamide (8h)

White solid; Yield: 92 %; M.P: 175.3 °C; Mol. formula: $C_{23}H_{27}N_5O_4S_2$; Mol. mass: 501.62 g/mol; IR (KBr, wave number, cm^{-1}) 2825 (Ar C-H), 1700 (C=O), 1600 (C=N), 1550 (Ar C=C), 1300 (CH_3), 1250 (S=O), 1150 (C-O-C), 725 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.23 (s, 1H, NH), 7.75 (d, *J* = 10.26 Hz, 2H, H-2', H-6'), 7.58 (d, *J* = 9.06 Hz, 2H, H-2", H-6"), 7.32-7.29 (m, 2H, H-2", H-5"), 7.08 (t, *J* = 8.3 Hz, 1H, H-4"), 7.03 (d, *J* = 10.26 Hz, 2H, H-3', H-5'), 3.93 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.87–3.83 (m, 2H, H_{eq}-2, H_{eq}-6), 3.46 (s, 3H, H-2'"), 2.68-2.62 (m, 1H, H-4), 2.59-2.55 (2H, H_{ax}-2, H_{ax}-6), 2.12-2.01 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.43 (C-4'), 163.11 (C-5""), 158.00 (C-3""), 157.69 (C-6""), 138.18 (C-1"), 132.13 (C-1'), 129.80 (C-3", C-5"), 128.84 (C-2', C-6'), 124.21 (C-4"), 119.78 (C-2", C-6"), 114.28 (C-3', C-5'), 55.61 (C-1'"), 45.49 (C-2, C-6), 36.45 (C-3'"), 32.12 (C-4), 30.11 (C-2'"), 29.13 (C-3, C-5).

2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(2-methylphenyl)acetamide (8i)

White amorphous solid; Yield: 92 %; M.P: 168.0°C; Mol. formula: $C_{24}H_{29}N_5O_4S_2$; Mol. mass: 515.64 g/mol; IR (KBr, wave number, cm^{-1}): 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1550 (Ar C=C), 1300 (CH_3), 1250 (S=O), 1150 (C-O-C), 750 (C-H): ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.70 (s, 1H, NH), 7.92 (d, *J* = 9.2 Hz, 1H, H-H-6"), 7.74 (d, *J* = 9.6 Hz, 2H, H-2', H-6'), 7.20-7.15 (m, 2H, H-4", H-5"), 7.07–7.02 (m, 3H, H-3', H-5', H-3"), 4.00 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.79–3.77 (m, 2H, H_{eq}-2, H_{eq}-6), 3.46 (s, 3H, H-2'"), 2.68-2.61 (m, 3H, H-4, H_{ax}-2, H_{ax}-6), 2.24 (s, 3H, H-4'"), 2.11-2.03 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.99 (C-4'), 163.08 (C-5""), 159.01 (C-3""), 157.73 (C-6""), 136.15 (C-1"), 130.42 (C-2"), 129.80 (C-2', C-6'),

129.29 (C-1'), 128.00 (C-6"), 126.42 (C-3"), 124.88 (C-5"), 122.39 (C-4"), 114.28 (C-3', C-5'), 55.61 (C-1'"), 45.33 (C-2, C-6), 36.04 (C-3'"), 31.82 (C-4), 30.03 (C-2'"), 29.07 (C–3, C-5), 18.11 (C-4'").

2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(3-methylphenyl)acetamide (8j)

Off white amorphous solid; Yield: 93 %; M.P: 155.0 ^{o}C; Mol. formula: $C_{24}H_{29}N_5O_4S_2$; Mol. mass: 515.64 g/mol; IR (KBr, wave number, cm^{-1}): 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1300 (CH_3), 1250 (S=O), 1100 (C-O-C), 725 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.11 (s, 1H, NH), 7.74 (d, J = 7.2 Hz, 2H, H-2', H-6'), 7.41 (br.s, 1H, H-2"), 7.36 (d, J = 8.2 Hz, 1H, H-6"), 7.18 (t, J = 7.8 Hz, 1H, H-5"), 7.02 (d, J = 8.8 Hz, 2H, H-3', H-5'), 6.91 (d, J = 7.5 Hz, 1H, H-4"), 3.92 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.86–3.84 (m, 2H, H_{eq}-2, H_{eq}-6), 3.46 (s, 3H, H-2'"), 2.64-2.57 (m, 1H, H-4), 2.57-2.53 (m, 2H, H_{ax}-2, H_{ax}-6), 2.33 (s, 3H, H-4'"), 2.10-2.00 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.41 (C-4'), 163.10 (C-5'""), 157.67 (C-3'""), 151.84 (C-6'""), 138.76 (C-1"), 138.05 (C-3"), 129.80 (C-2', C-6'), 129.70 (C-1'),128.66 (C-6"), 125.06 (C-2"), 120.38 (C-5"), 116.95 (C-4"), 114.29 (C-3', C-5'), 55.62 (C-1'"), 45.53 (C-2, C-6), 36.53 (C-3'"), 32.15 (C-4), 30.12 (C-2'"), 29.14 (C-3, C-5), 21.42 (C-4'").

2-[(5-{1-[(4-Methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4H-1,2,4-triazol-3-yl)sulfanyl]-N-(4-methylphenyl)acetamide (8k)

Off white amorphous solid; Yield: 86 %; M.P: 152.8 ^{o}C; Mol. formula: $C_{26}H_{33}N_5O_4S_2$; Mol. mass: 543.70 g/mol; IR (KBr, wave number, cm^{-1}) 2850 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1300 (CH_3), 1250 (S=O), 1100 (C-O-C), 725 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.09 (s, 1H, NH), 7.75 (d, J = 8.8 Hz, 2H, H-2', H-6'), 7.45 (d, J = 8.4 Hz, 2H, H-2", H-6"), 7.11 (d, J = 8.2 Hz, 2H, H-3", H-5"), 7.03 (d, J = 8.9 Hz, 2H, H-3', H-5'), 3.91 (s, 2H, H-3'"), 3.90 (s, 3H, H-1'"), 3.85–3.83 (m, 2H, H_{eq}-2, H_{eq}-6), 3.46 (s, 3H, H-2'"), 2.68-2.62 (m, 1H, H-4), 2.58-2.54 (m, 2H, H_{ax}-2, H_{ax}-6), 2.31 (s, 3H, H-4'"), 2.10-2.07(m, 2H, H_{eq}-3, H_{eq}-5), 2.03-2.00 (m, 2H, H_{ax}-3, H_{ax}-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.28 (C-4'), 163.09 (C-5'""), 157.65 (C-3'""), 151.86 (C-6'""), 135.60 (C-1"), 133.82 (C-4"), 129.81 (C-2', C-6'), 129.32 (C-3", C-5"), 129.79 (C-1'), 119.80 (C-2", C-6"), 114.28 (C-3', C-5'), 55.62 (C-1'"), 45.51 (C-2, C-6), 36.43 (C-3'"), 32.10 (C-4), 30.11 (C-2'"), 29.11 (C-3, C-5), 20.85 (C-4'").

RESULTS

Attempts were made by following the conventional as well as microwave assisted protocol in order to design the best economical methodology for the synthesis of a series of *N*-(substituted)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetamide based compounds. All the aimed compounds were successfully synthesized by conventional method but the time taken by every compound was long enough up to 9-19 hours which was very lazy and time taking synthetic strategy. The microwave assisted protocol for the synthesis of aimed compounds on the other hand was very attractive and authentic with respect to yield and time for the synthesis of designed series of compounds. The time taken for the synthesis of every compound at every step was ranged from 31-68 seconds (table 2) which was too short as compared to 9-19 hours spent by following the conventional methodology for the synthesis of all the target compounds. These finding made our minds that the microwave assisted methodology is best comparative to conventional method for the synthesis of 1,2,4-traizole based compounds having piperidine nucleus with potential pharmacological applications.

In the present work 4-methoxybenzene sulfonyl chloride (1) was treated with ethyl isonipecotate (2) for the synthesis of sulfonamide (3) in the presence of distilled water as a solvent. The sulfonamide (3) was refluxed with hydrazine monohydrate in the presence of methanol used as reaction medium in order to obtained compound 4. Compound 4 was refluxed with methyl isothiocyanate in ethanol first and then in 18% aq. potassium hydroxide (KOH) to change all the reaction mixture into final compound 5. A series of acetamides was reacted with compound 5 to design the final array of target compounds 8a-k, both by conventional as well as microwave assisted protocol. All the synthesized compounds were analyzed by thin layer chromatography to check their purity. Precipitation, filtration, recrystallization and solvent extraction techniques assisted us to acquire pure synthesized compounds in maximum yield. Proton nuclear magnetic resonance spectroscopy (^{1}H-NMR), carbon nuclear magnetic resonance spectroscopy (^{13}C-NMR) and infra-red (IR), the spectroscopic techniques were employed to characterize all the compounds and to confirm their structure.

DISCUSSION

The comparative synthetic study of compounds 8a-k (table 2) showed that these were synthesized just in few seconds by following the microwave assisted techniques and in hours by conventional synthetic strategy. On the other hand the synthetic yield with microwave assisted methodology was also very high comparative to that with conventional method. Time and yield are the key factors in the success of any project and we have achieved both of these goals by microwave assisted methodology. The short time synthesis is in the benefit of our project and thus emphasizes the importance of this strategy to serve the humanity in very fast and accurate style.

Table 1: Different *N*-substituted aryl groups

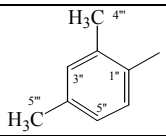
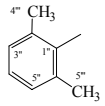
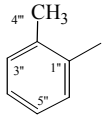
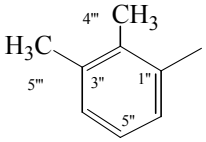
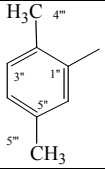
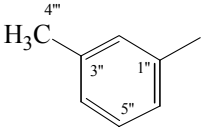
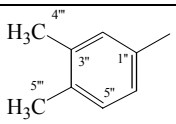
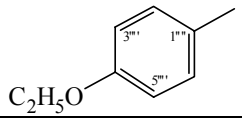
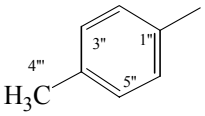
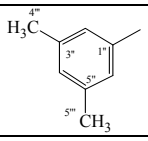
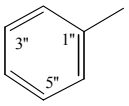
Comp.	R	Comp.	R	Comp.	R
8a		8e		8i	
8b		8f		8j	
8c		8g		8k	
8d		8h			

Table 2: Comparative study of conventional and microwave assisted methods for reaction time and % yield

Compounds	Reaction Time		Reaction Yield (%)	
	Conventional (hours)	Microwave (sec)	Conventional	Microwave
8a	14	33	70	89
8b	16	41	55	87
8c	11	42	58	95
8d	09	34	61	94
8e	17	36	66	95
8f	14	42	49	97
8g	19	31	73	90
8h	12	45	64	90
8i	11	68	72	92
8j	13	44	88	93
8k	17	32	44	86

Table 3: Enzyme inhibition results of the synthesized compounds (8a-k)

Compounds	IC_{50}±SEM (μM)		
	CA II inhibition	AChE inhibition	BChE inhibition
8a	17.7 ± 0.38	164.23±0.32	-
8b	20.30± 2.31	172.84±0.28	-
8c	23.59± 1.98	56.19±0.29	32.94±0.88
8d	51.72±1.54	98.25±0.29	-
8e	37.14± 1.09	93.74±0.21	-
8f	22.11± 1.68	315.47±0.27	-
8g	8.69 ± 0.38	11.87±0.19	26.01±0.55
8h	47.15± 1.39	21.43±0.91	40.11±1.46
8i	34.15 ± 1.34	46.25±0.15	-
8j	21.64 ± 0.15	127.36±0.26	113.24±0.35
8k	30.95 ± 0.98	32.85±0.18	-
Acetazolamide	0.12 ± 0.03		
Galantamine		4.0 ± 0.10	15.0 ± 0.67

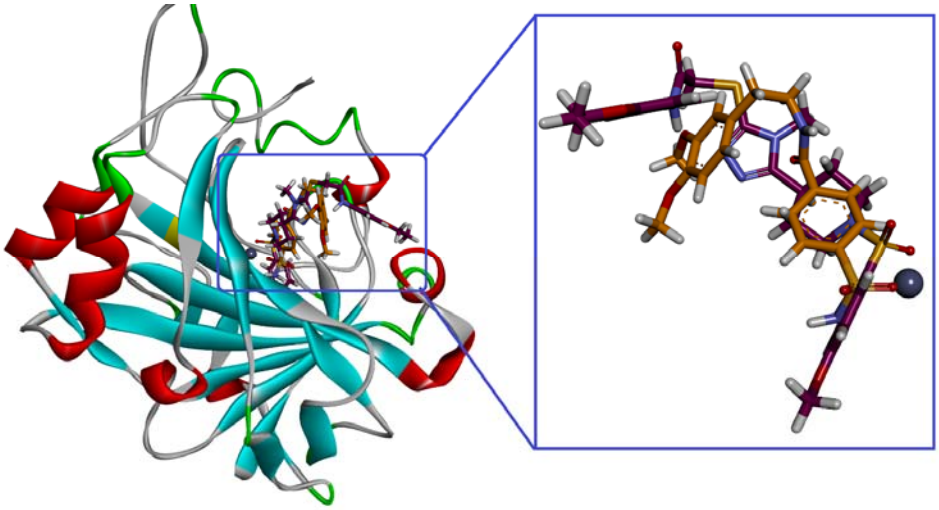


Fig. 1: In silico model of the superposed D7A and 8g in the active site of 1V9E.

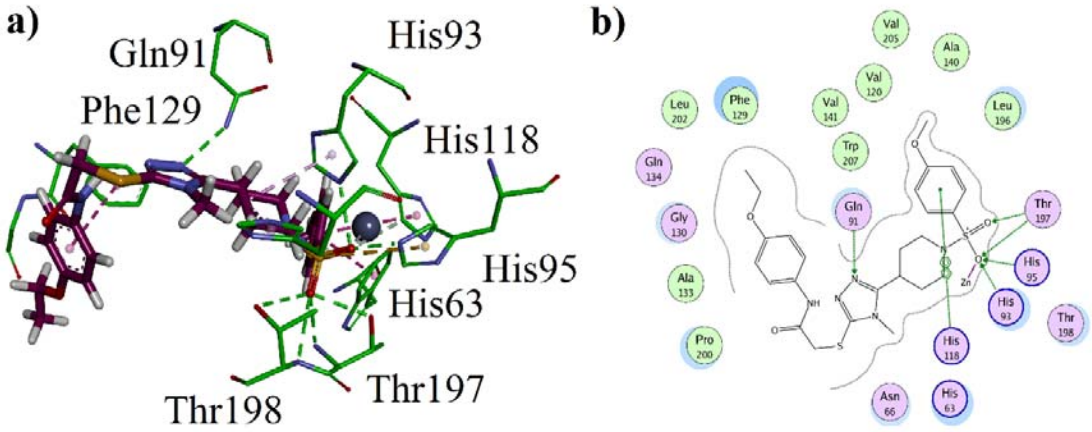


Fig. 2: (a) A close-up depiction of ribbon diagram of the docking pose of compound 8g in the binding site of 1V9E. Zinc ion is shown in blue-colored sphere while other key residues the active sites are represented as green stick model. Other pocket residues have been removed for better showing orientation and interactions; (b) 2D interactions of 8g and *b*CA-II generated by MOE software.

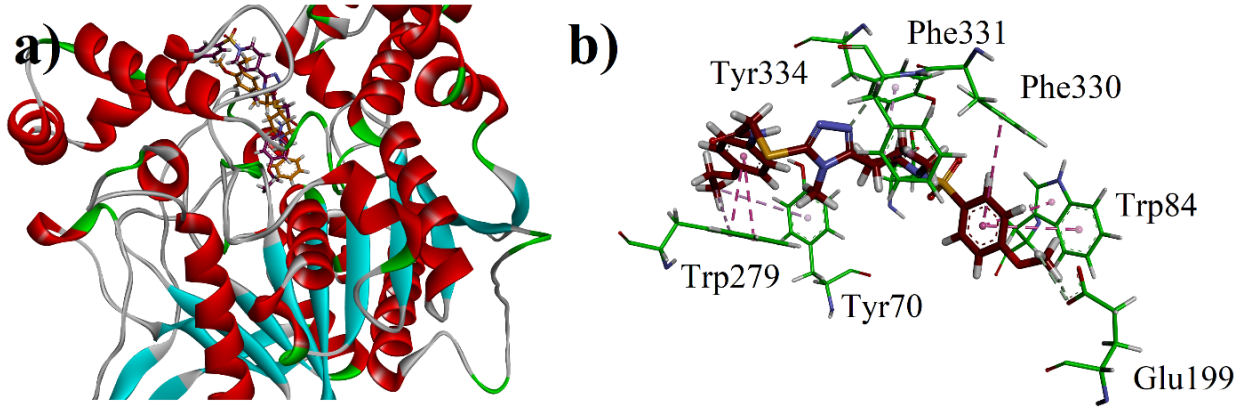
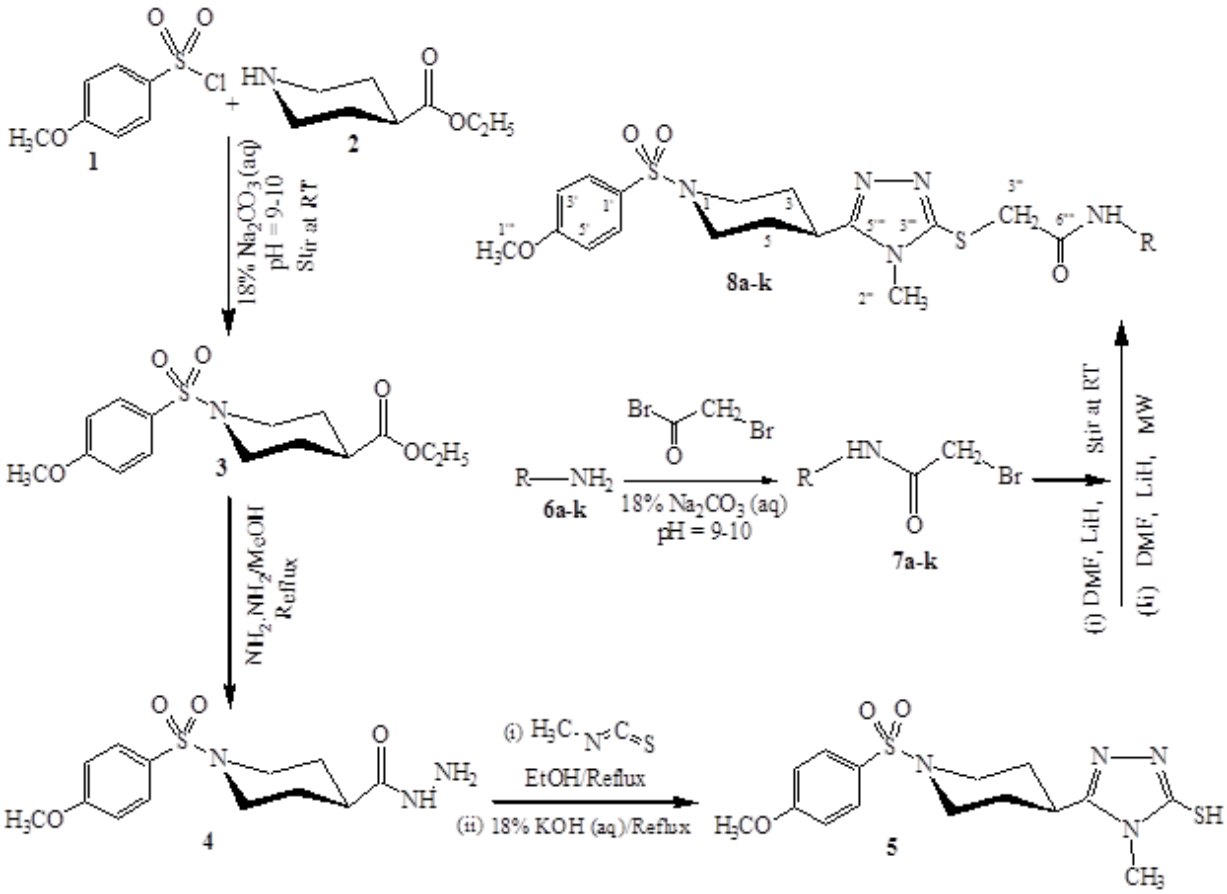


Fig. 3: (a) Computer generated model of compound 8g (purple) superimposed on donepezil (orange) in the binding site of 1EVE; (b) A close-up depiction of the docking pose of 8g in the active site of AChE (1EVE). The key residues the active sites are represented as green stick model. Other pocket residues have been removed for better showing orientation and interactions.



Scheme 1: Synthesis of *N*-(substituted)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetamide

The structural confirmation was elucidated by different spectroscopic techniques like ^{1}H-NMR and ^{13}C-NMR. The presence of various aromatic and aliphatic protons was confirmed by ^{1}H-NMR spectra. The four aromatic protons of sulfonyl moiety resonated as two doublets at δ (ppm) 7.74 and 7.03 with their *J* values of 8.2 Hz and 8.3 Hz respectively. While the remaining aromatic protons of benzene ring directly attached with amide group were confirmed by the peaks appearing at 7.71 (s, 1H, H-3") and 6.99-6.97 (m, 2H, H-5", H-6"). Three protons of methoxy appeared as singlet at 3.90 while three methyl protons of methyl group attached with the nitrogen atom of triazole ring resonated at 3.47 as a singlet. Two protons of methyl group directly attached with heteroatom were confirmed by the peak appearing at 3.99 (s, 2H, H-3'") and two methyl group attached with benzene ring were confirmed by the following peaks appearing at 2.29 (s, 3H, H-4'") and 2.18 (s, 3H, H-5'"). The presence of piperidine was justified by the four multiplet signals represented as 3.79–3.77 (m, 2H, H_{eq}-2, H_{eq}-6), 2.69-2.66 (m, 1H, H-4), 2.64-2.61 (m, 2H, H_{ax}-2, H_{ax}-6) and 2.08-2.01 (m, 4H, H_{eq}-3, H_{eq}-5, H_{ax}-3, H_{ax}-5). The available spectral data of ^{13}C-NMR spectroscopy was utilized to justify the presence of carbons as the back bone of structure of the discussed compound. The aromatic quaternary carbons present in the spectra were justified by the peaks appearing at 166.76 (C-4'), 163.08 (C-5""), 157.68 (C-3""), 151.78 (C-6""), 141.80 (C-1"), 134.62 (C-2"), 133.45 (C-4") and 129.52 (C-1') while the aromatic methine carbons of rings attached with sulfonyl group and amide group were confirmed by the peaks appearing at 129.80 (C-2', C-6'), 114.28 (C-3', C-5'), and 131.09 (C-6"), 126.92 (C-5"), 122.65 (C-3") respectively. The piperidine ring was justified by the presence of signals at 45.36 (C-2, C-6), 31.83 (C-4) and 29.06 (C-3, C-5). The discussed spectral information and supplementary material made us able to justify the discussed compound with name of *N*-(2,4-dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-methyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetamide.

Structure-activity relationship (SAR)

In vitro carbonic anhydrase inhibition potential

All the synthesized compounds (8a-k) were screened for their potential against bovine carbonic anhydrase II (*b*CA-II) enzyme. Acetazolamide was used as standard drug. The results of the CA-II inhibition are shown in table 3. Compounds 8a-f differs only in the position of two methyl groups at phenyl ring attached to nitrogen of acetamide functionality. The IC_{50} value of this set of compounds ranges from 17.7-51.72µM. Highest inhibition potential among these compounds was shown by 8a with IC_{50} value

of 17.7±0.38μM. The other synthesized compounds bear unsubstituted phenyl ring (8h) and mono-methyl substituted phenyl group (8i-k) at *o*-, *m*- and *p*-position. Highest inhibition potential among these compounds was shown by 8j with IC_{50} value of 21.64±0.15μM. All the compounds of this series of were good inhibitor of CA-II enzyme. Overall the excellent inhibition potential has been demonstrated by compound 8g. The best activity of this compound might be attributed to the presence of 4-ethoxyphenyl group. Compound 8g showed IC_{50} value of 8.69±0.38μM with respect to that of reference, 0.12±0.03μM.

In vitro acetyl cholinesterase (AChE) and butyryl cholinesterase (BChE) inhibition potential

All the synthesized compounds were further evaluated for their inhibition potential against AChE and BChE with galantamine as reference drug. Six compounds, 8a-f, have shown moderate potential to inhibit AChE. These compounds were found almost inactive against BChE (table 3). Compounds 8h-k showed moderate inhibition against both enzymes also. Among all the compounds, 8g bearing 4-ethoxyphenyl group was the most active one with IC_{50} values 11.87±0.19μM for AChE and 26.01±0.55μM for BChE. Compound 8g has shown excellent selectivity index (SI) of 2.19 (IC_{50} BChE/ IC_{50} AChE).

Docking studies

Docking studies on carbonic anhydrase

Molecular docking studies were carried out in the active site of bovine CA-II enzyme to predict the binding modes of the synthesized compounds. Crystal structure of *b*CA-II (PDB code 1V9E) was selected for this study. Docking simulations were carried out by using Molecular Operating Environment (MOE2016) software [Molecular Operating Environment (MOE), 2016.08; Chemical Computing Group ULC, 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, 2018]. Crystal structure of *b*CA-II is in Apo-form with no co-crystallized ligand. The enzyme was searched for its active site using MOE Site Finder. The active site was selected containing important residues and dummy atoms were created from the resulting alpha spheres. For the further validation of docking, co-crystallized ligand (D7A) from human CA-II (PDB code 3V7X) was also docked into the binding site of *b*CA-II and its interactions were analyzed. Binding mode analysis of the docked compounds revealed that active compounds coordinate well with Zn^{2+} and His63, His93, His95 and Thr197. The overlay of the most active compound 8g with D7A into the binding site of the 1V9E is shown in fig. 1. Computer generated three dimensional (3D) view of compound 8g is shown in fig. 2a-b. The fig. showed that compound fits well into the binding site and coordinates with Zn^{2+} and His63, His93, His95 and Thr197. A hydrogen bonding interaction was also found between nitrogen atom of the triazole ring and Gln91. Computed binding affinity and docking score for 8g is -10.8791 and -12.3894 kcal/mol respectively.

Docking studies on AChE

The synthesized compounds were docked into the active site of *Torpedo californica* (*Tc*AChE) using MOE software. X-ray crystal structure with PDB code 1EVE in complex with Anti-Alzheimer's drug donepezil was selected for this study. Re-docking process of native ligand (donepezil) was carried out for the validation of the docking process. It was noticed that active compounds establish π-π stacking interaction with Trp84 and Trp279. The binding orientation of most active AChE inhibitor 8g superimposed on donepezil is shown in fig. 3a. The analysis of binding interactions of 8g reveals that it forms π-π stacking interaction with dual binding site residue of AChE i.e. Trp84 and Trp279. Acetamide ring establishes π-π stacking interaction with Trp279, a tryptophan residue of peripheral anionic site (PAS). While, methoxyphenyl ring forms π-π stacking interaction with Trp84 of anionic subsite of the catalytic site (CAS). Another π-π stacking interaction was found between methoxyphenyl ring and Phe330 of aromatic gorge region. Inhibitor-enzyme complex is also stabilized by π-CH interactions (fig. 3b).

CONCLUSION

A library of compounds based on different heterocyclic moieties was successfully synthesized by using conventional as well as microwave assisted method. The comparative study on reaction time and % yield by microwave assisted as well as conventional method was carried out. All the compounds were characterized by the aid of spectroscopic techniques IR, ^{1}H-NMR, ^{13}C-NMR and EIMS. The whole array of synthesized compounds was screened against AChE, BChE and carbonic anhydrase. All the compounds were found active against AChE and carbonic anhydrase with the exception of BChE. The compound 8g was the outstanding against AChE, carbonic anhydrase and BChE with IC_{50} values 11.87±0.19, 8.69±0.38 and 26.01±0.55 μM respectively. The SAR was justified by the docking studies. After *in vivo* studies, the compounds could be considered as best anti-enzymatic agents against AChE and carbonic anhydrase enzymes.

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