In silico and BSA binding study of some new biological analogs of 1,2,4-triazole pendant with azinane through microwave and conventional synthesis

Naeem Akhtar Virk[1], Aziz-ur-Rehman[1,*], Muhammad Athar Abbasi[1], Sabahat Zahra Siddiqui[1], Asma Ashraf[2,*], Mehreen Lateef[3], Hira Javed[4], Javed Iqbal[1], Hira Khalid[5] and Shafi Ullah Khan[6]

[1]Department of Chemistry, Government College University, Lahore, Pakistan
[2]Department of Zoology, Government College University Faisalabad, Pakistan
[3]Multidisciplinary Research Laboratory, Bahria University Medical and Dental College, Bahria University Karachi, Pakistan
[4]Ganga Ram Hospital, Queen's Road, Mozang Lahore, Pakistan
[5]Department of Chemistry, Forman Christian College University, Lahore, Pakistan
[6]School of Pharmacy, MONASH University Malaysia, Jalan Lagoon Selatan, Bandar Sunway, Subang Jaya, Selangor, Malaysia

Abstract: Microwave and conventional techniques were employed to synthesize a novel array of compounds 7a-g with 1,2,4-triazole and piperidine rings having great biological importance. The microwave assisted method has a better operational scope with respect to time and yield comparative to the conventional method. ^{1}H-NMR, ^{13}C-NMR and IR techniques were employed to justify the structure of synthesized compounds. The antioxidant, butyrylcholinesterase inhibition and urease inhibition potential of every synthesized compound was evaluated. Every member of the synthesized series was found potent against mentioned activities. Compound 7g was the most active anti-urease agent having IC_{50} (µM) value 16.5±0.09 even better than the thiourea with an IC_{50} (µM) value of 24.3±0.24. The better urease inhibition potential of 7g was also elaborated and explained by docking and bovine serum albumin (BSA) binding studies.

Keywords: Acetamides, 1,2,4-triazole, piperidine, sulfonamide, docking, anti-urease activity.

INTRODUCTION

The population of the world especially in the big cities is increasing continuously and deteriorating the ecological as well as habitual conditions day by day. The immunity system of the humanity has been disturbed because of the development of active bacterial strains, viruses, enzymes and many more advanced diseases. The existing drugs have become almost the least effective (Grenet *et al.*, 2004). The current scenario demands the development of new drugs which can compete against all these issues effectively (Li *et al.*, 2014 and Upadhyay *et al.*, 2012). The drugs based on heterocyclic compounds are found to be effective drugs candidates. Among the various bioactive heterocyclic compounds, triazole is one of the best heterocyclic five-membered ring with excellent pharmacological history (Naik *et al.*, 2014). 1,2,4-Triazoles have been found as active part in different drugs including Anastrozole, Letrozole, Vorozole, Fluconazole, Itraconazole and Voriconazole which are acting as anti-inflammatory, anticancer, antimitotic, antifungal (Mishra *et al.*, 2006 and Kharb *et al.*, 2011), antiviral (Jones *et al.*, 1965), antioxidant (Ilango and Valentina, 2010), analgesic and antimicrobial agents (Shams and Hazzaa, 1974).

With the aim to synthesize highly biologically active functionalities, an array of hybrids (table 1) based on *N*-substituted-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl] acetamides (7a-g) was synthesized by following conventional and microwave-assisted synthesis. The comparative analysis of both methods was performed on the basis of synthetic time and yield. The study proved that the microwave assisted strategy is too good and effective in sense of less synthetic time and high yield of synthesized compounds (table 2). The characterization was carried out by various spectroscopic techniques (^{1}H-NMR, ^{13}C-NMR and IR) in order to justify the structures. The whole array (7a-g) was screened for inhibition potential against enzymes like urease and butyryl cholinesterase along with antioxidant potential. The results revealed that all the presented compounds have potential to tolerate the problems associated with the considered enzymes.

MATERIALS AND METHODS

General

The ^{1}H-NMR spectral information was recorded by Bruker spectrometers operating at 600 MHz while ^{13}C-NMR spectral details were availed by Bruker AM - 400 spectrometer (150 MHz) in $CDCl_3$, using TMS as an internal standard. The IR studies were done by Jasco - 320

**Corresponding author:* e-mail: asmabinm@gmail.com, azizryk@yahoo.com

-A spectrophotometer (wave number in cm^{-1}). The melting points of the series of compounds were observed by using Griffin and George apparatus. Reaction progress was confirmed by TLC and observed under UV lamp or iodine vapors. The chemical used for the synthesis of a series of target compounds were of brand Alfa Aeser and Sigma Aldrich supplied by local suppliers.

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

Ethyl 1-[(4-methoxyphenyl) sulfonyl]-4-piperidine carboxylate (1) was prepared by stirring the 4-methoxybenzenesulfonyl chloride (a: 0.04 mol) with ethyl isonipacotate (b: 0.04 mol) for green synthesis in aqueous medium with 10 % Na_2CO_3 solution. By using TLC, reaction completion was confirmed. The neutralization was attained by dil. HCl and product were obtained as a white precipitate by adding chilled water, filtered, washed and dried at room temperature.

Synthesis of 1-(4-methoxyphenylsulfonyl)piperidine-4-carbohydrazide (2)

By refluxing ethyl 1-[(4-methoxyphenyl)sulfonyl]-4-piperidinecarboxylate (1; 0.05 mol) and hydrazine hydrate for 4 hours in the presence of methanol as solvent, compound 2 was synthesized. Reaction completion was monitored by TLC. Finally the excess solvent was evaporated and crystals of the target compound were achieved.

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

1-(4-Methoxyphenylsulfonyl)piperidine-4-carbohydrazide (2) and phenyl isothiocyanate were refluxed for 1 hour to synthesize an intermediate of compound 3, its purity and reaction completion was analyzed through TLC. The reaction was further preceded for one hour by refluxing the mixture of precipitates of un-cyclized product with equimolar KOH. On completion of the reaction the pH was adjusted at 4-5 by addition of dil. HCl with continuous stirring. The target compound was precipitated out, filtered, washed and dried for further process.

Synthesis of N-(substituted)-2-bromoacetamides (6a-g)

Aralkyl/aryl amines (5a-g; 0.02 mol) were treated with 2-bromoacetyl bromide (4; 0.02 mol) in aqueous medium for 1-2 hour. The pH was maintained at 9-10 by the addition of 10% Na_2CO_3 solution. Amides in the precipitates form were obtained after filtration and washing the precipitates from the reaction mixture with distilled water and dried.

Synthesis of N-(substituted)-2-[(5-{1-[(4-methoxyphenyl) sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7a-g)

Conventional technique: Titled compounds were synthesized, by the reaction of the mixture of LiH and compound 3 (0.0005 mol) which was stirred in the presence of DMF for 30 minutes then added the equimolar quantities of acetamides (6a-g) and stirred at room temperature for 12 - 20 hours. The reaction progress was monitored through TLC. Desired compound was precipitated by the addition chilled water, filtered, washed and dried for analysis and further applications.

Microwave assisted method: For the synthesis of series of titled compound, the mixture of LiH and compound 3 (0.0005mol) was stirred in DMF for 30 minutes then added the equimolar amount of acetamides (6a-g). The reaction was preceded in the microwave for 34 - 80 seconds. The high yield and good purity obtained in less time made microwave assisted synthesis impressive. After confirmation of reaction completion, the target compounds were availed in the form of precipitates by using highly chilled water. The insoluble desired compound was filtered, washed and dried at room temperature for further analysis.

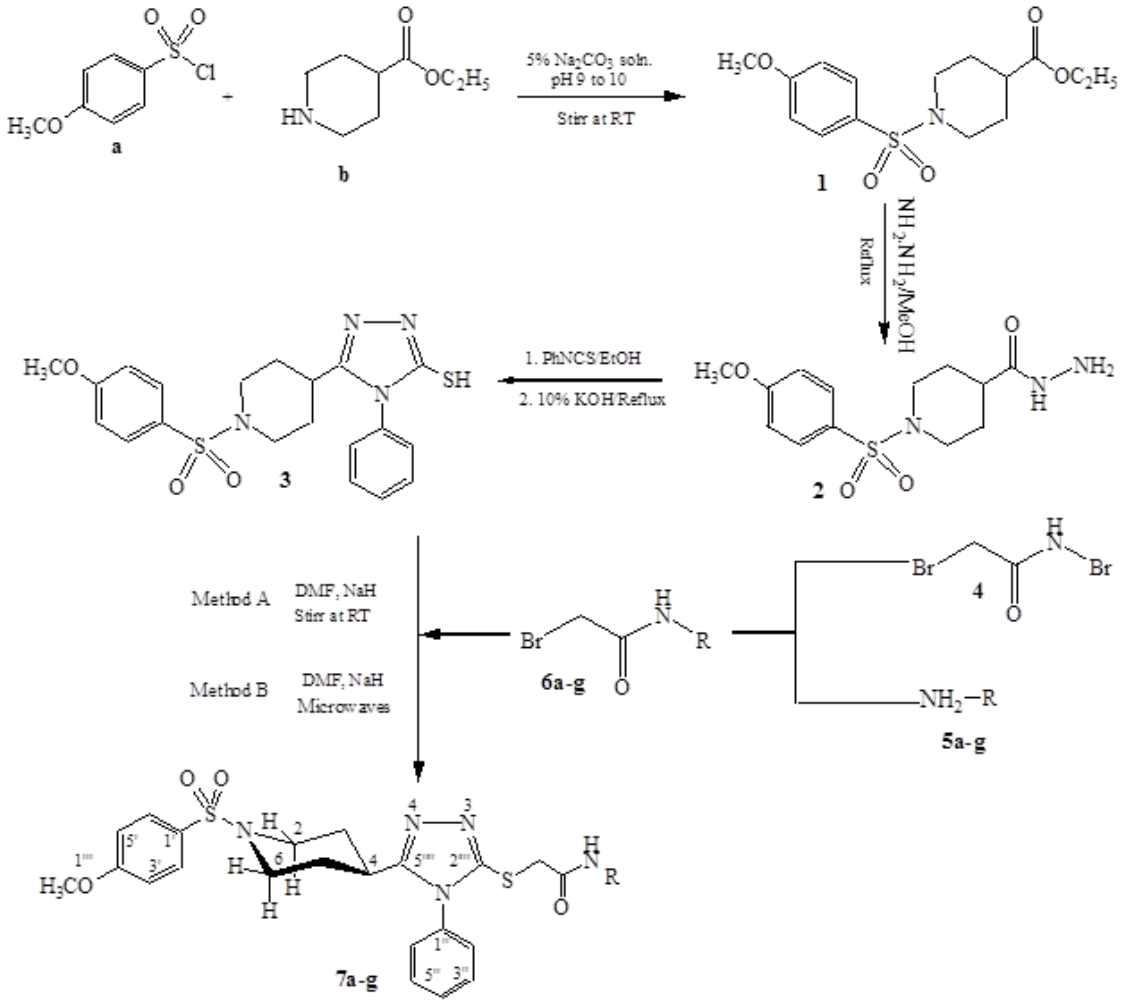
Antioxidant activity by DPPH method

The various concentrations of synthesized compounds (10 µL) were mixed with 90 µL of methanolic DPPH in 96 - well plates by making the total volume 100 µL following the reported method (Koleva *et al.*, 2002). A mixture of synthesized compounds and DPPH was mixed thoroughly, incubated for half an hour at 35 ^oC. Synergy HT BioTek® USA microplate reader has utilized to readout the absorbance at 517 nm. The standard utilized here was BHA (butylated hydroxyanisole). EZ-Fitz Perrella Scientific Inc. Amherst USA software was used to calculate the IC_{50} (µM) values. The radical scavenging activity was observed by noting the difference in absorbance in absorbance. The following formula was used to calculate the scavenging activity.

Percent scavenging activity = [100 - (Abs of test compound / Abs of control) x 100]

Butyryl cholinesterase inhibition assay

Butyryl cholinesterase is an enzyme possessing the serine hydrolysis. The variable amino residues available at the active sites of BChE are responsible for its specific action for the inhibition of this enzyme and substrate as well. The concerned enzyme is responsible for the conversion of acetylcholine to choline which is the important factor for neuromuscular junction. BChE produce choline by the catalysis of acetylcholine (Cygler *et al.*, 1993). It is observed that BChE found in higher concentration in the Alzheimer's plaques as compared to the concentration in the normal brain (Tougu, 2001). So by following the reported method (Ellman *et al.*, 1961) absorbance of a mixture of buffer solution of Na_2HPO_4, butyryl cholinesterase enzyme and synthesized compounds were calculated at 405 nm. The mixture then incubated and by the addition of butyryl thiocholine iodide (for BChE) and 10 µL, DTNB reaction gets started. After incubation absorbance again was noted at 405 nm by 96 - well plate reader Synergy HT, Biotek, USA. And finally EZ - Fit



Scheme 1: Synthesis of the library of heterocyclic acetamide derivatives based on azinane and 1,2,4-triazole.

Enzyme kinetics software (Perrella Scientific Inc. Amherst, USA) was employed to calculate the IC_{50} values.

Urease inhibition assay

The modified procedure reported by Mobley *et al.*, 1988, was performed to measure the action of synthesized compounds against urease enzyme (Mobley *et al.*, 1988). 85µL volume based on the 10µL sample as well as a buffer solution and 25µL of enzyme solution was incubated for 5 minutes at 37˚C. The whole mixture after addition of 40 µL stock solution of urea was incubated for a further 10 minutes. And finally, 115µL of a reagent (hypochlorite reagents) was added and incubated for 10 minutes for color development. By using 96 - well plate reader Synergy HT BioTek, USA, the absorbance was calculated at 625 nm. The calculations were made by using EZ -Fit Enzyme Kinetics Software (Perrella Scientific Inc. Amherst, USA).

Docking studies

Compounds 7a-g was docked to find out the binding interaction with urease. Protein structure of urease was downloaded from protein data bank using PDB ID: 3LA4 with a resolution of 2.05 A (Balasubramanian and Ponnuraj, 2010). Chem Draw Professional 15.0 was utilized to sketch the structure of potential inhibitors (Elmer *et al.*, 2017). Open Eye Scientific Software was used for the preparation of protein as well as ligand structures before docking. For docking calculations, FRED version 3.2.0 was used (FRED, version 3.0.0, 2013). The conformers of each ligand were generated by OMEGA 3.0.0 (OMEGA, version 2.4.6, 2013). We used default settings of OMEGA for generation of conformers. The active site of protein could be defined by the presence of all residues within 10 Å of nickel atoms and the non-standard residues (KCX and CME) in the urease. High dock resolutions were availed by FRED default parameters. 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 (BIOVIA, 2017).

BSA interactions using fluorescence measurements

Buffer solution (20 mM, pH 7.4) and bovine serum albumin (BSA) were purchased from Sigma-Aldrich (St

Table 1: Different *N*-substituted aryl/phenyl/aralkyl groups

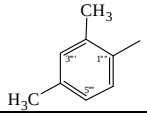
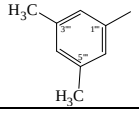
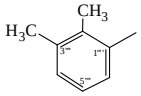
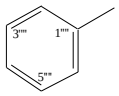
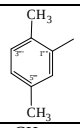
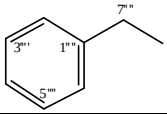
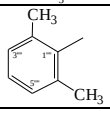
Compound	R	Compound	R
7a		7e	
7b		7f	
7c		7g	
7d			

Table 2: Comparative study of conventional and microwave assisted methods

Compounds	Reaction Time		Reaction Yield (%)	
	Conventional (hours)	Microwave (sec)	Conventional	Microwave
7a	17	37	66	92
7b	13	36	71	90
7c	20	56	53	93
7d	12	62	80	94
7e	17	53	74	86
7f	18	80	67	87
7g	15	75	79	91

Table 3: Biological potential of synthesized compounds

Compounds	IC_{50} (µM)		
	Antioxidant	Urease inhibition	BChE
7a	56.2 ± 0.42	36.2 ± 0.24	39.5 ± 0.09
7b	52.1 ± 0.12	39.5 ± 0.52	59.4 ± 0.02
7c	45.2 ± 0.36	32.4 ± 0.21	65.5 ± 0.24
7d	47.1 ± 0.14	42.1 ± 0.41	31.9 ± 0.36
7e	59.2 ± 0.36	30.2 ± 0.26	32.1 ± 0.13
7f	61.5 ± 0.11	36.5 ± 0.62	25.7 ± 0.08
7g	69.2 ± 0.45	16.5 ± 0.09	70.2 ± 0.32
BHA	44.2 ± 0.21		
Thiourea		24.3 ± 0.24	
Eserine			7.8 ± 0.24

Table 4: Stern-Volmer quenching constant, bimolecular quenching rate constant, binding constant and number of binding sites of compounds, warfarin and ibuprofen with BSA at 298K

Compounds	$K_{SV} \times 10^2$ (M^{-1})	$k_q \times 10^{10}$ (M^{-1} s^{-1})	K_a (L/mol)	N
7b	4.63	4.63	2.11×10^5	1.9
7d	2.38	2.38	6.24×10^2	1.1
7g	3.66	3.66	6.50×10^2	1.0
Warfarin			641000	1.07
Ibuprofen	3502	35	56870	1.26

Louis, USA). Fluorescence spectrophotometer (LS 55 Perkin) was employed to study the fluorescence intensities. Fluorescence quenching was calculated by fluorometric titration of a solution of BSA with stock solutions of the compounds 7a-g. After excitation at 295 nm, the intensity was recorded at 336 nm of the BSA solutions (Valstar *et al.*, 2000).

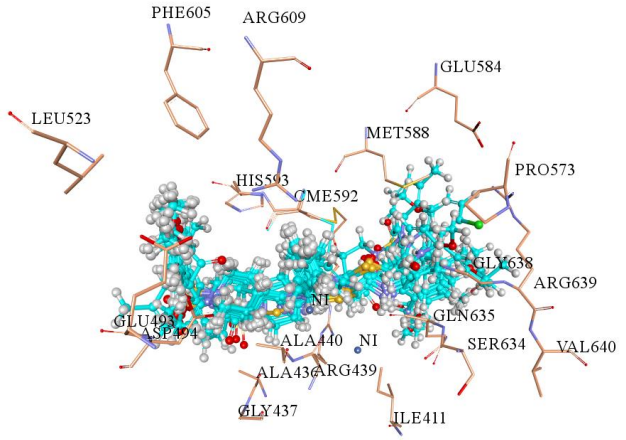


Fig. 1: Binding orientation of all synthetic compounds 7a-g within the active site of urease. Amino acid residues are shown in golden color stick while ligands are shown in different colors superimposed within the active site

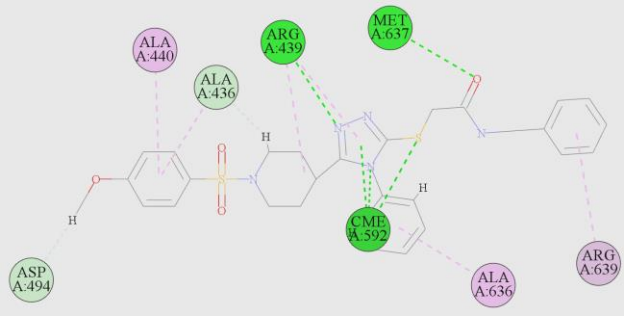


Fig. 2: 2D Binding interaction of compound 7g within the active site of urease. Hydrogen bonding is shown in green dotted line while π–alkyl interactions are shown in light pink dotted line

Spectral characterization of synthesized compounds

N-(2,4-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7a)

Light pink amorphous solid; yield: 90%; M.P: 117.6°C, Molecular formula: $C_{30}H_{33}N_5O_4S_2$; Molecular mass: 591.74 g/mol; IR (KBr, wave number, cm^{-1}) 2900 (Ar C-H), 1700 (C=O), 1600 (C=N), 1500 (Ar C=C), 1400 (CH_3), 1300 (S=O), 1250 (C-O-C), 730 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 8.17 (s, 1H, NH), 7.78 (d, *J* = 7.8 Hz, 1H, H-6''''), 7.70 (d, *J*=7.2 Hz, 2H, H-2', H-6'), 7.60-7.53 (m, 3H, H-3", H-4", H-5"), 7.26 -7.21(m, *J*=7.7 Hz, 3H, H-2", H-6", H-5''''), 7.00 (s 1H, H-3''''), 6.98 (d, *J* =7.6 Hz, 2H, H-3', H-5'), 3.95 (s, 2H, H-2'''), 3.87 (s, 3H, H- 1'''), 3.74-3.70 (m, 2H, H_e-2, H_e-6), 2.53-2.51(m, 1H, H-4), 2.34-2.29 (m, 2H, H_a-2, H_a-6), 2.28 (s,3H, H-3'''), 2.25 (s, 3H, H-4''') 2.04 -1.98 (m, 2H, H_e-3, H_e-5), 1.87-1.85 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.87 (C-4'), 162.99 (C-5''''), 158.04 (C-3''''), 152.72 (C -6''''), 133.55 (C-1''''), 133.56 (C-1"), 132.39 (C-2''''), 131.12 (C-6''''), 130.77(C-4''''), 130.45 (C -3", C-5"), 130.19 (C-4"), 129.76 (C-2", C- 6"), 129.33 (C-5''''), 126.96 (C-2', C-6'), 125.90(C-1'), 122.50(C-3''''), 114.20 (C-3', C-5'), 55.59 (C-1'''), 45.38 (C-2, C-6), 35.62 (C-2'''), 31.78 (C-4), 29.46(C-3, C-5), 20.84 (C-3'''), 18.08 (C-4'''); EIMS(*m/z*): 591,$[M]^+$, 371 $[C_{19}H_{21}N_3O_3S]^+$, 295$[C_{13}H_{16}N_2O_3S]^+$, 255$[C_{12}H_{17}NO_3S]^+$, 171$[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 106 $[C_8H_{10}]^+$, 66 $[C_5H_6]^+$; Ana. Calcd for $C_{30}H_{33}N_5O_4S_2$: C, 60.89; H, 5.62; N, 11.84; O, 10.82; S, 10.84; found C, 60.67; H, 5.69; N, 11.74; O, 10.66; S, 10.82.

N-(2,3-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7b)

Light pink amorphous solid; yield: 85%; M.P: 110.5°C; Molecular formula: $C_{25}H_{31}N_5O_4S_2$; Molecular 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), 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, 3.1 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'), 3.97 (s, 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-3'''), 2.20 (s, 3H, H-4'''), 2.03-2.01 (m, 2H, H_e-3, H_e-5), 1.87-1.87 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 167.01 (C-4'), 163.00 (C-5''''), 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), 35.67 (C-2'''), 31.96 (C-4), 29.44 (C-3, C-5), 20.60(C-3'''), 13.83 (C-4'''); EIMS (*m/z*): 591,$[M]^+$, 371$[C_{19}H_{21}N_3O_3S]^+$, 295 $[C_{13}H_{16}N_2O_3S]^+$, 255 $[C_{12}H_{17}NO_3S]^+$, 171 $[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 106 $[C_8H_{10}]^+$, 52 $[C_4H_4]^+$; Ana. Calcd for $C_{30}H_{33}N_5O_4S_2$: C, 60.89; H, 5.62; N, 11.84; O, 10.82; S, 10.84; found C, 60.68; H, 5.64; N, 11.72; O, 10.63; S, 10.84.

N-(2,5-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7c)

Off white amorphous solid; yield: 85%; M.P: 104.5°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), 1375 (CH_3), 1250 (S=O), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.62 (s, 1H, NH), 7.79

(s,1H, H-6''''), 7.68 (d, *J* = 10.3 Hz, 2H, H-2', H-6'), 7.58-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.23-7.21 (m, 2H, H-2'', H-6''), 6.98 (d, *J* = 10.2 Hz, 2H, H-3', H-5'), 7.94 (d, *J* = 9.4 Hz, 1H, H-4''''), 3.96 (s, 2H, H-2'''), 3.88 (s, 3H, H-1'''), 3.71-3.70 (m, 2H, H_e-2, H_e-6), 2.54-2.51(m, 1H, H-4), 2.40-2.36 (m, 2H, H_a-2, H_a-6), 2.33 (s, 3H, H-3'''), 2.26 (s, 3H, H-4'''), 2.02-1.97 (m, 2H, H_e-3, H_e-5), 1.87-1.84 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 167.02 (C-4'), 164.01 (C-5''''), 158.05 (C-3''''), 151.60 (C-6''''), 132.81(C-1''''), 131.66 (C-1''), 131.88 (C-2''''), 130.75 (C-6''''), 130.42 (C-3'', C-5''), 130.25 (C-4''), 129.75 (C-2'', C-6''), 128.52 (C-5''''), 126.96 (C-2', C-6'), 125.68 (C-1'), 124.44 (C-3''''), 122.95 (C-4''''), 114.21 (C-3', C-5'), 55.56 (C-1'''), 45.26 (C-2, C-6), 38.02 (C-2'''), 32.33 (C-4), 29.38 (C-3, C-5), 21.99 (C-3'''), 17.68 (C-4'''); EIMS (*m/z*): 591, $[M]^+$, 371 $[C_{19}H_{21}N_3O_3S]^+$, 295 $[C_{13}H_{16}N_2O_3S]^+$, 255 $[C_{12}H_{17}NO_3S]^+$, 171 $[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 106 $[C_8H_{10}]^+$, 52 $[C_4H_4]^+$; Ana. Calcd for $C_{30}H_{33}N_5O_4S_2$: C, 60.89; H, 5.62; N, 11.84; O, 10.82; S, 10.84; found C, 60.73; H, 5.67; N, 11.70; O, 10.61; S, 10.82.

N-(2,6-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7d)

Off white amorphous solid; yield: 87%; M.P: 126.0^{o}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), 1350 (CH_3), 1280 (S=O), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.32 (s, 1H, NH), 7.68 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 7.61-7.58 (m, 3H, H-3'', H-4'', H-5''), 7.23-7.21(m, 2H, H-2'', H-6''), 7.11-7.06 (m, 3H, H-3'''', H-4'''', H-5''''), 6.98 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 3.99 (s, 2H, H-2'''), 3.86 (s, 3H, H-1'''), 3.73-3.71 (m, 2H, H_e-2, H_e-6), 2.54-2.51(m, 1H, H-4), 2.39-2.35 (m, 2H, H_a-2, H_a-6), 2.17 (s, 6H, H-3''', H-4'''), 2.02-1.99 (m, 2H, H_e-3, H_e-5), 1.88-1.85 (m, 2H, H_a-3,H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 167.09 (C-4'), 163.00 (C-5''''), 158.00 (C-3''''), 152.85 (C-6''''), 135.27 (C-1''''), 133.84 (C-1''), 132.45 (C-2'''', C-6''''), 130.81 (C-4''), 130.49 (C-3'', C-5''), 129.74 (C-2'', C-6''), 128.10 (C-2', C-6'), 128.00 (C-1'), 127.24 (C-4''''), 126.93 (C-3'''', C-5''''), 114.21 (C-3', C-5'), 55.57 (C-1'''), 45.32 (C-2, C-6), 34.92 (C-2'''), 31.89 (C-4), 29.34 (C-3, C-5), 18.18 (C-3''', C-4'''); EIMS (*m/z*): 591, $[M]^+$, 371 $[C_{19}H_{21}N_3O_3S]^+$, 295 $[C_{13}H_{16}N_2O_3S]^+$, 255 $[C_{12}H_{17}NO_3S]^+$, 171 $[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 106 $[C_8H_{10}]^+$, 66 $[C_5H_6]^+$; Ana. Calcd for $C_{30}H_{33}N_5O_4S_2$: C, 60.89; H, 5.62; N, 11.84; O, 10.82; S, 10.84; found C, 60.71; H, 5.62; N, 11.66; O, 10.60; S, 10.83.

N-(3,5-Dimethylphenyl)-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7e)

Pink amorphous solid; yield: 86%; M.P: 111.2^{o}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), 1575 (C=N), 1500 (Ar C=C), 1325 (CH_3), 1250 (S=O), 1150 (C-O-C), 725 (C-H); ^{1}H-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 9.63 (s, 1H, NH), 7.79 (s,1H, H-2''''), 7.68 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.60-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.23-7.21 (dd, *J* = 10.8 Hz, 2H, H-2'', H-6''), 7.07 (d, *J* = 7.6, 1H, H-6''''), 6.99 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 6.89 (d, *J* = 7.56 Hz, H-5''''), 3.96 (s, 2H, H-2'''), 3.88 (s, 3H, H-1'''), 3.72-3.69 (m, 2H, H_e-2, H_e-6), 2.55-2.51 (m, 1H, H-4), 2.39-2.35 (m, 2H, H_a-2, H_a-6), 2.33 (s, 3H, H-3'''), 2.26 (s,3H, H-4'''), 2.04-1.97 (m, 2H, H_e-3, H_e-5), 1.87-1.84 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.89 (C-4'), 162.99 (C-5''''), 158.06 (C-3''''), 152.69 (C-6''''), 136.20 (C-1''''), 135.96 (C-1''), 132.37 (C-2''''), 130.77 (C-6''''), 130.45 (C-3'', C-5''), 130.24 (C-4''), 129.76 (C-2'', C-6''), 127.80 (C-3''''), 126.95 (C-2', C-6'), 126.07 (C-1'), 125.61 (C-4''''), 122.88 (C-5''''), 114.21 (C-3', C-5'), 55.60 (C-1'''), 45.34 (C-2, C-6), 35.68 (C-2'''), 31.77 (C-4), 29.36 (C-3, C-5), 21.16 (C-3'''), 17.74 (C-4'''); EIMS (*m/z*): 591, $[M]^+$, 371 $[C_{19}H_{21}N_3O_3S]^+$, 295 $[C_{13}H_{16}N_2O_3S]^+$, 255 $[C_{12}H_{17}NO_3S]^+$, 171 $[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 106 $[C_8H_{10}]^+$, 52 $[C_4H_4]^+$; Ana. Calcd for $C_{30}H_{33}N_5O_4S_2$: C, 60.89; H, 5.62; N, 11.84; O, 10.82; S, 10.84; found C, 60.66; H, 5.71; N, 11.68; O, 10.61; S, 10.83.

2-[(5-{1-[(4-Methoxyphenyl) sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl) sulfanyl]-N-phenylacetamide (7f)

Off white amorphous solid; yield: 87 %; M.P: 122.2^{o}C; Molecular formula: $C_{28}H_{29}N_5O_4S_2$; Molecular mass: 563.69 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), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.27 (s, 1H, NH), 7.69 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 7.62 (d, *J* = 7.8, 2H, H-2'''', H-6''''), 7.59-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.33 (t, *J*=7.8 Hz, 2H, H-3'''', H-5''''), 7.26-7.22 (m, 2H, H-2'', H-6''), 7.11 (t, *J* = 7.3 Hz, 1H, H-4''''), 6.99 (d, *J*=8.7 Hz, 2H, H-3', H-5'), 3.89 (s, 2H, H-2'''), 3.88 (s, 3H, H-1'''), 3.77-3.75 (m, 2H, H_e -2, H_e-6), 2.53-2.49 (m, 1H, H- 4), 2.32-2.28 (m, 2H, H_a-2, H_a-6), 2.06-2.00 (m, 2H, H_e-3, H_e-5), 1.88-1.85 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.58 (C-4'), 163.01 (C-5''''), 157.99 (C-3''''), 152.88 (C-6''''), 138.23 (C-1''''), 132.37 (C-1''), 130.81 (C-4''), 130.45 (C-3'', C-5''), 129.75 (C-2'', C-6''), 128.89 (C-2', C-6'), 127.76 (C-1'), 126.95 (C-3'''', C-5''''), 124.20 (C-4''''), 119.78 (C-2'''', C-6''''), 114.22 (C-3', C-5'), 55.61 (C-1'''), 45.50 (C-2, C-6), 36.11 (C-2'''), 32.04 (C-4), 29.40 (C-3, C-5); EIMS (*m/z*): 563, $[M]^+$, 371 $[C_{19}H_{21}N_3O_3S]^+$, 295 $[C_{13}H_{16}N_2O_3S]^+$, 255 $[C_{12}H_{17}NO_3S]^+$, 171 $[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 92 $[C_8H_{10}]^+$, 52 $[C_4H_4]^+$; Ana. Calcd for $C_{28}H_{29}N_5O_4S_2$: C, 59.66; H, 5.19; N, 12.42; O, 11.35; S, 11.38; found C, 59.64; H, 5.18; N, 12.48; O, 11.32; S, 11.35.

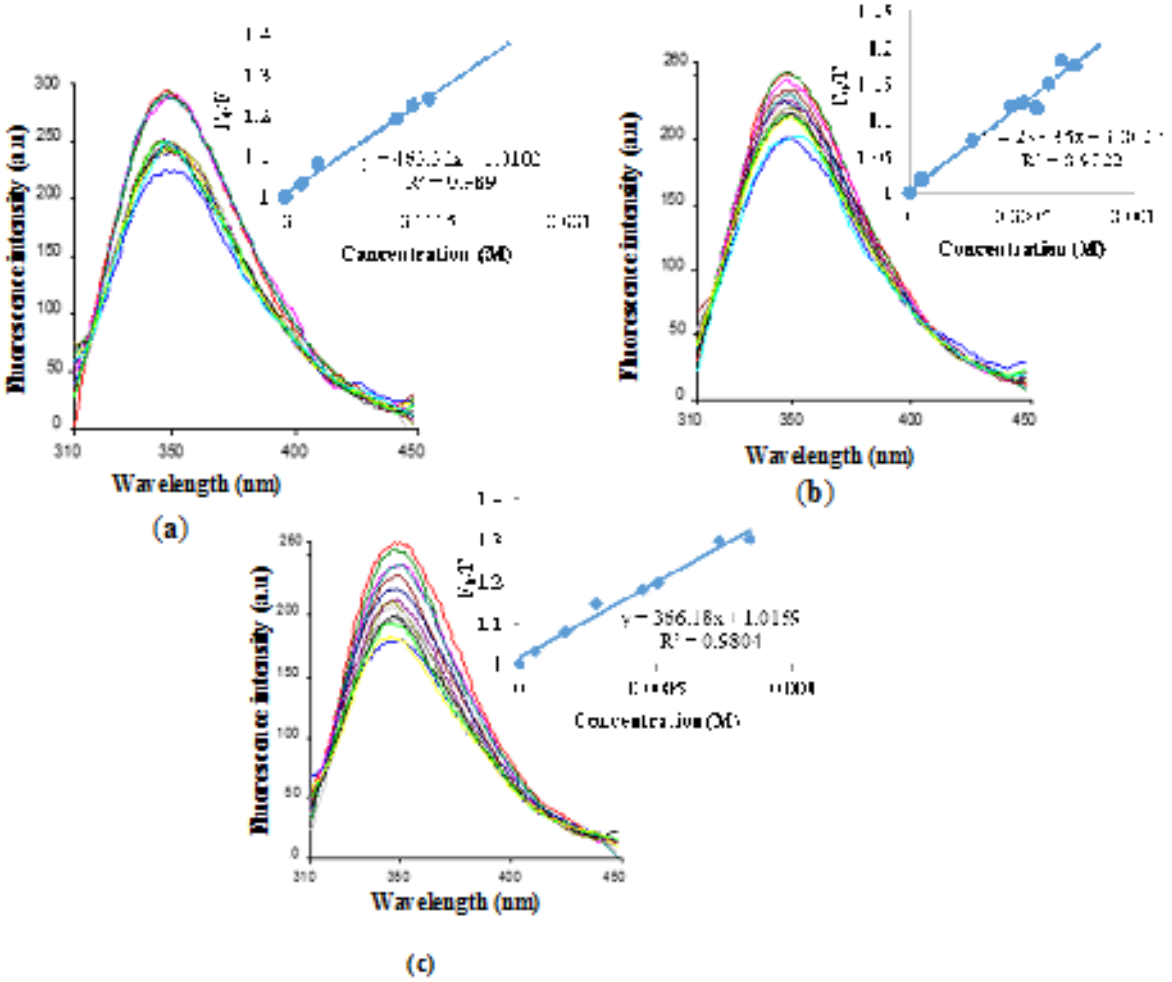


Fig. 3: Fluorescence graph of BSA in the presence of (a) Compound 7b (b) Compound 7d (c) Compound 7g (Insets show the Stern-Volmer plots).

N-Benzyl-2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4H-1,2,4-triazol-3-yl)sulfanyl]acetamide (7g)

White amorphous solid; yield: 89%; M.P: 122.0°C; Molecular formula: $C_{29}H_{31}N_5O_4S_2$; Molecular mass: 577.71 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), 1150 (C-O-C), 750 (C-H); ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 7.68 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.59-7.54 (m, 3H, H-3", H-4", H-5"), 7.33-7.30 (m, 3H, H-3"", H-4"", H-5""), 7.27 (d, *J* = 7.5 Hz, 2H, H-2", H-6"), 7.18 (d, *J* = 7.6 Hz, 2H, H-2"", H-6""), 6.98 (d, *J* = 8.6 Hz, 2H, H-3', H-5'), 4.47 (d, *J* = 6.0 Hz, 2H, H-3'"), 3.88 (s, 3H, H-1'"), 3.82 (s, 2H, H-2'"), 3.74-3.72 (m, 2H, H_e-2, H_e-6), 2.51-2.47 (m, 1H, H-4), 2.36-2.31 (m, 2H, H_a-2, H_a-6), 2.02-1.96 (m, 2H, H_e-3, H_e-5), 1.86-1.83 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.20 (C-4'), 163.03 (C-5""), 157.79 (C-3""), 153.21 (C-6""), 136.05 (C-1""), 132.52 (C-1"), 130.67 (C-4"), 130.36 (C-3", C-5"), 129.74 (C-2", C-6"), 128.50 (C-3"", C-5""), 127.49 (C-2', C-6'), 127.20 (C-1'), 126.99 (C-4""), 126.98 (C-2"", C-6""), 114.21 (C-3', C-5'), 55.57 (C-1'"), 45.39 (C-2, C-6), 43.65 (C-3'"), 34.99 (C-2'"), 31.93 (C-4), 29.36 (C-3, C-5); EIMS (*m/z*): 577, $[M]^+$, 371 $[C_{19}H_{21}N_3O_3S]^+$, 295 $[C_{13}H_{16}N_2O_3S]^+$, 255 $[C_{12}H_{17}NO_3S]^+$, 171 $[C_7H_7O_3S]^+$, 108 $[C_7H_8O]^+$, 78 $[C_6H_6]^+$, 52 $[C_4H_4]^+$; Ana. Calcd for $C_{29}H_{31}N_5O_4S_2$: C, 60.29; H, 5.41; N, 12.12; O, 11.08; S, 11.10; found C, 60.27; H, 5.40; N, 12.10; O, 11.02; S, 11.07.

RESULTS

A series of unique hybrids 7a-g bearing heterocyclic 1,2,4-triazole nucleus was synthesized. The different varying groups are listed in table 1. All the designed compounds have been obtained in better yield by microwave assisted technique comparative to the conventional methodology. IR, ^{1}H-NMR and ^{13}C-NMR spectroscopic techniques were employed to confirm the purity and structures of synthesized compounds. All the compounds were evaluated for various biological applications. All the compounds were found to be potent antioxidant and anti-enzymatic agents and a few of them showed excellent anti-urease activity.

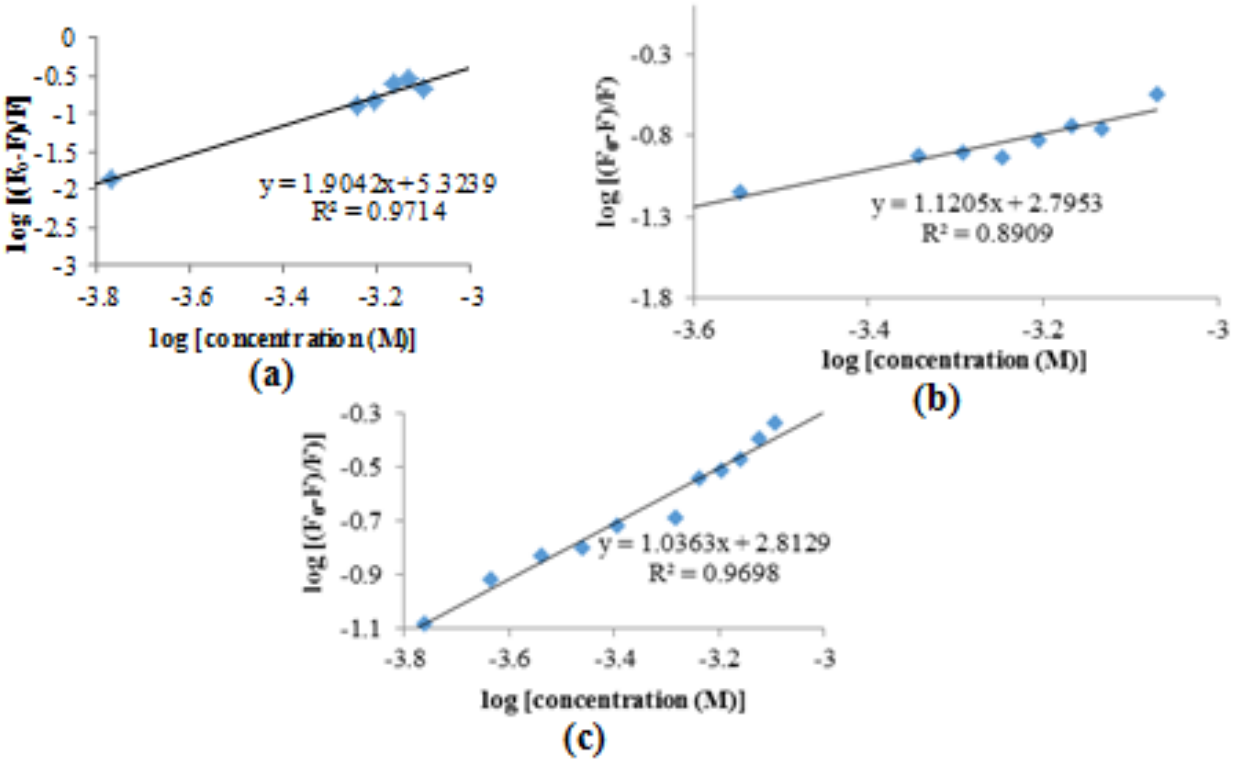


Fig. 4: Fluorescence graph double reciprocal plots of binding of BSA with (a) Compound 7b (b) Compound 7d (c) Compound 7g

DISCUSSION

Chemistry

Compound 7a was selected for single compound discussion. The selected compound was an amorphous solid of light pink color, 90% yield and 117.6°C melting point. The presence of various functionalities was justified by the following peaks appeared at 2900, 1700, 1600, 1500, 1400, 1300, 1250 and 730 cm^{-1} obtained by IR spectroscopy. The ^{1}H-NMR spectral data were used to confirm the presence of aromatic as well as aliphatic protons. The four protons of an aromatic ring attached with sulfonyl group were justified by two doublet peaks appeared at 7.70 and 6.98. The protons of aromatic attached with triazole ring were justified by two multiplet signal 7.60 - 7.53 and 7.26-7.21. Similarly, the protons of an aromatic ring attached with amide group were justified by a doublet, a singlet and a multiplet signal appeared at 7.78, 7.26-7.21 and 7.00 respectively. The nine protons of piperidine ring were justified by five multiplet peaks at 3.74-3.70, 2.53-2.51, 2.34 - 2.29, 2.04-1.98 and 1.87- 1.85 respectively. While the -CH_2 group attached to the heteroatom, three protons of a methoxy group and six protons of two substituted methyl groups were justified by four singlet peaks appeared at 3.95, 3.87, 2.28 and 2.25 respectively. The position of all the peaks is well justified in spectral data. On the similar pattern all the carbon were justified by peaks appeared at 166.87 (C - 4'), 162.99 (C - 5''''), 158.04 (C - 3''''), 152.72 (C - 6''''), 133.55 (C - 1''''), 133.56 (C - 1''), 132.39 (C-2''''), 131.12 (C - 6''''), 130.77 (C - 4''''), 130.45 (C - 3'', C - 5''), 130.19 (C - 4''), 129.76 (C - 2'', C - 6''), 129.33 (C - 5''''), 126.96 (C - 2', C - 6'), 125.90 (C - 1'), 122.50 (C - 3''''), 114.20 (C - 3', C - 5'), 55.59 (C - 1'''), 45.38 (C - 2, C - 6), 35.62 (C - 2'''), 31.78 (C- 4), 29.46 (C - 3, C-5), 20.84 (C - 3'''), 18.08 (C - 4''') for are all the quaternary, aromatic as well as aliphatic carbon atoms obtained by ^{13}C-NMR spectra. By following the given detailed spectral analysis we were able to justify the structure of the synthesized compound with the name of *N*-(2,4-dimethyl phenyl)-2-[(5-{1-[(4-methoxyphenyl) sulfonyl]-4-piperidinyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl) sulfanyl] acetamide. All the synthesized compounds were justified by using a similar pattern of studies and spectroscopic information.

The whole library was comparatively synthesized by using conventional as well as microwave assisted techniques and very effective information was obtained. The findings revealed that the microwave assisted synthesis is very fast and productive. All the compounds were synthesized just in 36-80 seconds with 86-94% yield by this method. The same array of compounds was synthesized in 12-18 hours with 53-80% yield by following the conventional methodology. So it was important to have knowledge that the microwave assisted technique is a very effective synthetic technique in terms of time and yield.

Antioxidant activity

The synthesized compounds (7a-g) were found very active antioxidant agents (table 3). Compounds 7a, 7b, 7c and 7d possessed the comparable potential to that of BHA (butylated hydroxyanisole) utilized as standard. These compounds showing better potential among the screened series of compounds could be due to di-substituted aromatic ring. The highest antioxidant potential was observed by compound 7c because of methyl group substitution at ortho position.

Urease inhibition potential

All the compounds were tested against urease enzyme and the observed results were very impressive (table 3). All the compounds were outstanding in their behavior to act as anti-urease agents as compared to the standard utilized. Compound 7g was found outstanding in their urease inhibition potential as compared to thiourea used as a standard. Their best potential could be understandable by the IC_{50} (µM) value of 16.5±0.09µM as compared to thiourea with an IC_{50} (µM) value of 24.3±0.24µM. So it might be a positive addition in the form of newly synthesized compounds for the inhibition of urease enzyme.

Butyryl cholinesterase (BChE) inhibition potential

The AChE inhibition potential of the compounds 7a-g against butyryl cholinesterase enzyme, was tested with reference of Eserine (table 3). The findings revealed that all the compounds were active with variable range. A few compounds were found to have good potential for this enzyme. The following compounds 7a, 7d, 7e and 7f were found good in potential among the tested compounds. The highest potential was observed by compound 7f as presented by the IC_{50} (µM) values given in table 3 among the synthesized compounds. The highest potential possessed by compound 7f was might be due to the absence of bulky groups attached amidic aromatic ring.

Docking studies

Molecular docking studies of all compounds 7a-g were carried out to find the putative binding orientation of these compounds within the active site of the target protein. Superimposition of all best-docked compounds within the active site of urease is shown in fig. 1. Detail binding interactions of compound 7g having an IC_{50} (µM) value of 16.5±0.09 was analyzed and illustrated in fig. 2. Binding interaction analysis of compound 7g has illustrated that the triazole moiety in compound 7g formed hydrogen bonds with Arg439 as shown in fig. 2. The triazole ring in all compound also exhibited hydrogen bonding with non-amino acid residue CME 592. Amino acid Met637 was also involved in making hydrogen bonding with formamide moiety of compound 7g. Other hydrophobic π–alkyl interactions were formed between amino acid residue Ala440, Ala636 and Arg639. Methoxy group within compound 7g was involved in making carbon-hydrogen interaction with Asp494 as shown in fig. 2.

BSA Binding studies

The emission spectrum of BSA was recorded at 295 nm after excitation to investigate the changes occurred in the structure of BSA to evaluate the binding of synthesized compounds with BSA (table 4). At λ_{max} = 336 nm BSA shows strong emission. The binding of synthesized compounds was judge by the decrease in fluorescence intensities. Fluorescence quenching could be dynamic or static (Gelamo *et al.*, 2002). Stern-Volmer equation was used to explain the mode of action for quenching (Equation 1) (Wang *et al.*, 2013; Hu *et al.*, 2012).

$$F_o/F = K_{SV}[Q] + 1 = k_q \tau 0[Q] + 1 \quad (1)$$

The value of scattering collision quenching rate constant (2×10^{10} M^{-1} s^{-1}) is less than the k_q value showing that the quenching process by synthesized compounds followed the static mechanism (figs. 3 and fig. 4).

CONCLUSION

An array of acetamide derivatives based on piperidine and 1,2,4-triazole was synthesized by following the comparative conventional and microwave assisted synthetic techniques. Microwave-assisted protocol was proved to be very attractive with respect to high yield and purity achieved within a few seconds. All the compounds were screened against various enzymes and observed that every compound was active against every enzyme with variable potential. All the compounds showed good potential against urease enzyme comparative to anti-oxidant activity and butyryl cholinesterase enzyme. Compound 7g was the most active anti-urease agent with higher inhibition potential as compared to the standard utilized. This compound could be an effective addition to inhibit the action of the urease enzyme after its further biological evaluations including *in vivo* study.

ACKNOWLEDGEMENTS

The authors acknowledge the Higher Education Commission of Pakistan for financial support.

REFERENCES

Balasubramanian A and Ponnuraj K (2010). 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**: 274-283.

BIOVIA DS. BIOVIA Discovery Studio Client, v16. 1.0. 15350, San Diego: Dassault Systems, 2017.

Cygler M, Schrag JD, Sussman J, Harel LM, Silman I and Gentry MK (1993). Relationship between sequence conservation and three-dimensional structure in a large

family of esterases, lipases and related proteins. *Protein Sci*., **2**: 366-382.

Ellman GL, Courtney KD, Andres V and Featherstone RM (1961). A new and rapid colorimetric determination of acetyl cholinesterase activity. *Biochem. Pharmacol*., **7**: 88-95.

Elmer P (2017). Chem Bio Draw Profeesional Version (15.0.0.106). Cambridge Soft Waltham, MA, USA.

FRED, version 3.0.0. Santa Fe, NM, USA: OpenEye Scientific Software, Inc.; 2013. Available from www.eyesopen.com.

Gelamo EL, Silva CHTP, Imasato H and Tabak M (2002). Interaction of bovine (BSA) and human (HSA) serum albumins with ionic surfactants: spectroscopy and modelling. *Biochim. Biophys. Acta.*, **1594**: 84-99.

Grenet K, Guillemot D, Jarlier V, Moreau B, Dubourdieu S, Ruimy R, Armand LL, Brau P and Andremont A (2004). Antibacterial resistance, Wayampis Amerindians, French Guyana. *Emerg. Infect. Dis.*, **10**: 1150-1153.

Hu M, Wang X, Wang H, Chai Y, He Y and Song G (2012). Fluorescence spectroscopic studies on the interaction of gemini surfactant 14-6-14 with bovine serum albumin. *J. Lumin.*, **27**: 204-210.

Ilango K and Valentina P (2010). Synthesis and biological activities of novel 1,2,4-triazolo-[3,4-b]-1,3,4-thiadiazoles. *Der. Pharm. Chem*., **2**: 16-22.

Jones DH, Slack R, Squires S and Wooldridge KRH (1965). Antiviral chemotherapy.1. The activity of pyridine and quinoline derivatives against neurovaccinia in mice. *J. Med. Chem.*, **8**: 676-680.

Kharb R, Sharma PC and Yar MS (2011). Pharmacological significance of triazole scaffold. *J. Enzyme Inhib. Med. Chem*., **26**: 1-21.

Koleva II, Van Beek TA, Linssen JPH, de Groot A and Evstatieva LN (2002). Screening of plant extracts for antioxidant activity: a comparative study on three testing methods. *Phytochem. Anal.*, **13**: 8-17.

Li k, Schurig-Briccio LA, Feng X, Upadhyay A, Pujari V, Lechartier B, Fontes FL, Yang H, Rao G, Zhu W, Gulati A, No J H, Cintra G, Bogue S, Liu YL, Molohon K, Orlean P, Mitchell DA, Junior LF, Ren F, Sun H, Jiang T, Li Y, Guo RT, Cole ST, Gennis RB, Crick DC and Oldfield E (2014). Multitarget drug discovery for tuberculosis and other infectious diseases. *J. Med. Chem.*, **57**: 3126-3139.

Mishra P, Upmanyu N and Mehta A (2006). In vitro anti-microbial activity of some 5-aryl-4-(substituted amino)-3-mercapto-1,2,4- triazole. *Indian J. Microbiol*., **46**: 401-404.

Mobley HL, Cortesia MJ, Rosenthal LE and Jones BD (1988). Characterization of urease from Campylobacter pylori. *J. Clin. Microbiol.*, **26**: 831-836.

Naik M, Ghorpade S, Jena LK, Gorai G, Narayan A, Guptha S, Sharma S, Dinesh N, Kaur P, Nandishaiah R, Bhat J, Balakrishnan G, Humnabadkar V, Ramachandran V, Naviri LK, Khadtare P, Panda MI and Chatterji N (2014). 2-Phenylindole and Arylsulphonamide: Novel scaffolds bactericidal against Mycobacterium tuberculosis. *ACS Med. Chem. Lett.*, **5**: 1005-1009.

OMEGA, version 2.4.6. Santa Fe, NM, USA: OpenEye Scientific Software; 2013. Available from:www.eyesopen.com.

Shams ESA and Hazzaa AAB (1974). Synthesis of compounds with potential fungicidal activity. *Pharmazie*., **29**: 761-763.

Tougu V (2001). Mechanism of catalysis and inhibition. *Curr. Med. Chem*., **1**: 155-170.

Upadhyay K, Manvar A, Rawal K, Joshi S, Trivedi J, Chaniyara R and Shah A (2012). Evaluation of structurally diverse benzoazepines clubbed with coumarins as Mycobacterium tuberculosis agents. *Chem. Biol. Drug. Des.*, **80**: 1003-1008.

Valstar A, Almgren M, Brown W and Vasilescu M (2000). The interaction of bovine serum albumin with surfactants studied by light scattering. *Langmuir.*, **16**: 922-927.

Wang Y, Jiang X, Zhou L, Yang L, Xia G, Chen Z and Duan M (2013). Synthesis and binding with BSA of a new gemini surfactant. *Colloids Surf A Physicochem. Eng. Asp.*, **436**: 1159-1169.