Synthesis, *in vitro* and *in silico* studies of *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols as cholinesterase inhibitors

Muhammad Arfan[1], Sabahat Zahra Siddiqui[1*], Muhammad Athar Abbasi[1], Aziz-ur-Rehman[1], Syed Adnan Ali Shah[2,3], Muhammad Ashraf[4], Jameel Rehman[4], Rahman Shah Zaib Saleem[5], Hira Khalid[6], Rashid Hussain[6] and Uzman Khan[7]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]College of Natural Sciences, Department of Biological Science, Kongju National University, Gongju, South Korea
[3]Faculty of Pharmacy & Atta-ur-Rahman Institute for Natural Products Discovery (AuRIns), Level 9, FF3, Universiti Teknologi MARA, Puncak Alam Campus, Bandar Puncak Alam, Selangor Darul Ehsan, Malaysia
[4]Department of Chemistry, The Islamia University of Bahawalpur, Bahawalpur, Pakistan
[5]Department of Chemistry & Chemical Engineering, SBA School of Sciences & Engineering, Lahore University of Management Sciences, Opposite Sector-U, DHA, Lahore, Pakistan
[6]Department of Chemistry, Forman Christian College Lahore (A Chartered University), Lahore, Pakistan
[7]SGS Laboratories, Thoker Niaz Baig, Lahore, Pakistan

Abstract: The research was aimed to unravel the enzymatic potential of sequentially transformed new triazoles by chemically converting 4-methoxybenzoic acid *via* Fischer's esterification to 4-methoxybenzoate which underwent hydrazinolysis and the corresponding hydrazide (1) was cyclized with phenyl isothiocyanate (2) *via* 2-(4-methoxybenzoyl)-*N*-phenylhydrazinecarbothioamide (3); an intermediate to 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-thiol (4). The electrophiles; alkyl halides 5(a-g) were further reacted with nucleophilic *S*-atom to attain a series of *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g). Characterization of synthesized compounds was accomplished by contemporary spectral techniques such as FT-IR, ^{1}H-NMR, ^{13}C-NMR and EI-MS. Excellent cholinesterase inhibitory potential was portrayed by 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole; 6g against AChE (IC_{50}; 38.35±0.62μM) and BChE (IC_{50}; 147.75±0.67μM) enzymes. Eserine ($IC_{50;}$ 0.04±0.01μM) was used as reference standard. Anti-proliferative activity results ascertained that derivative encompassing long straight chain substituted at *S*-atom of the moiety was the most potent with 4.96 % cell viability (6g) at 25 μM and with 2.41% cell viability at 50μM among library of synthesized derivatives. *In silico* analysis also substantiated the bioactivity statistics.

Keywords: 1,2,4-Triazole, phenyl isothiocyanate, alkyl halides, cholinesterase inhibition, anti-proliferative activity, docking study.

INTRODUCTION

The compounds known to human in early ages were various heterocyclic structural forms; many heterocycles were used as medicine to cure various diseases (Tuberculosis, Malaria, neglected tropical diseases i.e. Leishmaniasis and Chagas diseases). Purine or pyrimidine bases fused with triazole core were exemplary e.g. *S*-triazolopyrimidines, as latent therapeutic agents. Both simple and fused triazole systems were synthesized after their introduction in chemical industry as fog inhibitors, in photographic suspensions and some usefulness as herbicides and convulsants (Siddiqui *et al.,* 2011, Khan *et al.,* 2012, Saad *et al.,* 2011 and Ghochikyan *et al.,* 2011).

1,2,4-Triazole is an aromatic structure, stabilized by resonance as shown below (fig. 1) (Koteleviskii *et al.,* 2001).

The triazole nucleus has two hybrid structures, each expressing a specific tautomeric arrangement. The attachment of *H*-atom to *N*-atom of triazole ring decide whether it is 1,2,4-triazole or 1,3,4-triazole (fig. 2). These both forms are designed as 1,2,4-triazole (1-*H* form) or 1,3,4-triazole (4-*H* form) (Obot *et al*., 2012).

Due to its purity and chemical configuration, 4-amino-1,2,4 triazole is used as an intermediate to obtain desired bioactive analogues with outstanding yields. e.g. 3-amino-1*H*-1,2,4-triazole have herbicidal and defoliant properties and regarded as catalase inhibitor (Konorev *et al.,* 1993) which is used to block certain ethanol-induced conducting effects (Aspberg *et al.,* 1993). Certain enantiomers of triazoles comprising of oxazolidine rings are considered to be active against *Candida albicans* infections in mice (Papakonstantinou *et al*., 2002). Some triazole derivatives act as angiotensin II receptor antagonists (Naito *et al.,* 1996). Additionally, numerous 1,2,4-triazole derivatives have been described as

**Corresponding author:* e-mail: sabahat.gcu@gmail.com

fungicidal, insecticidal (Chiu *et al.*, 1998), anti-microbial (Yasmin *et al.*, 2008), anti-asthmatic (Goss *et al*., 2004), anti-convulsants, anti-depressants agents (Chen *et al.*, 1997), and plant growth controllers. Furthermore, it is testified that pharmaceutical agents having triazole moieties, such as Vorozole (VII), Letrozole (VIII), and Anastrozole (IX), seemed to be precisely effective aromatase inhibitors against breast cancer cells (Ugale *et al*., 2016) (fig. 3). 1,2,4-Triazoles work together intensely with heme (Fe^{2+}) and aromatic substitutions on the triazoles are precise effective for interacting with the active site of aromatase (Takaoka *et al*., 1994).

The derivatives of *S*-triazolopyrimidines are used as latent therapeutic agents (Shukla *et al*., 2014); some 3-amino-1,2,4-triazole, 3-mercapto-1,2,4triazole, and 3-nitro-1,2,4-triazole derivatives possess anti-thyroid activity. 1,2,4-Triazoles are amphoteric in nature and form salts with acid as well as base. Over the last few spans, considerable interest in synthesis and characterization of 1,2,4-triazole has been developed because of their potent activity as biological pharmaceuticals (Sahoo *et al*., 2013). This heterocyclic moiety and its derivatives hold broadly divergent activities e.g., bacteriostatic, bactericidal (Al-Khuzaie et al., 2014), anti-fungal (Jess *et al*., 2014), muscle relaxant, tranquilizing (Hou *et al.*, 2011), anti-carcinogenic (Baviskar *et al*., 2012 and Arul *et al*., 2014), anti-inflammatory (Mousa *et al.*, 2012), diuretic (Hanif *et al*., 2012), anti-viral (Jassim *et al*., 2011), tuberculostatic (Meenaxi *et al*., 2011) and anti-human immunodeficiency virus (HIV) (Jawad *et al*., 2016). A highly potent drug, 1-*β*-D-ribofuranosyl-[1*H*]-1,2,4triazole-3-carboxamide (Virazol; Glycosylated triazole VII) (Panchal *et al*., 2011) is very effective against DNA and RNA-viruses (Jerald *et al*., 2015). In fibric industry, 3-amino-1,2,4-triazole has been used as a commercial defoliant (Gudelj *et al.*, 2011). Furthermore, some triazole derivatives are also used in inks to attain smooth writing properties e.g. 3-amino-5-mercapto-1,2,4-triazole. Acid fading of dyestuff (Abdul-Hameed *et al*., 2014) is inhibited by *N*-benzylated aminotriazoles. Alzheimer's disease (AD) is most globally widespread neurodegenerative disorder which can be countered only by sedative treatment and to inhibit acetylcholinesterase (AChE) in order to increase concentration of acetylcholine in synaptic cleft (Susimaire *et al*., 2016). Computational chemistry and biological potential have fast-tracked the drug discovery system and helpful in designing new active drug candidates. Molecular docking is very effective in determination of active site for interaction between drugs and receptors and in understanding the binding orientation of drug, its affinity for target protein (Pushpan *et al*., 2012 and Bondock *et al*., 2012). Generally, proteins are responsible for the effective action of various drugs which could be justified through the binding of typical drug with the proteins in living system. The binding of drugs with the protein gave the idea about the effectiveness of the drug, so studies of synthesized drugs–protein binding interaction are the active area of research (Macaaev *et al*., 2005).

Our current research work and literature (Tarikogullari *et al*., 2016, Bulut *et al*., 2006) mentioned on 1,2,4-triazoles' inhibiting cholinesterases by interfering mitosis and denaturing serum protein reversibly. A new series of pharmaceutically valuable scaffolds of 1,2,4-triazoles encompassing 4-methoxyphenyl moiety were synthesized and were evaluated for their inhibitory potential against cholinesterases and anti-proliferative activity was also estimated along with docking.

MATERIALS AND METHODS

Materials and instruments

Analytical grade chemicals and solvents involved in research work were procured from Sigma Aldrich & Alfa Aesar. The reaction were monitored and completion was confirmed by thin layer chromatography (TLC) utilizing various percentage of mobile phase (*n*-hexane: ethyl acetate), visualized under 254 nm UV spectral lines. Open tube capillary method was used to determine the melting points of compounds on Griffin & George melting point. Jasco-320-A spectrophotometer (wave number in cm^{-1}) in KBr recorded IR spectra. EIMS were measured on Jeol MS 600H-1 instrument having the data system. ^{1}H (600 MHz) and ^{13}C (150 MHz) NMR were recorded on Bruker system to elucidate the structure of synthesized products.

Synthesis

5-(4-Methoxyphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (4)

4-Methoxybenzohydrazide (16.6 g; 0.1 mol; 1) was refluxed in methanolic solution of phenyl isothiocyanate (12 mL; 0.1 mol; 2) for 15 min., after which the reaction contents were cooled down, to precipitate 2-(4-methoxybenzoyl)-*N*-phenylhydrazinecarbothioamide (3). Precipitates were filtered, washed with methanol, air-dried and were refluxed for 6 h and cyclized in 30 mL 10 % NaOH solution. After reaction completion, the reaction mixture was acidified with few aliquots of HCl (pH 2-3) to achieve 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (4) which was washed with access of distilled water and recrystallized from absolute ethanol.

S-Alkylated 5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiols 6(a-g)

5-(4-Methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (0.2 g; 0.7 mmol; 4) solublized in *N*,*N*-dimethylformamide in a 250ml round bottom flask along with catalytic amount of lithium hydride (0.0008 g; 0.1 mmol) was stirred for 0.5 h at RT, different alkyl halides [0.2-0.3 g, 0.7 mmol; 5(a-g)] were then added to the mixture which was further stirred for 7-8 h at room temperature. After reaction completion, the products were

Table 1: Alkyl halides utilized in synthesize of *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g).

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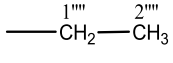
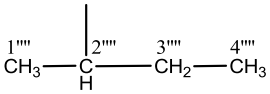
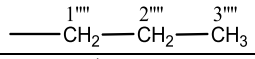
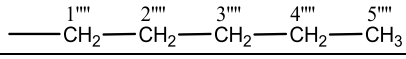
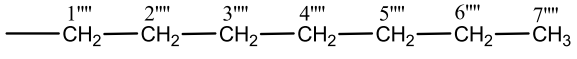
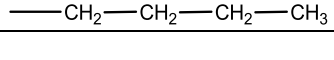
6a		6e	
6b		6f	
6c		6g	
6d			

Table 2: AChE/BChE assay of *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g).

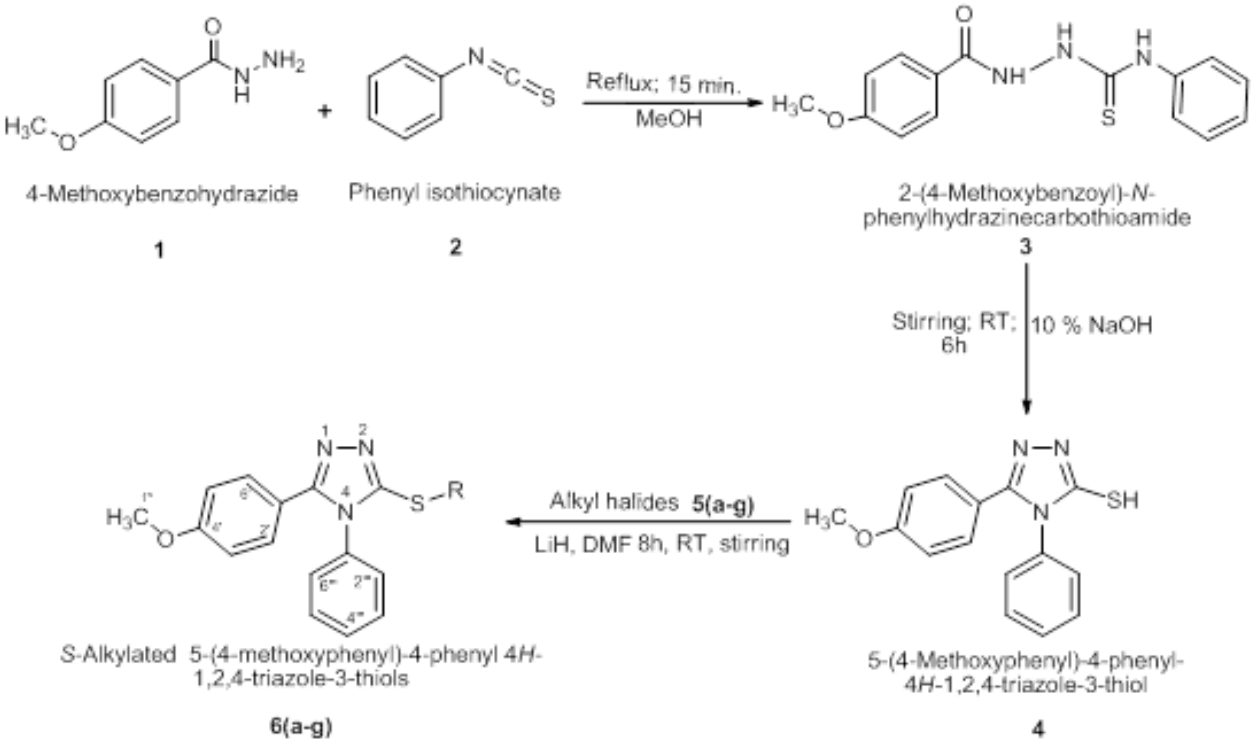
No.	Codes	Enzyme Inhibition Assay			
		(AChE)		(BChE)	
		Inhibition (%) 0.5 mM	IC_{50} (μM)	Inhibition (%) 0.5 mM	IC_{50} (μM)
1.	6a	7.38±0.25	-	59.32±0.63	342.17±0.56
2.	6b	13.25±0.24	-	61.87±0.58	291.68±0.52
3.	6c	25.43±0.36	-	63.42±0.54	260.34±0.47
4.	6d	8.79±0.23	-	37.64±0.45	-
5.	6e	5.34±0.24	-	64.26±0.78	258.41±0.71
6.	6f	22.45±0.36	-	35.43±0.42	-
7.	6g	90.35±0.78	38.35±0.62	80.79±0.75	147.75±0.67
	Eserine	91.27±1.17	0.04±0.001	91.27±1.17	0.04±0.001

Table 3: *In-silico* analysis of 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (**6g**) with active binding site interactions against Acetylcholinesterase (AChE)

Acetylcholinesterase Binding Interactions of 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole						
Code	B.E (KJ/mol)	H-Bonding		Arene-Arene Interactions	Electrostatic Interactions	Hydrophobic Interactions
		Interacting Residues	Distance (Å)			
6g	-7.40	Tyr124:OH---O:Anisole ring	3.59	Tyr341---Anisole Ring	Asp74 with Anisole Ring	Trp286---N-Phenyl ring
		Ser293:OH---N:Triazole ring	3.16	Phe295---Anisole ring	Trp286 with Sulfur	Phe297---Anisole Ring
		Ser293:OH---N:Triazole ring	3.56		Glu292 with Sulfur	Phe338---Anisole ring
		Tyr337:OH---O:Anisole ring	3.15			Phe295---Anisole ring
		Tyr341:OH---O:Anisole ring	4.65		Pro290 with Sulfur	Leu289---Heptythio
					Glu202 with Anisole Ring	

Table 4: SRB assay of *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g)

Sr. No.	Codes	% age cell viability					
		25 μM			50 μM		
		Mean	SD	N	Mean	SD	N
1	6a	87.67	18.76	2	65.91	10.86	2
2	6b	78.51	7.70	2	63.71	10.30	2
3	6c	84.54	11.79	2	68.98	1.22	2
4	6d	33.99	6.29	2	10.63	5.00	2
5	6e	60.44	3.13	2	33.41	1.40	2
6	6f	60.51	1.86	2	32.90	1.48	2
7	6g	4.96	0.98	2	2.41	0.72	2



Scheme 1: Schematic representation for synthesis of *S*-Alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g)

precipitated by quenching reaction mixture in ice cold water and in some cases extracted *via* solvent extraction using chloroform as an organic phase to afford *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g) in good yields.

Biological activity

Cholinesterase activity

The cholinesterase (acetyl/butyryl) assay was performed by method reported by (Ellman *et al*., 1961). 100 μL of reaction mixture having 60 μL phosphate buffer (50 mM) maintained at PH 7.7, 10 μL of enzyme (0.005 unit well^{-1}) and 10 µL (0.5 mM well^{-1}) of compound under study was pre-read at 405 nm. Further contents were pre-incubated at 37 ^{0}C for 10 min. and reaction was initiated by addition of 10 μL acetyl/butyryl thiocholine iodide (substrate) and 10 μL of DTNB after which absorbance was determined at 405 nm after 15 min. of incubation at 37^{o}C. Readings were performed in triplicate with controls. Eserine was used as reference standard.

Inhibition (%) = [(B-S)/B] ×100

whereas,

B and S are absorbance for the blank and samples respectively. The IC_{50} was calculated was calculated with the assistance of EZ-Fit Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA).

Anti-proliferative (SRB) activity

The anti-proliferative activity of the compounds against HCT 116 human colon cancer cell line was evaluated by Sulforhodamine B (SRB) assay (Al-Samaraie *et al*., 2006, Vichai and Kirtikara, 2006).

After trypsinized, calculation and dilution of HCT 116 cells to 15k cellsmL^{-1}, 1500 cellwell^{-1} conc. was produced by addition of 100 μL diluted cells into every well of 96 well culture dishes (SPL life science®). which were incubated for 1 day with 5% CO_2 at 37^{o}C. DMSO was used as control, 50 & 25mM conc. of compounds was used for treating seeds cells in screening. Cytotoxicity assay was calculated after incubation of cells for three days at 37^{o}C. Fixation of cells was made by the addition of Cl_3CCOOH/well. The dishes were swept and desiccated with H_2O several times after 2 h incubation. Each well is supplemented with 0.06% SRB dye (100μL) after 30 min. incubation at 25^{o}C. Diluted CH_3COOH was used for washing of surplus SRB dye. The dye in each cells was solubilized in 10 *m*M *Tris* base pH 10.5 solution after 5 min. shaking. Absorbance of solubilized dye was determined at 490 nm wavelength.

Anti-proliferation (%) = [(B-S)/B] ×100

Where as,

B and S are absorbance for the blank and samples respectively.

Molecular docking

The AChE crystal structure (PDB accession code, *1B41*) (Kryger et al., 2000) was retrieved from the PDB. The missing residues in the crystal structure were constructed

by using the program, UCSF Chimera 1.6.16 (Pettersen *et al*., 2004). The pdb file (PDB accession code, *2WID*) was retrieved from protein databank and its missing residues were constructed by aligning it to the other pdb file (PDB accession code, *1P0P*). All water molecules were removed from the retrieved crystal structures using the program, VMD 1.9 (William *et al*; 1994). Both AChE and BChE were allowed to dock to experimentally synthesize 13 active compounds including parent compounds. The 3D structures of all compounds were constructed in pdb format and subsequently optimized at semi empirical RM1 level of theory using by the program Gabedit (Allouche AR et al; 2010) and MOPAC 2012, (Kryger *et al*., 2000) respectively. The docking study of all compounds was accomplished by the software, Auto-Dock Vina, (Trott O; 2010 using built in Lamarckian genetic algorithm method. A total of 10 runs were performed for each docking and rests of parameters were set to default values.

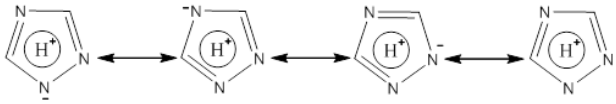


Fig. 1: Resonance forms of 1,2,4-triazlole (I-IV)

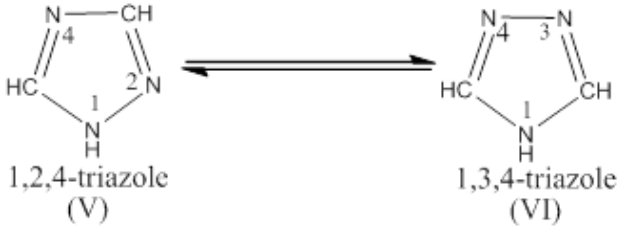


Fig. 2: Tautomeric forms of 1,2,4-triazole and 1,3,4-triazole

The search space was restricted to a grid box size of 46 x 46 x 46 in x, y and z dimensions, respectively, centered on the binding site of protein with x, y, and z coordinates of 120.491, 106.059 and -136.443 Å, respectively. All the docking runs were performed on Intel (R) Core (TM) i7-5500U CPU @ 2.40 GHz of Lenovo origin, with 8.0 GB DDR RAM. Auto-Dock Vina was compiled and run under Linux Ubuntu 64-bit.

RESULTS

The synthetic methodology is mentioned in experimental for synthesis of *S*-alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol and its derivatives [Scheme 1, table 1; 6(a-g)]. The research work has been premeditated to explore biological potential *S*-alkylated-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols against cholinesterase along with useful anti-proliferative activity.

Chemistry

The synthesis was initiated by esterification of 4-methoxybenzoic acid *via* Fischer's esterification method to ethyl-4-methoxybenzoate which underwent hydrazinolysis with hydrazine hydrate to yield 4-methoxybenzohydrazide. Benzohydrazide was refluxed with phenyl isothiocynate for 15 min. in MeOH to yield precipitates of intermediate 2-(4-methoxybenzoyl)-*N*-phenylhydrazinecarbothioamide as intermediate which were cyclized under refluxed and hydrolyzed in aqueous NaOH to achieve 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol. Further, the parent 1,2,4-triazole-3-thiol was alkylated utilizing a series of alkyl halides with catalytic amount LiH which act as base in aprotic medium provided by *N*,*N*-dimethylformaide; DMF to afford target compounds 6(a-g) in excellent yields and purity. All synthesized compounds were characterized by contemporary spectral techniques e.g. IR, EI-MS, ^{1}H-NMR and ^{13}C-NMR to elucidate their structures.

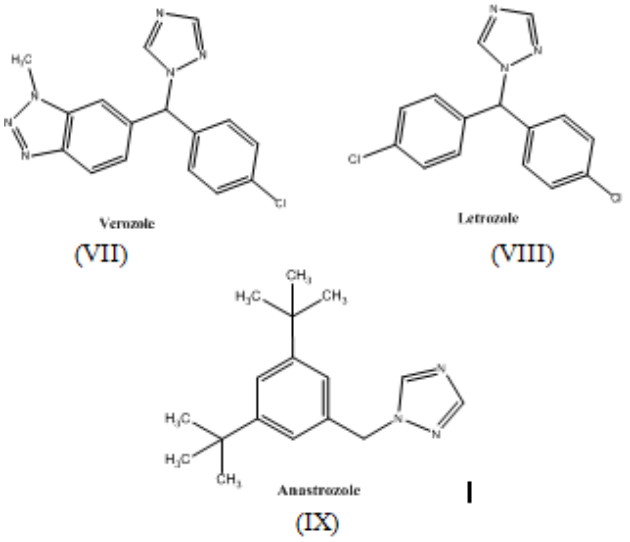


Fig. 3: Pharmaceutically active commercially available triazoles (VII-IX)

Spectral characterization

5-(4-Methoxyphenyl)-4-phenyl-4H-1,2,4-triazole-3-thiol (4)

White amorphous powder: Yield: 90%; M.P: 272-273^{0}C; Molecular formula: $C_{15}H_{13}N_3OS$; Molecular weight: 283 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2938 (Ar. C-H str.), 2354 (S-H), 1608 (C=N), 1408 (C=C), 1256 (C-O), 1177 (C-N), 1023 (C-S), 835 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 12.25 (s, 1H, -SH), 7.48-7.44 (m, 3H, H-3‴ to H-5‴), 7.35 (d, J = 10.5 Hz, 2H, H-2′ & H-6′), 7.31 (d, J = 8.7 Hz, 2H, H-2‴ & H-6‴), 6.80 (d, J = 10.5 Hz, 2H, H-3′ & H-5′), 3.89 (s, 3H, -OCH$_3$-1″); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 168.3 (C-4′), 161.0 (C-5), 151.1 (C-3), 134.3 (C-1‴), 129.5 (C-2′ & C-6′), 129.5 (C-4‴), 129.4 (C-3‴ & C-5‴), 128.1 (C-2‴ &C-6″), 117.4 (C-1′), 113.8 (C-3′ & C-5′), 55.0 (C-1″); EI-MS: *m/z* 283 $[C_{15}H_{13}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^{+}$ $[M-1]^{\bullet+}$, 250 $[C_{15}H_{12}N_3O]^{+}$, 224 $[C_{14}H_{12}N_2O]^{+}$, 210 $[C_{14}H_{12}NO]^{+}$, 147 $[C_8H_7N_2O]^{+}$, 133 $[C_8H_7NO]^{+}$, 77 $[C_6H_5]^{+}$, 73 $[CHN_2S]^{+}$, 51 $[C_4H_3]^{+}$.

3-(Ethylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6a)

White amorphous powder: Yield: 74 %; M.P: 132^{0}C; Molecular formula: $C_{17}H_{17}N_3OS$; Molecular weight: 311 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2944 (Ar. C-H str.), 1610 (C=N), 1439 (C=C), 1258 (C-O), 1172 (C-N), 1027 (C-S), 829 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.52-7.50 (m, 3H, H-3‴ to H-5‴), 7.36 (d, J = 8.7 Hz, 2H, H-2′ & H-6′), 7.24 (dd, J = 2.1, 7.8 Hz, 2H, H-2‴ & H-6‴), 6.80 (d, J = 8.7 Hz, 2H, H-3′ & H-5′), 3.79 (s, 3H, -OCH_3-1″), 3.28 (q, J = 7.3 Hz, 2H, -CH_2-1‴), 1.44 (t, J = 7.4 Hz, 3H, -CH_3-2‴); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 160.57 (C-4′), 154.72 (C-5), 152.37 (C-3), 134.56 (C-1‴), 129.85 (C-2′ & C-6′), 129.70 (C-4‴), 129.59 (C-3‴ & C-5‴), 127.45 (C-2‴ &C-6‴), 119.21 (C-1′), 113.92 (C-3′ & C-5′), 55.23 (C-1″), 26.96 (C-1‴′), 14.73 (C-2‴′); EI-MS: *m/z* 311 $[C_{17}H_{17}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^+$, 224 $[C_{14}H_{12}N_2O]^+$, 210 $[C_{14}H_{12}NO]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

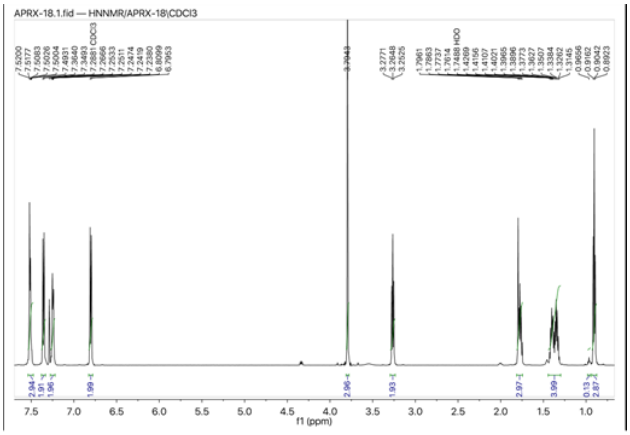


Fig. 4: ^{1}H-NMR of 3-(*n*-pentylthio)-5-(4-methoxyphenyl)- 4-phenyl-4*H*-1,2,4-triazole (6f).

3-(n-Propylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6b)

White amorphous solid: Yield: 78 %; M.P: 96 ^{0}C; Molecular formula: $C_{18}H_{19}N_3OS$; Molecular weight: 325 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2970 (Ar. C-H str.), 1614 (C=N), 1433 (C=C), 1256 (C-O), 1175 (C-N), 1026 (C-S), 838 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.52-7.51 (m, 3H, H-3‴ to H-5‴), 7.35 (d, J = 8.4 Hz, 2H, H-2′ & H-6′), 7.24 (br.d, J = 6.1 Hz, 2H, H-2‴ & H-6‴), 6.80 (d, J = 8.4 Hz, 2H, H-3′ & H-5′), 3.79 (s, 3H, -OCH_3-1″), 3.25 (t, J = 7.2 Hz, 2H, -CH_2-1‴), 1.81 (sex., J = 7.3 Hz, 2H, -CH_2-2‴), 1.03 (t, J = 7.3 Hz, 3H, -CH_3-3‴); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 160.56 (C-4′), 154.70 (C-5), 152.57 (C-3), 134.6 (C-1‴), 129.83 (C-2′ & C-6′), 129.70 (C-4‴), 129.62 (C-3‴ & C-5‴), 127.47 (C-2‴ &C-6‴), 119.25 (C-1′), 113.91 (C-3′ & C-5′), 55.22 (C-1″), 34.55 (C-1‴′), 22.77 (C-2‴′), 13.25 (C-3‴′); EI-MS: *m/z* 325 $[C_{18}H_{19}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^+$, 210 $[C_{14}H_{12}NO]^+$, 133 $[C_8H_7NO]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

3-(iso-Propylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6c)

White amorphous powder: Yield: 80 %; M.P: 151 ^{0}C; Molecular formula: $C_{18}H_{19}N_3OS$; Molecular weight: 325 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2969 (Ar. C-H str.), 1616 (C=N), 1434 (C=C), 1253 (C-O), 1177 (C-N), 1025 (C-S), 840 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.51-7.49 (m, 3H, H-3‴ to H-5‴), 7.35 (d, J = 8.4 Hz, 2H, H-2′ & H-6′), 7.24 (br.d, J = 7.6 Hz, 2H, H-2‴ & H-6‴), 6.80 (d, J = 8.46 Hz, 2H, H-3′ & H-5′), 3.95 (hept., J = 7.2 Hz, 2H, -CH_2-1‴), 3.79 (s, 3H, -OCH_3-1″), 1.43 (d, J = 6.6 Hz, 6H, -CH_3-2‴); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 160.57 (C-4′), 154.54 (C-5), 151.97 (C-3), 134.69 (C-1‴), 129.76 (C-2′ & C-6′), 129.63 (C-4‴), 129.60 (C-3‴ & C-5‴), 127.47 (C-2‴ & C-6‴), 119.28 (C-1′), 113.90 (C-3′ & C-5′), 55.25 (C-1″), 38.68 (C-1‴′), 23.47 (C-2‴′); EI-MS: *m/z* 325 $[C_{18}H_{19}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^+$, 210 $[C_{14}H_{12}NO]^+$, 133 $[C_8H_7NO]^+$, 77 $[C_6H_5]^+$, 51 $[C_4H_3]^+$.

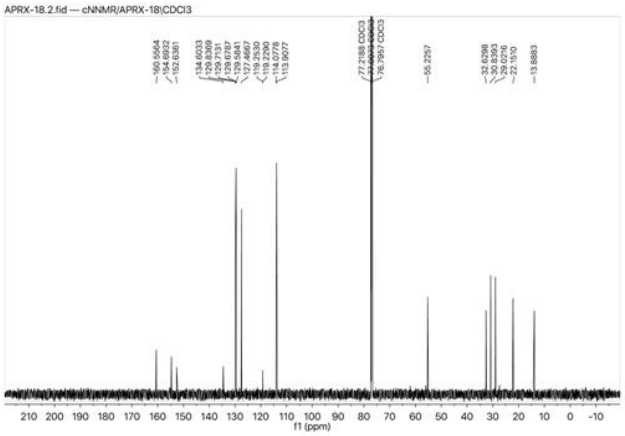


Fig. 5: ^{13}CNMR of 3-(*n*-pentylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6f).

3-(n-Butylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6d)

White amorphous powder: Yield: 73%; M.P: 115^{o}C; Molecular formula: $C_{19}H_{21}N_3OS$; Molecular weight: 339 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2937 (Ar. C-H str.), 1615 (C=N), 1433 (C=C), 1255 (C-O), 1178 (C-N), 1023 (C-S), 834 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.57-7.51 (m, 3H, H-3‴ to H-5‴), 7.40 (d, J = 8.2 Hz, 2H, H-2′ & H-6′), 7.34 (dist.dd, J =2.0, 8.16 Hz, 2H, H-2‴ & H-6‴), 6.80 (d, J=8.2 Hz, 2H, H-3′ & H-5′), 3.79 (s, 3H, -OCH_3-1″), 3.27 (t, J=8.76 Hz, 2H, -CH_2-1‴), 1.75(quint., J = 8.7 Hz, 2H, -CH_2-2‴), 1.46 (sex., J=8.7 Hz, 2H, -CH_2-3‴), 0.94 (t, J=8.8 Hz, 3H, -CH_3-4‴); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 160.57 (C-4′), 154.69 (C-5), 152.62 (C-3), 134.4 (C-1‴), 129.82 (C-2′ & C-6′), 129.66 (C-4‴), 129.58 (C-3‴ & C-5‴), 127.47 (C-2‴ &C-6‴), 119.27 (C-1′), 113.91 (C-3′ & C-5′), 55.22 (C-1″), 32.34 (C-1‴′), 31.36 (C-2‴′), 21.82 (C-3‴′), 13.5 (C-4‴′); EI-MS: *m/z* 339 $[C_{19}H_{21}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^{\bullet+}$, 250 $[C_{15}H_{12}N_3O]^+$, 210 $[C_{14}H_{12}NO]^+$, 133 $[C_8H_7NO]^+$, 89 $[C_4H_9S]^+$, 77 $[C_6H_5]^+$, 57 $[C_4H_9]^+$, 51 $[C_4H_3]^+$.

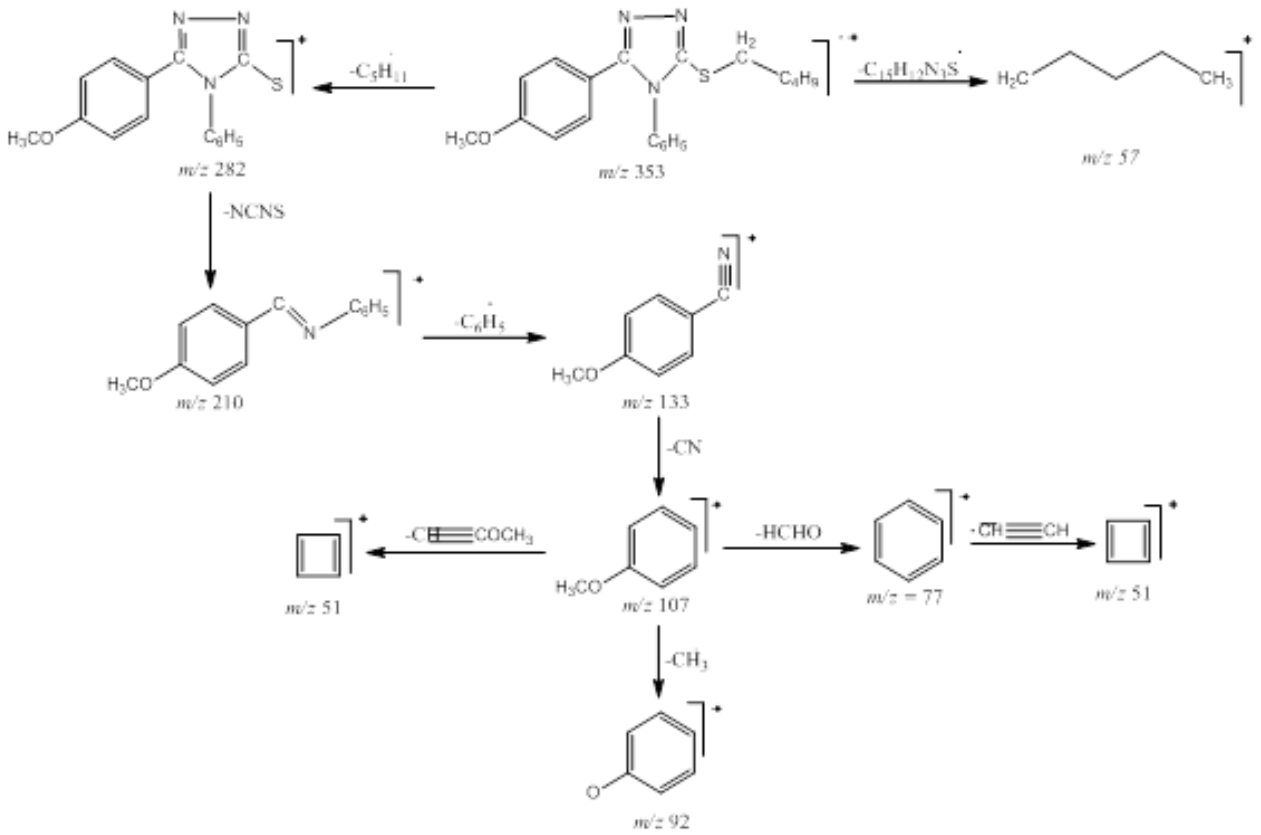


Fig. 6: Proposed EI-MS fragmentation pattern of 3-(*n*-pentylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6f).

3-(sec-Butylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6e)

Off-white amorphous Powder: Yield: 67 %; M.P: 110°C; Molecular formula: $C_{19}H_{21}N_3OS$; Molecular weight: 339 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2935 (Ar. C-H str.), 1617 (C=N), 1435 (C=C), 1257 (C-O), 1177 (C-N), 1025 (C-S), 836 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.52-7.49 (m, 3H, H-3‴ to H-5‴), 7.41 (d, J = 8.5 Hz, 2H, H-2′ & H-6′), 7.24 (dd, J = 2.0, 8.4 Hz, 2H, H-2‴ & H-6‴), 6.80 (d, J = 8.5 Hz, 2H, H-3′ & H-5′), 3.79 (s, 3H, OCH_3-1″), 1.77 (quint., J = 7.7 Hz, 2H, CH_2-3‴), 1.67 (sex., J = 7.0Hz, 1H, CH-2‴), 1.42 (d, J = 6.9 Hz, 3H, CH_3-1‴), 0.99 (t, J = 7.3 Hz, 3H, CH_3-4‴); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 160.54 (C-4′), 154.57 (C-5), 152.05 (C-3), 134.72 (C-1‴), 129.75 (C-2′ & C-6′), 129.56 (C-3‴ & C-5‴), 128.51 (C-4‴), 127.61 (C-2‴ &C-6‴), 119.30 (C-1′), 113.89 (C-3′ & C-5′), 55.26 (C-1″), 45.16 (C-2‴), 29.74 (C-3‴), 20.97 (C-1‴), 11.25 (C-4‴); EI-MS: *m/z* 339 $[C_{19}H_{21}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^+$, 250 $[C_{15}H_{12}N_3O]^+$, 210 $[C_{14}H_{12}NO]^+$, 133 $[C_8H_7NO]^+$, 89 $[C_4H_9S]^+$, 77 $[C_6H_5]^+$, 57 $[C_4H_9]^+$, 51 $[C_4H_3]^+$.

3-(n-Pentylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6f)

White amorphous powder: Yeild: 75%; M.P: 117°C; Molecular formula: $C_{20}H_{23}N_3OS$; Molecular weight: 353 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2944 (Ar. C-H str.), 1620 (C=N), 1443 (C=C), 1260 (C-O), 1173 (C-N), 1026 (C-S), 830 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.52-7.50 (m, 3H, H-3‴ to H-5‴), 7.35 (d, J = 8.8 Hz, 2H, H-2′ & H-6′), 7.24 (dist.dd, J = 3.3, 7.8 Hz, 2H, H-2‴ & H-6‴), 6.79 (d, J = 8.8 Hz, 2H, H-3′ & H-5′), 3.79 (s, 3H, -OCH_3-1″), 3.26 (t, J = 7.4 Hz, 2H, -CH_2-1‴), 1.77 (quint., J = 7.8 Hz, 2H, -CH_2-2‴), 1.40 (quint., J = 6.8 Hz, 2H, -CH_2-3‴), 1.36 (sex., J = 7.0 Hz, 2H, -CH_2-4‴), 0.90 (t, J = 7.2 Hz, 3H, -CH_3-5‴); ^{13}C-NMR (150 MHz, $CDCl_3$): δ 160.55 (C-4′), 154.69 (C-5), 152.63 (C-3), 134.60 (C-1‴), 129.83 (C-2′ & C-6′), 129.67 (C-4‴), 129.58 (C-3‴ & C-5‴), 127.46 (C-2‴ & C-6‴), 119.23 (C-1′), 113.90 (C-3′ & C-5′), 55.22 (C-1″), 32.62 (C-1‴), 30.83 (C-3‴), 29.02 (C-2‴), 22.15 (C-4‴), 13.88 (C-5‴); EI-MS: *m/z* 353 $[C_{20}H_{23}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^+$, 210 $[C_{14}H_{12}NO]^+$, 133 $[C_8H_7NO]^+$, 77 $[C_6H_5]^+$, 57 $[C_4H_9]^+$, 51 $[C_4H_3]^+$.

3-(n-Heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6g)

White amorphous powder; Yield: 70%; M.P: 103°C; Molecular formula: $C_{22}H_{27}N_3OS$; Molecular weight: 381 gmol^{-1}; IR (KBr), V_{max} (cm^{-1}): 2934 (Ar. C-H str.), 1615 (C=N), 1441 (C=C), 1256 (C-O), 1171 (C-N), 1023 (C-S), 828 (C-H bend); ^{1}H-NMR (600 MHz, $CDCl_3$): δ 7.52-7.49 (m, 3H, H-3‴ to H-5‴), 7.35 (d, J = 8.7 Hz, 2H, H-2′ & H-6′), 7.24 (dd, J = 2.2, 7.8 Hz, 2H, H-2‴ & H-6‴),

6.80 (d, *J* = 8.7 Hz, 2H, H-3′ & H-5′), 3.79 (s, 3H, -OCH_3-1″), 3.26 (t, *J* = 7.4 Hz, 2H, -CH_2-1‴), 1.76 (quint., *J* = 7.3 Hz, 2H, -CH_2-2‴), 1.40 (quint., *J* = 6.7 Hz, 2H, -CH_2-3‴), 1.27-1.25 (m, 6H, -CH_2-4‴ to 6‴), 0.90 (t, *J* = 7.6 Hz, 3H, -CH_3-7‴); ^{13}C-NMR (150 MHz, $CDCl_3$): *δ* 160.55 (C-4′), 154.69 (C-5), 152.64 (C-3), 134.59 (C-1‴), 129.83 (C-2′ & C-6′), 129.67 (C-4‴), 129.58 (C-3‴ & C-5‴), 127.46 (C-2‴ & C-6‴), 119.24 (C-1′), 113.90 (C-3′ & C-5′), 55.22 (C-1″), 32.67 (C-1⁗), 31.66 (C-5⁗), 29.32 (C-2⁗), 28.73 (C-4⁗), 28.66 (C-3⁗), 22.54 (C-6⁗), 14.02 (C-7⁗); EI-MS: *m/z* 381 $[C_{22}H_{27}N_3OS]^{\bullet+}$ $[M]^{\bullet+}$, 282 $[C_{15}H_{12}N_3OS]^+$, 210 $[C_{14}H_{12}NO]^+$, 133 $[C_8H_7NO]^+$, 77 $[C_6H_5]^+$, 57 $[C_4H_9]^+$, 51 $[C_4H_3]^+$.

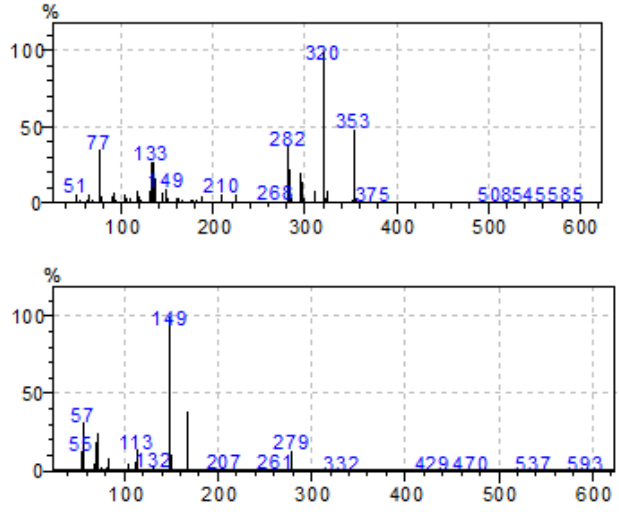


Fig. 7: EIMS spectra of 3-(*n*-pentylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6f).

Biological activity

Moreover, they were screened for their anti-enzymatic potential and their % age inhibition and IC_{50} values were recorded to evaluate their potency against cholinesterases. Few of them were found to be potent when compared to standard; Eserine. The docking studies were found to be in concordance with the bioactivity results substantiating the insertion of the *n*-heptyl group on the thiol position of triazole. The 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole bonded with the amino acid residues of the enzyme active pocket through almost all portions of the molecule of 6g via H-bonding, arene-arene, electrostatic and hydrophobic interaction which played a major role for inhibiting acetycholinesterase.

DISCUSSION

Among the whole series of synthesized bioactive pharmaceutical candidates compound 6f was elaborated for comprehensive discussion on structural elucidation *via* contemporary spectral techniques. The absorption peaks of IR spectrum appeared at 2944, 1620, 1443, 1260, 1173, 1026, 830 for C-H, C=N, C=C, C-O, C-N, C-S, -CH_3 groups, respectively. Furthermore, ^{1}H-NMR and ^{13}CNMR spectral data established the structure *via* number of counting the protons and carbon atoms in their respective spectra.

The most downfield signal observed in aromatic region was a multiplet at *δ* 7.52-7.50 for 3 protons (H-3‴- H-5‴), and a dist. doublet at *δ* 7.24 for (H-2‴ & H-6‴) having *J* of (3.3, 7.8 Hz) for *meta* and *ortho* coupling of protons which confirmed the presence of phenyl ring. An A_2B_2 spin system was observed as *di*-orthocoupled doublets at *δ* 7.35 for (H-2' & H-6') & another at *δ* 6.79 (for (H-3' & H-5') having *J* (8.8 Hz) which established 4-substituted phenyl moiety. The aliphatic region possessed a singlet at *δ* 3.79 for -OCH_3 group protons, a triplet at *δ* 3.26 for methylene protons CH_2-1"", two quintets at *δ* 1.77 and *δ* 1.40 for 2 methylene groups at 2"" & 3"" respectively, a sextet at *δ* 1.36 for CH_2-4"" and the most shielded triplet was observed at *δ* 0.90 for terminal CH_3-5"" which confirmed the attachment of *n*-pentyl chain with the thiol group of the triazole moiety (fig. 4) which was additionally established by ^{13}C-NMR spectrum (fig. 5). The most downfield signal was observed at 160.55 for methine carbon (C-4′) of phenyl ring bearing -OCH_3 group. The quaternary methine carbons (C-5 & C-3) of the 1,2,4-triazole moiety showed downfield peaks at *δ* 154.69 and 152.63 respectively. The rest of the carbons showed peaks at *δ* 134.60, 129.83, 129.67, 129.58, 127.46, 119.23, 113.90 for the aromatic carbons C-1‴, C-2′ & C-6 ′, C-4‴, C-3‴ & C-5‴, C-2‴ & C-6‴, C-1′, C-3′ & C-5′ confirming the presence of two aromatic (unsubstituted phenyl & 1,4-diusubstituted phenyl) rings. In the up field region of the spectrum the first signal observed was for -OCH_3-1″ group 55.22. Further four signals were observed for -CH_2 groups join to *S*-atom of triazole moiety at 32.62, 30.83, 29.02, 22.15 for C-1⁗, C-3⁗, C-2⁗, C-4⁗ respectively and finally the most upfield signal resonated at 13.88 for terminal CH_3-5⁗ methyl group carbon. EIMS spectrum deduced the molecular formula ($C_{20}H_{23}N_3OS$; 8f) of synthesized derivative. It exhibited $[M]^{\cdot+}$ peak at *m/z* 353 which cleaved into two major fragments one at *m/z* 57 for *n*-pentyl cation; $[C_5H_{11}]^+$ and other at *m/z* 282 $[C_{15}H_{12}N_3OS]^+$ cation by loss of *n*-pentyl radical which undergo multistep fragmentation to yield methoxyphenyl radical cation at *m/z* 107 which generated phenyl radical cation at *m/z* 77 *via* removal of neutral HCHO molecule which eventually lost acetylene molecule to generate cyclobutadiene radical cation at *m/z* 51. EIMS spectrum and proposed mass fragmentation pattern is sketched in (fig. 6 & 7). The cumulative spectral data lead to the designation of compound as 3-(pentylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (8f). Other *S*-substituted derivatives were categorized in the similar manner.

Biological assays

Acetyl/Butyryl Cholinesterase assay

The synthesized *S*-substituted of 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-thiols; 6(a-g) were screened

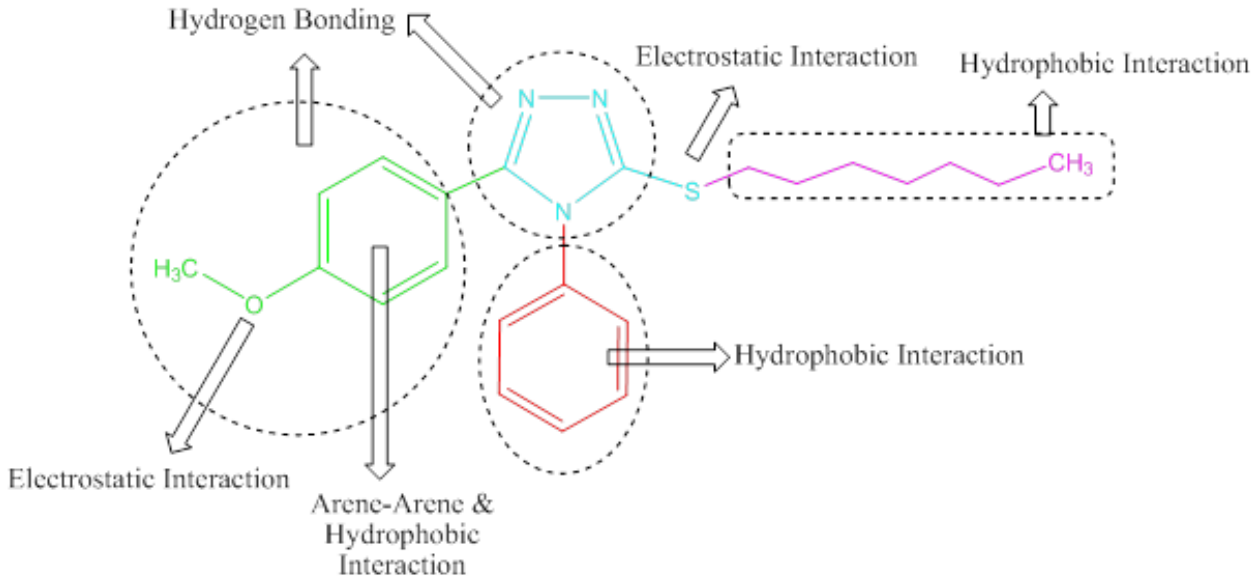


Fig. 8: Possible active sites within molecule with kind of interactions observed in *In-Silico* analysis of 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6g)

against acetylcholinesterase enzyme (AChE) to assess their inhibitory potential and results are tabulated as % age inhibition and IC_{50} values in (table 2). All results were calculated as mean of 3 experiments. It was revealed that 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole showed IC_{50} having value (38.35±0.62 μM; 6g) good activity compared to standard Eserine (0.04±0.001 μM). Compounds 6a-6f were found to be inactive against AChE enzyme.

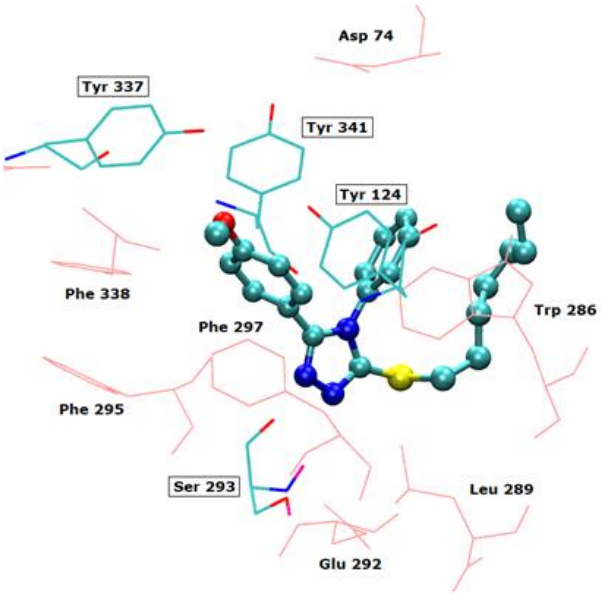


Fig. 9: *In Silico* analysis (3D image) of most potent inhibitor of acetyl cholinesterase enzyme; 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4H-1,2,4-triazole (6g)

The synthesized compounds were also screened against butyrylcholinesterase enzyme (BChE) to assess their potential. The results are tabulated as % age inhibition and IC_{50} values (table 2). All results were calculated in triplicate. It was revealed from results that 3-(ethylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6a), 3-(*n*-propylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6b), 3-(*iso*propylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6c), 3-(*sec*-butylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6e), 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6g) showed moderate to poor activity with IC_{50} range of [147.75±0.67 μM - 342.17±0.56 μM] compared to standard Eserine (0.04±0.001 μM). Compounds 8d, 8f are to be found inactive compounds against BChE enzyme.

In-Silico analysis

The 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole bonded with the amino acid residues of the enzyme active pocket through various important fragments of the molecule making 6g a potent candidate for acetycholinesterase inhibition (table 3 & fig. 8).

The Tyr124 residue bonded with the *O*-atom of the anisole ring with bond distance of 3.59 Å and Ser293 with *N*-atom of the triazole ring with bond distance of 3.16 Å *via* H-bonds. The *N*-atom of the triazole ring and *O*-atom of the anisole ring also bonded strongly *via* hydrogen bonding with Ser293, Tyr337 and Try341 with bond distance 3.56 v, 3.15 Å and 4.65 Å respectively.

Arene-arene interaction was observed with the anisole ring with Tyr341 and Phe295 amino acid residues of

acetylcholinesterase. The anisole ring and S-atom of the derivative displayed electrostatic interactions with Asp74, Trp286 and Glu292 respectively. The S-atom of the triazole-3-thiol ring and anisole ring electrostatically interacted with Pro290 and Glu202 respectively.

The hydrophobic interactions was observed between *N*-atom of the phenyl ring and anisole ring with Trp286, Phe297 and Phe338. Finally Leu289 showed non-polar hydrophobic interactions with the *n*-heptylthio group. Interactions occurred at bond energy -7.40 KJ/mol. The 3D image of docking with acetylcholinesterase enzyme is shown as (fig. 9).

Anti-proliferative (SRB) assay

S-Alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazol-3-thiols; 6(a-g) were tested for anti-proliferative activity through a 3-day SRB assay at 2 concentrations (25 μM and 50 μM) using HCT 116 cell line in which compounds showed moderate to very good activities (table 4). The compounds with an alkyl chain on the *S*-atom showed activity which can be related to the length of the size chain and steric bulk. In general, the longer the alkyl chain, better was the activity (8g> 8d> 8e> 8b > 8a). The activity of the compounds with no alpha-branching was better than the compounds with alpha branching (8b > 8c and 8d> 8e). Overall, 3-(heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole (6g) was the most potent with 4.96 % cell viability at 25 μM and with 2.41 % cell viability at 50 μM.

CONCLUSION

S-Alkylated 5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole-3-thiols 6(a-g) were synthesized as potential targets for treatment of Alzheimer's disease. The structures were elucidated by modern spectral techniques. Cholinesterase assay displayed that 3-(*n*-heptylthio)-5-(4-methoxyphenyl)-4-phenyl-4*H*-1,2,4-triazole showed excellent acetyl/butyryl cholinesterase inhibitory potential (IC_{50} value; 38.35±0.62; 6g), (IC_{50} value; 147.75±0.67; 6g) respectively and can be the future potent candidate to counter this neuro-degenerative disorder. Moreover, 6g was the best compound in the tested library with 2.41 % cell viability at 50 μM.

REFERENCES

Abdul Hameed A and Hassan F (2014). Synthesis, Characterization and Antioxidant Activity of Some 4 Amino-5-phenyl-4H-1,2,4-triazole-3-thiol Derivatives. *Int. J. App. Sci. Tech.*, **4**: 202-211.

Al-Khuzaie MGA and Al-Majidi SMH (2014). Synthesis, Characterization and Evaluation Antimicrobial Activity of Some New substituted 2-Mercapto-3-Phenyl-4(3H) Quinazolinone. *Iraqi J. Sci.*, **55**: 582-593.

Allouche AR (2010). Software News and Updates: Gabedit A Graphical User Interface for Computational Chemistry Softwares. *J. Comput. Chem.*, **32**: 174-18.

Al-Samaraie WK, Al-Rahbi SO and Al-Nasiri AN (2006). Formulation and evaluation of new dosage forms with patent graecorum. *Al-Mustansiriyah J. Pharm. Sci*., **1**: 16-24.

Arul K and Smith AA (2014). Synthesis and *in vitro* Anticancer Evaluation of Some Novel 1,2,4-Triazole Derivatives. *The Experiment*, **21**: 1439-1452.

Aspberg A, Soderback M and Tottmar O (1993). Increase in catalase activity in developing rat brain cell reaggregation cultures in the presence of ethanol. *Biochem. Pharmacol.*, **46**: 1873-6.

Baviskar BA, Khadabadi SS, Deore SL and Shiradkar MR (2012). Synthesis of clubbed Triazolyl Indeno[1,2C]Isoquinolines as an Novel Anticancer Agent. *Der Pharmacia Sinica*, **3**: 24-30.

Bondock S, Adel S, Etman HA and Badria FA (2012). Synthesis and Antitumor Evaluation of Some New 1,3,4-oxadiazole-based Heterocycles. *Eur. J. Med. Chem*., **48**: 192–199.

Bulut N, Kocyigit UM, Gecibesler IH, Dastan T, Taslimi HKP, Dastan SD, Gulcin I and Cetin A (2006). Synthesis of some novel pyridine compounds containing bis-1,2,4-triazole/thiosemicarbazide moiety and investigation of their antioxidant properties, carbonic anhydrase and acetylcholinesterase enzymes inhibition profiles. *J. Biochem. Mol. Toxicol.*, **32**: e22006.

Chen S, Kao YC and Laughton CA (1997). Binding characteristics of aromatase inhibitors and phytoestrogens to human aromatase. *J. Steroid Biochem.*, **61**(3-6): 107-115.

Chiu SHL and Huskey SEW (1998). ASPET N-glucuronidation of xenobiotics symposium. *Drug Metab. Dispos.*, **26**: 838-847.

Ellman G.L, Courtney K.D, Andres V and Featherstone RM (1961). A New and Rapid Colorimetric Determination of Acetyl cholinesterase Activity. *Biochem. Pharm*., **7**: 88-95.

Ghochikyan TV, Samvelyan MA, Arutyunyan EV, Arutyunyan VS, Avetisyan AA and Malakyan MG (2011). Synthesis and Antioxidant Activity of New 1,2,4-Triazole Derivatives. *Pharm. Chem. J*., **44**: 525-527.

Goss PE and Strasser WK (2004). Aromatase inhibitors for chemoprevention. *Best Pract. Res. Clin. Endocrinol. Metab*., **18**: 113.

Gudelj M, Fruhwirth GO, Paar A, Lottspeich F, Robra KH, Cavaco-Paulo A and Gubitz GM (2001). A CatalasePeroxidase from a Newly Isolated Thermoalkaliphilic Bacillus sp. with Potential for the Treatment of Textile Bleaching Effluents. *Extremophiles*, **5**: 423-429.

Hanif M, Saleem M, Hussain MT, Rama NH, Zaib S, Aslam MAM, Jones PG and Iqbal J (2012). Synthesis,

urease inhibition, antioxidant and antibacterial studies of some 4-amino-5-aryl-3*H*-1,2,4-triazole-3thiones and their 3,6-disubstituted 1,2,4-triazolo[3,4b]1,3,4-thiadiazole derivatives. *J. Braz. Chem. Soc*., **23**: 854-860.

Hou YP, Sun J, Pang ZH, Lv PC, Li DD, Yan L, Zhang HJ, Zheng EX, Zhao J and Zhu HL (2011). Synthesis and Antitumor Activity of 1,2,4-Triazoles Having 1,4Benzodioxan Fragment as A Novel Class of Potent Methionine Aminopeptidase Type II Inhibitors," *Bioorg. Med. Chem.*, **19**: 5948-5954.

Jassim IK, Fayad AA and Jassim WK (2011). Synthesis and characterization of some substituted heterocyclic compounds and evaluation of biological activity. *Kerbala J. Pharma. Sci*., **2**: 228-240.

Jawad KS and Yusra HA (2016). Chemistry of 1,2,4-triazole. *Int. J. Sci. & Res*., **5**: 1411-1423.

Jerald F, Giang D, Charles DM, Troy CG and Kyung-Ho P (2015). Highly quantum efficient Phosphorescent sky blue emitters based on diasterreomeric Iridium(III) Complexes of atropisomeric 5-Aryl-4H 1,2,4-Triazole Ligand. *Pubs. acs. org/ organometallics*, **34**: 3665-9.

Jess S, Kildea S, Moody A, Rennick G, Murchie AK and Cooke LR (2014). European Union Policy on Pesticides: Implications for Agriculture in Ireland. *Pest. Mang. Sci.*, **70**: 1646-1654.

Khan SI, Badshah A, Aslam M and Akhtar MS (2012). Synthesis and Antibacterial Activities of Substituted 4-Amino-1,2,4-triazole-3-thiols. *Med. Chem. and Drug Discovery*, **3**: 116-121.

Konorev EA, Struck AT, Baker JE, Ramanujam S, Thomas JP, Radi R and Kalyanaraman B (1993). Intercellular catalase inhibition does not predispose rat heart to ischemia-reperfusion and hydrogen peroxide-induced injuries. *Free Rad. Res. Commun.*, **19**: 397-407.

Koteleviskii SI and Prezhdo OV (2001). Aromaticity Indices revisited: Refinement and Application to Certain Five Membered Ring Heterocycles. *Tetrahedron*, **57**: 5715-5729.

Kryger G, Harel M, Giles K, Toker L, Velan B, Lazar A, Kronman C, Barak D, Ariel N, Shafferman A, Silman I and Sussman JL (2000). Structures of recombinant native and E202Q mutant human acetylcholinesterase complexed with the snake-venom toxin fasciculin-II. *Acta. Crystallogr. D.*, **56**, 1385-1394.

Macaaev F, Rusu V, Pogreboni S, Gudima A, Stingaci E, Vlad I, Shvets N, Kandemirli F, Dimogloa A, and Reynolds R (2005). Synthesis of novel 5-aryl-2-thio-1,3,4-oxadiazoles and the study of their structure, anti-mycobacterial activities. *Bioorg. Med. Chem*., **13**: 4842–4850.

Meenaxi MM, Ainapure R, Patil PB and Bhat AR (2011). Triazolone and their derivatives for anti-tubercular Activities. *Asian J. Res. Chem*., **4**: 1050-1054.

Mousa MN and Al-jadaan SAN (2012). Evaluation of the Anti-Inflammatory Activity and Ulcerogenic Liability of 5-(3-Chloro-1-benzothien-2-yl)-4-phenyl-4H-1,2,4triazole-3-thiol. *Bas. J. Vet. Res.*, **11**: 122-127.

Naito Y, Akahoshi F, Takeda S, Okada T, Kajii M, Nishimura H, Sugiura M, Fukaya C and Kagitani Y (1996). Synthesis and pharmacological activity of triazole derivatives inhibiting eosinophilia. *J. Med. Chem.*, **39**: 3019-29.

Obot IB and Johnson AS (2012). Ab initio, DFT and TDDFT Electronic Absorption Spectra Investigations on 3,5-Diamino-1,2,4-triazole. *Elixir Comp. Chem.*, **43**: 6658-6661.

Panchal AD, Kunjadia PD and Patel PM (2011). Synthesis and Biological Evaluation of Chalcone Derivatives Linked Triazoles. *Int. J. Pharma. Sci. Drug Res.*, **3**: 331-337.

Papakonstantinou-Garoufalias S, Pouli N, Marakos P and Chytyroglou-Ladas A (2002). Synthesis antimicrobial and antifungal activity of some new 3 substituted derivatives of 4-(2,4-dichlorophenyl)-5-adamantyl-1H-1,2,4-triazole. *Farmaco.*, **57**: 973-7.

Pettersen EF, Goddard TD, Huang CC, Couch GS, Greenblatt DM, Meng EC and Ferrin TE (2004). UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput. Chem.*, **25**: 1605-1612.

Pushpan P, Poojary B, Chikkanna C and Sunil KB (2012). Oxadiazole a nucleus with versatile biological behavior. *Eur. J. Med. Chem*., **53**: 203-210.

Saad HA, Osman NA and Moustafa AH (2011). Synthesis and Analgesic Activity of Same Triazoles and Pyrazoles. *Molecules*, **16**: 10187-10201.

Sahoo S, Patwari PK, Kumar MCB and Setty CM (2013). Synthesis and Biological Activity of Certain Mannich Bases Derivatives from 1,2,4-Triazoles," *Iranian J. Pharma. Sci.*, **9**: 51-60.

Shukla PK, Soni N, Verma A and Jha AK (2014). Synthesis, characterization and *in vitro* biological evaluation of a series of 1,2,4-triazoles derivatives & triazole based Schiff's bases. *Der Pharm. Chem*, **6**: 153-160.

Siddiqui N, Ashan W, Shamsher Alam M, Jain S and Akhtar J (2011). Triazoles as potential bioactive agents. *Int. J. Pharm. Rev. & Res*., **8**: 161-169.

Susimaire PM, Talita PCC, Adriana FLV, Carmen LC, Ana Martínez and Ivone C (2016). Novel Triazole-quinoline derivatives as selective dual binding site acetylcholinesterase inhibitors. *Molecules*, **21**: 193-205.

Takaoka M, Manabe S, Yamoto T, Matsunuma N, Masuda H and Goto N (1994). Comprehensive study of goitrogenic actions of 3-substituted 1,2,4-triazoles in rats. *J. Vet. Med. Sci.*, **56**: 341-346.

Tarikogullar AH, Cizmecioglu MM, Saylam M, Parlar S, Alptuzun V and Soyer Z (2016). Synthesis and cholinesterase inhibitory activity of some phenylacetamide derivatives bearing 1*H*-pyrazole or 1*H*-1,2,4-triazole. *Marmara. Pharm. J.*, **20**: 21-27.

Trott O and Olson AJ (2010). Auto Dock Vina: Improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem.*, **31**: 455-461.

Ugale MR, Berad BN and Bhiwgade SM (2016). Synthesis, structural and biological studies of N-(3-tetra-O-acetyl-*B*-D-glucopyranosyl)-1H(1,2,4-thiadizole(3,4-C) thiadiazol-5(3H) ylidene Arylamine. *Int. J. Sci. and Eng. Res.*, **7**: 1405-1409.

Vichai V and Kirtikara K (2006). Sulforhodamine B colorimetric assay for cytotoxicity screening. *Nature Protocols*, **1**: 1112-1116.

William H, Andrew D and Klaus S (1996). VMD: Visual molecular dynamics. *J. Mol. Graphics*, **14**: 33-38.

Yasmin U, Esra D, Kemal S, Mustafa ER and Sengul AK (2008). Synthesis and antimicrobial evaluation of novel Di-trazole and 4-arylidene amino 4,5-dihydro-1H-[1,2,4-] triazole-5-one derivatives. *Turk. J. Chem.*, **32**: 441-455.