

BSA Binding, molecular docking and *in vitro* biological screening of some new 1, 2, 4-triazole heterocycles bearing azinane nucleus

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Abstract: A series of new compounds (5a-q), derived from 5-(1-(4-nitrophenylsulfonyl) piperidin-4-yl)-4-phenyl-4*H*-1,2,4-triazole-3-thiol (3) were proficiently synthesized to evaluate their biological activities. 1-(4-Nitrophenylsulfonyl) piperidine-4-carbohydrazide (2) was refluxed with phenylisothiocyanate to yield an adduct which was cyclized to compound 3 by reflux reaction with 10 % potassium hydroxide. The targeted compounds 5a-q, were synthesized by stirring alkyl/aralkyl halides (4a-q) and compound 3 in a polar aprotic solvent. ¹H-NMR, ¹³C-NMR, EI-MS and IR spectral techniques were employed to confirm the structures of all the synthesized compounds. The compounds were biologically evaluated for BSA binding studies followed by anti-bacterial, anti-inflammatory and acetylcholinesterase (AChE) activities. The active sites responsible for the best AChE inhibition were identified through molecular docking studies. Compound 5e bearing 4-chlorobenzyl moiety found most active antibacterial and anti-inflammatory agent among the synthesized compounds. The whole library of synthesized compounds except compounds 5d and 5f was found highly active for AChE inhibition and recommended for *in vivo* studies so that their therapeutic applications may come in utilization.

Keywords: Sulfonamide, triazole, azinane, anti-inflammatory, acetyl cholinesterase inhibition

INTRODUCTION

The need of hour is to inaugurate new biologically active compounds for curing many infectious diseases which are still a thought-provoking problem. The resistance of microbial pathogens against multi-drugs system insisted upon the scrutinization of new anti-microbial compounds which can combat the resistive pathogens clinically (Kaplanickli *et al.*, 2008).

The triazole moieties have become the major part of many important drugs including anti-inflammatory, anti-cancer (Anastrozole, Letrozole, Vorozole), anti-mycotic (fluconazole, itraconazole) and anti-fungal drugs (Voriconazole) (Mishra *et al.*, 2006; Kharb *et al.*, 2010). During the last few years, heterocyclic compounds and especially 1,2,4-triazoles have attained a lot of attention due to their biomedical applications (Mathew *et al.*, 2006). 1,2,4-Triazole ring includes a broad spectrum of biological activities like anti-cancer (Sztanke *et al.*, 2008), anti-tumoral (Demirbaş *et al.*, 2002), anti-inflammatory (Unangst *et al.*, 1992; Mullican *et al.*, 1993), antiviral (Jones *et al.*, 1965), anti-oxidant (Ilango and Valentina,

2010), analgesic (Sughen and Yoloye, 1978), anti-microbial (Shams and Hazzaa, 1974), anti-convulsant and anti-depressant activity (Oruc *et al.*, 2004). Piperidine ring has developed much interest among the other heterocyclic systems due to their wide range of biological applications such as anti-cancer (Sahu *et al.*, 2013), anti-microbial (Gopalakrishnan *et al.*, 2009; Ramalingan *et al.*, 2004), antimycobacterial (Das *et al.*, 2008) and anti-hypertensive activities (Zhang *et al.*, 2009).

Blood plasma protein serum albumin plays an important and key role in the distribution of drugs through the circulatory system and it also ensures their storage. Generally, the blood serum albumin (BSA) binding interaction with the drug candidates affects the drugs dispensation volume and apparently its elimination rate. In this regard, the structural features of the drugs responsible for drug-protein interaction will suggest the therapeutic effectiveness of the drug systems (Karami *et al.*, 2017).

It is conveyed from the literature that the modifications in moieties attached to heterocyclic compounds boost their biological activities at a considerable extent (Kharb *et al.*,

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2010). So the heterocyclic compounds are utilized and modified at large scale to attain the desired functions. The present study has been conducted to synthesize new molecules bearing 1,2,4-triazole and piperidine rings. Both of these moieties might enhance bio-active potential and BSA binding strength of synthesized compounds for their maximum effectiveness. 5-(1-(4-Nitrophenylsulfonyl)piperidin-4-yl)-4-phenyl-4H-1,2,4-triazole-3-thiol (3) and its derived compounds were synthesized and subjected to anti-bacterial, anti-inflammatory and AChE inhibition activities supported by BSA binding and computational analysis.

MATERIALS AND METHODS

The chemicals of Sigma Aldrich and Alfa Aesar were utilized during experimental work and their supply was assured by a local supplier. The Purity of synthesized compounds and the completion of reactions were confirmed by thin layer chromatography that was performed with silica gel coated on the aluminum plate. A mixture of *n*-hexane: ethyl acetate was utilized as mobile phase and UV₂₅₄ lamp assisted in viewing the clear spots of compounds. Melting points of compounds were determined by Gallenkemp apparatus and were uncorrected. The spectral data of ¹H-NMR and ¹³C-NMR were recorded on Bruker spectrometer working at 600 MHz and 150 MHz, respectively, in a CDCl₃ solvent. IR and EIMS spectra were recorded by utilizing JMS-HX-110 spectrometer and Jasco-320-A spectrophotometer respectively. IR data was recorded by KBr pellet method.

Synthesis of ethyl 1-(4-nitrophenylsulfonyl)piperidin-4-carboxylate (1)

Ethyl 1-(4-nitrophenylsulfonyl) piperidin-4-carboxylate (1) was synthesized by stirring ethyl isonipecotate (0.389 mol; b) with 4-nitrobenzenesulfonyl chloride (6.12g, 0.0389 mol; a) at room temperature for five hours in water (50mL). The pH of the reaction mixture was maintained up to 9-10 by 18% sodium carbonate solution during the reaction. At completion, precipitates of a product were obtained by adding cold water (20mL) and dilute HCl (3-4 mL) to adjust a pH of 5-6. Obtained precipitates of the product were filtered, washed and dried at room temperature.

Synthesis of 1-(4-nitrophenylsulfonyl)piperidin-4-carbohydrazide (2)

Hydrazine monohydrate (0.07mol) and compound 1 (0.07mol) was refluxed in methanol (40mL) for eight hours. After completion of the reaction, excess chilled distilled water (100mL) was added gradually followed by gentle shaking to obtain the title compound. Precipitates were filtered, washed with *n*-hexane and dried.

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

Phenyl isothiocyanate (5mL, 0.0304 mol) and compound 2 (10g, 0.0304 mol) were refluxed in methanol (25mL) for two hours. After the completion of the reaction, off-white uncyclized precipitates were obtained that were filtered and dried. These uncyclized precipitates were cyclized by refluxing with 10% KOH (35mL). At completion, dil. HCl was added to adjust pH of 4-5 and continuously stirred until the precipitates have appeared. The precipitates of compound 3 were filtered, washed and dried.

General synthesis of 4-(5-(aralkyl/alkylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5a-q)

Compound 3 (0.2g, 0.00044mol) was activated by LiH (0.00044 mol) on stirring for half an hour in DMF (15 mL). Then an equimolar amount of aralkyl/alkyl halides (4a-q) was added and stirred for 4-9 hours. Excess chilled distilled water (50mL) was added to the reaction mixture slowly on continuous shaking. The resulting precipitates were filtered, washed and dried. In case of no precipitation, extraction by chloroform was performed. Afterward, the chloroform was evaporated to obtain the pure compounds.

Antibacterial activity (in vitro) assay

The reported method was employed to evaluate the antibacterial activity of the synthesized molecules (Kaspady *et al.*, 2009; Yang *et al.*, 2006). Five bacterial strains namely *B. subtilis* (+), *S. aureus* (+), *E. coli* (-), *P. aeruginosa* (-) and *S. typhi* (-) were employed to calculate the antibacterial activity. Ofloxacin was used as reference standard.

Anti-inflammatory activity (in vitro) assay

The reported method was employed to evaluate the anti-inflammatory activity of the synthesized molecules (Govindappa *et al.*, 2011). Ibuprofen was used as reference standard.

Acetyl cholinesterase inhibition (in vitro) assay

Acetyl cholinesterase enzyme inhibition was studied following the reported method (Ellman *et al.*, 1961). The standard used to estimate the percent inhibition was eserine.

STATISTICAL ANALYSIS

All the calculations were made in triplicate and statistical analysis was performed by Microsoft Excel 2010. Results were presented as mean ± SEM.

BSA binding studies using fluorescence measurements

Fluorometric titration was used for the calculation of BSA quenching constant. BSA solution in phosphate buffer (20 mM, pH 7.4) and synthesized compounds solution in (1 mg/mL in the CDCl₃ solvent) were used to carry out the

fluorometric titration (Wu *et al.*, 2011). Ibuprofen and Warfarin were the site markers.

Molecular docking simulations

The docking study of two compounds, 5l and 5i was performed to understand the factors responsible for their active behaviour against acetyl cholinesterase enzyme. 5l posed better activity than that of 5i. This different activity behaviour was understood by the substituent attached to thio group as shown by the docked fig. This understanding was availed by the 3D structures of both compounds which were formed by Sybyl-X1.3/SKETCH module (Jain and Dock, 2007), followed by energy minimization by applying Tripos force field with Gasteigere Hückel atomic charge. Co-crystal structure of human acetylcholine esterase (AChE) complex with donepezil (PDB entry: 4EY7) was selected as the target receptor for molecular docking study. Surfex-Dock (SFXC) algorithm implemented in SYBYL-X 1.3 (Chohan *et al.*, 2016) was used to dock 5l and 5i to the active site of 4EY7. At first, co-crystallized water molecules were removed and necessary hydrogens were added. The model was then undergone to energy minimization and the atom types were assigned according to AMBER 7 FF99 force field. A *protomol* was generated by the mode of donepezil in 4EY7. The additional starting conformations for each molecule were set to twenty. Other parameters were established using default values. The C-score calculation was kept on. The best binding conformation was retrieved on the basis of C-score as well as the ligand-receptor mode of interactions.

Spectral characterization of the synthesized compounds

4-(5-(3-Bromobenzylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5a)

Light brown extract; Yield: 80%; Molecular formula: $C_{26}H_{24}BrN_5O_4S_2$; Molecular mass: 614.53 gmol^{-1} ; IR (KBr, ν_{max} , cm^{-1}): 3085 (Ar, C-H), 1669 (C=N), 1607 (Ar, C=C), 1535 (N=O), 1387 (S=O), 1057 (C-N), 617 (C-S); $^1\text{H-NMR}$ (CDCl_3 , 600 MHz, δ (ppm)): 7.53-7.48 (m, 3H, H-3'''-H-5'''), 7.47 (d, $J = 9.6 \text{ Hz}$, 2H, H-3'', H-5''), 7.37 (s, 1H, H-2'''), 7.36 (d, $J = 7.9 \text{ Hz}$, 2H, H-2''', H-6'''), 7.21 (d, $J = 10.0 \text{ Hz}$, 1H, H-4'''), 7.12 (t, $J = 7.9 \text{ Hz}$, 1H, H-5'''), 7.97 (d, $J = 8.0 \text{ Hz}$, 1H, H-6'''), 6.66 (d, $J = 8.7 \text{ Hz}$, 2H, H-2'', H-6''), 4.14 (s, 2H, H-7'''), 3.67-3.64 (m, 2H, $H_{eq-2'}$, $H_{eq-6'}$), 2.46-2.42 (m, 1H, H-4'), 2.30 (dt, $J = 2.8$, 11.6 Hz, 2H, $H_{ax-2'}$, $H_{ax-6'}$), 1.96 (dq, $J = 3.9$, 10.8 Hz, 2H, $H_{eq-3'}$, $H_{eq-5'}$), 1.81-1.69 (m, 2H, $H_{ax-3'}$, $H_{ax-5'}$); $^{13}\text{C-NMR}$ (CDCl_3 , 150 MHz, δ (ppm)): δ 162.5 (C-5), 157.7 (C-3), 150.9 (C-4''), 150.5 (C-1'''), 143.2 (C-1'''), 139.0 (C-3'''), 132.9 (C-1'''), 132.0 (C-3'', C-5''), 131.9 (C-2'''), 130.5 (C-4'''), 130.2 (C-2'', C-6''), 130.0 (C-6'''), 129.7 (C-2'''), 127.5 (C-4'''), 127.1 (C-5'''), 114.0 (C-3'''), 111.9 (C-2'', C-6'''), 45.4 (C-2', C-6'), 36.7 (C-4'), 29.3 (C-3', C-5'), 18.4 (C-7'''); EIMS (m/z): 616 $[\text{M}+2]^+$, 614 $[\text{M}]^+$, 386 $[\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_4\text{S}]^+$, 295 $[\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_4\text{S}]^+$, 270

$[\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_4\text{S}]^+$, 186 $[\text{C}_6\text{H}_4\text{NO}_4\text{S}]^+$, 170 $[\text{C}_7\text{H}_7\text{Br}]^+$, 123 $[\text{C}_6\text{H}_5\text{NO}_2]^+$, 65 $[\text{C}_5\text{H}_5]^+$, 51 $[\text{C}_4\text{H}_4]^+$.

4-(5-(4-Bromobenzylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5b)

Off white amorphous solid; Yield: 81 %; M.P.: 151-153 °C; Molecular formula: $C_{26}H_{24}BrN_5O_4S_2$; Molecular mass: 614.53 gmol^{-1} ; IR (KBr, ν_{max} , cm^{-1}): 3123 (Ar C-H), 1652 (C=N), 1622 (Ar C=C), 1516 (N=O), 1415 (S=O), 1087 (C-N), 618 (C-S); $^1\text{H-NMR}$ (CDCl_3 , 600 MHz, δ (ppm)): 7.50-7.46 (m, 3H, H-3'''-H-5'''), 7.37 (d, $J = 8.4 \text{ Hz}$, 2H, H-3'', H-5''), 7.18 (d, $J = 8.4 \text{ Hz}$, 2H, H-2''', H-6'''), 7.00 (d, $J = 9.1 \text{ Hz}$, 2H, H-2'', H-6''), 6.66 (d, $J = 8.7 \text{ Hz}$, 2H, H-2'' H-6''), 6.58 (d, $J = 8.4 \text{ Hz}$, 2H, H-3''', H-5'''), 4.11 (s, 2H, H-7'''), 3.64 (br.s, 2H, $H_{eq-2'}$, $H_{eq-6'}$), 2.47-2.43 (m, 1H, H-4'), 2.37-2.29 (m, 2H, $H_{ax-2'}$, $H_{ax-6'}$), 1.99-1.93 (m, 2H, $H_{eq-3'}$, $H_{eq-5'}$), 1.81-1.78 (m, 2H, $H_{ax-3'}$, $H_{ax-5'}$); $^{13}\text{C-NMR}$ (CDCl_3 , 150 MHz, δ (ppm)): δ 157.6 (C-3), 151.1 (C-4''), 150.5 (C-1'''), 137.0 (C-5), 135.8 (C-1'''), 133.0 (C-4'''), 131.9 (C-1'''), 131.7 (C-3'', C-5''), 131.6 (C-4'''), 130.8 (C-2'', C-6''), 127.1 (C-2''', C-6'''), 121.6 (C-3''', C-5'''), 114.1 (C-3''', C-5'''), 111.9 (C-2'', C-6'''), 45.4 (C-2', C-6'), 45.3 (C-7'''), 36.4 (C-4'), 29.3 (C-3', C-5'); EIMS (m/z): 616 $[\text{M}+2]^+$, 614 $[\text{M}]^+$, 386 $[\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_4\text{S}]^+$, 295 $[\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_4\text{S}]^+$, 270 $[\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_4\text{S}]^+$, 186 $[\text{C}_6\text{H}_4\text{NO}_4\text{S}]^+$, 170 $[\text{C}_7\text{H}_7\text{Br}]^+$, 123 $[\text{C}_6\text{H}_5\text{NO}_2]^+$, 65 $[\text{C}_5\text{H}_5]^+$, 51 $[\text{C}_4\text{H}_4]^+$.

4-(5-(2-Methylbenzylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5c)

Dark brown sticky product; Yield: 82%; Molecular formula: $C_{27}H_{27}ClN_5O_4S_2$; Molecular mass: 549.66 gmol^{-1} ; IR (KBr, ν_{max} , cm^{-1}): 3096 (Ar C-H), 1688 (C=N), 1603 (Ar C=C), 1546 (N=O), 1385 (S=O), 1046 (C-N), 643 (C-S); $^1\text{H-NMR}$ (CDCl_3 , 600 MHz, δ (ppm)): 7.66 (d, $J = 8.4 \text{ Hz}$, 2H, H-2''', H-6'''), 7.61-7.59 (m, 1H, H-4'''), 7.55 (d, $J = 7.2 \text{ Hz}$, 2H, H-3''', H-5'''), 7.49 (d, $J = 8 \text{ Hz}$, 2H, H-3'', H-5''), 7.22 (d, $J = 6.8 \text{ Hz}$, 2H, H-2'', H-6''), 7.20-7.09 (m, 4H, H-3'''-H-6'''), 4.60 (s, 2H, H-7'''), 3.65 (br.s, 2H, $H_{eq-2'}$, $H_{eq-6'}$), 2.93 (s, 3H, H-8'''), 2.68-2.62 (m, 1H, H-4'), 2.34-2.32 (m, 2H, $H_{ax-2'}$, $H_{ax-6'}$), 2.28-2.27 (m, 2H, $H_{eq-3'}$, $H_{eq-5'}$), 1.87 (br.s, 2H, $H_{ax-3'}$, $H_{ax-5'}$); $^{13}\text{C-NMR}$ (CDCl_3 , 150 MHz, δ (ppm)): δ 157.9 (C-3), 152.0 (C-4''), 151.5 (C-1'''), 137.9 (C-5), 136.2 (C-1'''), 132.0 (C-1'''), 131.2 (C-4'''), 130.9 (C-3'', C-5''), 129.6 (C-2'', C-6''), 128.1 (C-2''', C-6'''), 127.3 (C-4'''), 126.2 (C-2'''), 123.6 (C-4'''), 122.9 (C-3'''), 114.8 (C-3''', C-5'''), 113.1 (C-5'''), 45.6 (C-2', C-6'), 44.2 (C-7'''), 35.7 (C-4'), 32.3 (C-7'''), 30.1 (C-3', C-5'); EIMS (m/z): 549 $[\text{M}]^+$, 386 $[\text{C}_{18}\text{H}_{18}\text{N}_4\text{O}_4\text{S}]^+$, 295 $[\text{C}_{12}\text{H}_{13}\text{N}_3\text{O}_4\text{S}]^+$, 270 $[\text{C}_{11}\text{H}_{14}\text{N}_2\text{O}_4\text{S}]^+$, 186 $[\text{C}_6\text{H}_4\text{NO}_4\text{S}]^+$, 123 $[\text{C}_6\text{H}_5\text{NO}_2]^+$, 105 $[\text{C}_8\text{H}_9]^+$, 79 $[\text{C}_6\text{H}_7]^+$, 65 $[\text{C}_5\text{H}_5]^+$.

4-(5-(3-Chlorobenzylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5d)

Dark brown sticky product; Yield: 86%; Molecular formula: $C_{26}H_{24}ClN_5O_4S_2$; Molecular mass: 570.08 gmol^{-1}

¹; IR (KBr, ν_{max} , cm^{-1}): 3096 (Ar C-H), 1688 (C=N), 1603 (Ar C=C), 1546 (N=O), 1385 (S=O), 1046 (C-N), 643 (C-S); ¹H-NMR (CDCl_3 , 600 MHz, δ (ppm)): 7.55-7.49 (m, 3H, H-3'''-H-5'''), 7.48 (d, $J = 9.6$ Hz, 2H, H-3'', H-5''), 7.36 (s, 1H, H-2'''), 7.39 (d, $J = 8.9$ Hz, 2H, H-2''', H-6'''), 7.31 (d, $J = 10$ Hz, 1H, H-4'''), 7.12 (t, $J = 7.9$ Hz, 1H, H-5'''), 7.97 (d, $J = 8$ Hz, 1H, H-6'''), 6.66 (d, $J = 8.8$ Hz, 2H, H-2'', H-6''), 4.14 (s, 2H, H-7'''), 3.67-3.64 (m, 2H, H_{eq}-2', H_{eq}-6'), 2.46-2.42 (m, 1H, H-4'), 2.32 (dt, $J = 2.8, 11.6$ Hz, 2H, H_{ax}-2', H_{ax}-6'), 1.96 (dq, $J = 3.9, 10.8$ Hz, 2H, H_{eq}-3', H_{eq}-5'), 1.81-1.79 (m, 2H, H_{ax}-3', H_{ax}-5'); ¹³C-NMR (CDCl_3 , 150 MHz, δ (ppm)): δ 157.6 (C-3), 151.4 (C-4''), 150.5 (C-1''), 136.4 (C-5), 135.1 (C-3'''), 133.5 (C-1'''), 132.8 (C-1''), 130.5 (C-3'', C-5''), 129.8 (C-2'', C-6''), 128.7 (C-4'''), 128.0 (C-2'''), 129.0 (C-2''', C-6'''), 124.3 (C-4'''), 114.0 (C-3''', C-5'''), 112.0 (C-6'''), 111.8 (C-6''), 45.4 (C-2', C-6'), 36.4 (C-4'), 31.9 (C-7'''), 29.3 (C-3', C-5'); EIMS (m/z): 572 [M+2]⁺, 570 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 125 [C₇H₇Cl]⁺, 123 [C₆H₅NO₂]⁺, 65 [C₃H₅]⁺, 51 [C₄H₄]⁺.

4-(5-(4-Chlorobenzylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5e)

Light green amorphous solid; Yield: 83%; M.P.: 116-117 °C; Molecular formula: C₂₆H₂₄ClN₅O₄S₂; Molecular mass: 570.08 gmol⁻¹; IR (KBr, ν_{max} , cm^{-1}): 3098 (Ar C-H), 1668 (C=N), 1586 (Ar C=C), 1516 (N=O), 1396 (S=O), 1078 (C-N), 616 (C-S); ¹H-NMR (CDCl_3 , 600 MHz, δ (ppm)): 7.53-7.50 (m, 3H, H-3'''-H-5'''), 7.33 (d, $J = 8.1$ Hz, 2H, H-3'', H-5''), 7.24 (d, $J = 8.2$ Hz, 2H, H-2''', H-6'''), 7.03 (d, $J = 7.3$ Hz, 2H, H-2'', H-6''), 6.66 (d, $J = 8.1$ Hz, 2H, H-2'', H-6''), 6.58 (d, $J = 8.4$ Hz, 2H, H-3''', H-5'''), 4.35 (s, 2H, H-7'''), 3.67-3.65 (m, 2H, H_{eq}-2', H_{eq}-6'), 2.50-2.48 (m, 1H, H-4'), 2.34-2.29 (m, 2H, H_{ax}-2', H_{ax}-6'), 1.97-1.95 (m, 2H, H_{eq}-3', H_{eq}-5'), 1.81-1.79 (m, 2H, H_{ax}-3', H_{ax}-5'); ¹³C-NMR (CDCl_3 , 150 MHz, δ (ppm)): δ 157.6 (C-3), 151.4 (C-4''), 150.5 (C-1''), 136.4 (C-5), 135.1 (C-4'''), 133.5 (C-1'''), 132.8 (C-1''), 130.5 (C-3'', C-5''), 129.8 (C-2'', C-6''), 129.0 (C-2''', C-6'''), 128.7 (C-3''', C-5'''), 124.3 (C-4'''), 114.0 (C-3''', C-5'''), 112.0 (C-2''', C-6'''), 45.4 (C-2', C-6'), 36.4 (C-4'), 31.9 (C-7'''), 29.3 (C-3', C-5'); EIMS (m/z): 572 [M+2]⁺, 570 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 125 [C₇H₇Cl]⁺, 123 [C₆H₅NO₂]⁺, 65 [C₃H₅]⁺, 51 [C₄H₄]⁺.

1-(4-Nitrophenylsulfonyl)-4-(4-phenyl-5-(3-phenylpropylthio)-4H-1,2,4-triazol-3-yl)piperidine (5f)

Light brown amorphous solid; Yield: 87 %; M.P.: 134-135 °C; Molecular formula: C₂₈H₂₉N₅O₄S₂; Molecular mass: 563.69 gmol⁻¹; IR (KBr, ν_{max} , cm^{-1}): 3096 (Ar C-H), 1688 (C=N), 1603 (Ar C=C), 1546 (N=O), 1385 (S=O), 1046 (C-N), 643 (C-S); ¹H-NMR (CDCl_3 , 600 MHz, δ (ppm)): 7.63-7.57 (m, 3H, H-3'''-H-5'''), 7.53 (d, $J = 7.6$ Hz, 2H, H-2''', H-6'''), 7.40 (d, $J = 6$ Hz, 2H, H-3'', H-5''), 7.25 (t, $J = 8.0$ Hz, 1H, H-4'''), 7.16 (t, $J = 8$ Hz, 2H, H-

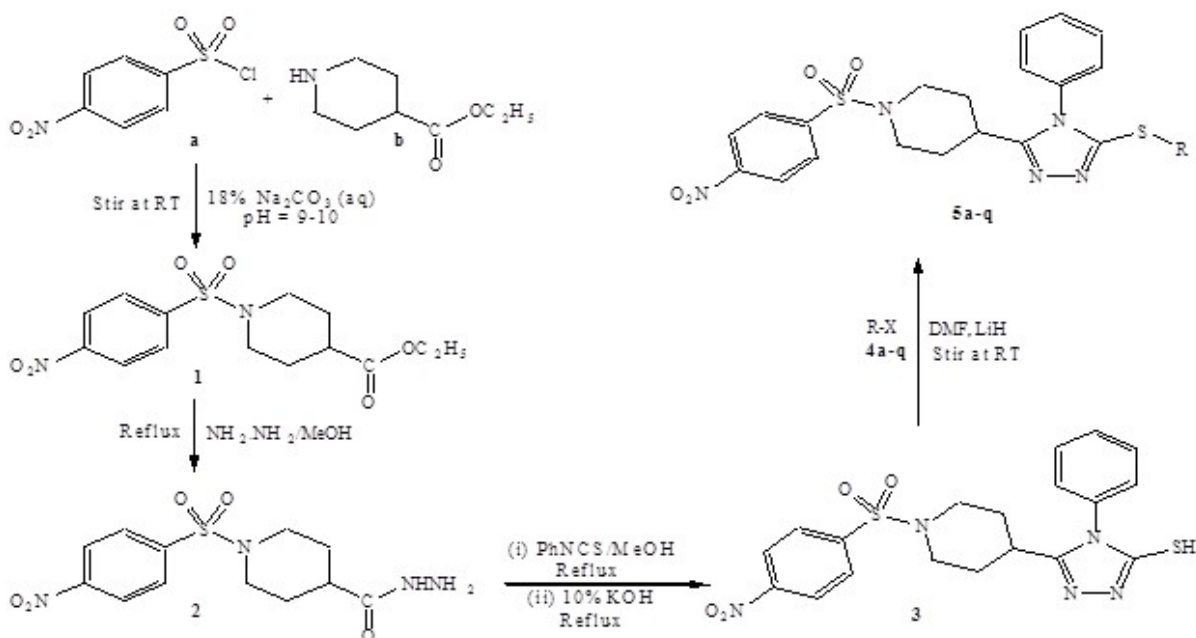
3'', H-5'''), 7.11 (d, $J = 8$ Hz, 2H, H-2'', H-6''), 6.96 (d, $J = 7.2$ Hz, 2H, H-2'', H-6''), 3.65 (t, $J = 6.4$ Hz, 2H, H-9'''), 3.37 (t, $J = 6.4$ Hz, 2H, H_{eq}-2', H_{eq}-6'), 2.75 (t, $J = 7.6$ Hz, 2H, H-7'''), 2.69 (t, $J = 7.6$ Hz, 2H, H_{ax}-2', H_{ax}-6'), 2.43 (br.s, 1H, H-4'), 2.15 (qui, $J = 7.2$ Hz, 2H, H-8'''), 2.05-2.03 (m, 2H, H_{eq}-3', H_{eq}-5'), 1.89-1.88 (m, 2H, H_{ax}-3', H_{ax}-5'); ¹³C-NMR (CDCl_3 , 150 MHz, δ (ppm)): δ 156.9 (C-3), 152.1 (C-4''), 151.3 (C-1''), 137.2 (C-5), 133.9 (C-1'''), 131.8 (C-1'''), 130.6 (C-3'', C-5''), 129.5 (C-2'', C-6''), 128.7 (C-2''', C-6'''), 128.4 (C-3''', C-5'''), 127.1 (C-3'''), 126.3 (C-4'''), 114.3 (C-3''', C-5'''), 113.2 (C-2''', C-6'''), 46.3 (C-2', C-6'), 35.8 (C-4'), 32.4 (C-7'''), 30.2 (C-9'''), 29.8 (C-7'''), 29.3 (C-3', C-5'); EIMS (m/z): 563 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 119 [C₉H₁₁]⁺, 106 [C₈H₁₁]⁺, 65 [C₃H₅]⁺.

4-(5-(Ethylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5g)

Light yellow amorphous solid; Yield: 86%; M.P.: 198-200 °C; Molecular formula: C₂₁H₂₃N₅O₄S₂; Molecular mass: 473.57 gmol⁻¹; IR (KBr, ν_{max} , cm^{-1}): 3075 (Ar C-H), 2569 (S-H), 2154 (C-N-C), 1654 (C=N), 1722 (C=O), 1593 (Ar C=C), 1538 (N=O), 1398 (S=O), 1123 (C-N), 629 (C-S); ¹H-NMR (CDCl_3 , 600 MHz, δ (ppm)): 7.54-7.52 (m, 3H, H-3'''-H-5'''), 7.50 (d, $J = 8.6$ Hz, 2H, H-3'', H-5''), 7.17 (d, $J = 9.6$ Hz, 2H, H-2''', H-6'''), 6.66 (d, $J = 9.2$ Hz, 2H, H-2'', H-6''), 3.67-3.64 (m, 2H, H_{eq}-2', H_{eq}-6'), 3.18 (q, $J = 7.4$ Hz, 2H, H-1'''), 2.52-2.48 (m, 1H, H-4'), 2.34 (dt, $J = 2.8, 8.11$ Hz, 2H, H_{ax}-2', H_{ax}-6'), 1.99-1.96 (m, 2H, H_{eq}-3', H_{eq}-5'), 1.83-1.80 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.37 (t, $J = 7.3$ Hz, 3H, H-2''); ¹³C-NMR (CDCl_3 , 150 MHz, δ (ppm)): δ 157.3 (C-3), 152.0 (C-4''), 150.5 (C-1''), 133.3 (C-5), 130.2 (C-1'''), 130.1 (C-3'', C-5''), 129.8 (C-2'', C-6''), 127.2 (C-2''', C-6'''), 124.5 (C-4'''), 114.0 (C-3''', C-5'''), 45.4 (C-2', C-6'), 31.9 (C-4'), 29.3 (C-3', C-5'), 26.8 (C-1'''), 14.7 (C-2''); EIMS (m/z): 473 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 51 [C₄H₄]⁺, 29 [C₂H₅]⁺.

1-(4-Nitrophenylsulfonyl)-4-(4-phenyl-5-(n-propylthio)-4H-1,2,4-triazol-3-yl)piperidine (5h)

Light yellow amorphous solid; Yield: 87 %; M.P.: 240-241 °C; Molecular formula: C₂₂H₂₅N₅O₄S₂; Molecular mass: 487.60 gmol⁻¹; IR (KBr, ν_{max} , cm^{-1}): 3133 (Ar C-H), 1665 (C=N), 1612 (Ar C=C), 1522 (N=O), 1456 (S=O), 1123 (C-N), 603 (C-S); ¹H-NMR (CDCl_3 , 600 MHz, δ (ppm)): 7.54-7.52 (m, 3H, H-3'''-H-5'''), 7.49 (d, $J = 8.6$ Hz, 2H, H-3'', H-5''), 7.17 (d, $J = 9.5$ Hz, 2H, H-2''', H-6'''), 6.66 (d, $J = 8.6$ Hz, 2H, H-2'', H-6''), 3.67-3.63 (m, 2H, H_{eq}-2', H_{eq}-6'), 3.14 (t, $J = 7.3$ Hz, 2H, H-1'''), 2.49-2.47 (m, 1H, H-4'), 2.33 (dt, $J = 2.9, 11.5$ Hz, 2H, H_{ax}-2', H_{ax}-6'), 1.99-1.95 (m, 2H, H_{eq}-3', H_{eq}-5'), 1.83-1.80 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.74 (sex, $J = 7.3$ Hz, 2H, H-2''), 0.96 (t, $J = 7.3$ Hz, 3H, H-3'''); ¹³C-NMR (CDCl_3 , 150 MHz, δ (ppm)): δ 157.3 (C-3), 152.2 (C-4''), 150.5 (C-1''), 133.3



Scheme 1: Synthesis of 4-(5-(aralkyl/alkylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl) piperidine (5a-q)

Table 1: Different substituted aralkyl/alkyl groups

Comp.	R	Comp.	R
5a		5j	$\text{---H}_2\text{C---CH}_2\text{---CH}_2\text{---CH}_3$ 1''' 2''' 3''' 4'''
5b		5k	$\text{---H}_2\text{C---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_3$ 1''' 2''' 3''' 4''' 5'''
5c		5l	$\begin{array}{c} 5''' \text{ CH}_3 \\ \\ \text{---HC---CH}_2\text{---CH}_2\text{---CH}_3 \\ 1''' \quad 2''' \quad 3''' \quad 4''' \end{array}$
5d		5m	$\text{---H}_2\text{C---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_3$ 1''' 2''' 3''' 4''' 5''' 6''' 7'''
5e		5n	$\text{---H}_2\text{C---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_2\text{---CH}_3$ 1''' 2''' 3''' 4''' 5''' 6''' 7''' 8'''
5f		5o	$\text{---H}_2\text{C---CH}_2\text{---Cl}$ 1''' 2'''
5g	$\text{---H}_2\text{C---CH}_3$ 1''' 2'''	5p	$\text{---H}_2\text{C---CH}_2\text{---CH}_2\text{---Br}$ 1''' 2''' 3'''
5h	$\text{---H}_2\text{C---CH}_2\text{---CH}_3$ 1''' 2''' 3'''	5q	
5i	$\begin{array}{c} 3''' \text{ CH}_3 \\ \\ \text{---HC---CH}_3 \\ 1''' \quad 2''' \end{array}$		

Table 2: % Inhibition of antibacterial activity

Compounds	% Inhibition				
	Gram positive bacteria		Gram negative bacteria		
	<i>B. subtilis</i> (+)	<i>S. aureus</i> (+)	<i>E. coli</i> (-)	<i>P. aeruginosa</i> (-)	<i>S. typhi</i> (-)
5a	-	-	-	-	-
5b	16.6	-	16.31	-	3.52
5c	25.76	5.34	19.13	-	-
5d	29.89	3.14	14.03	-	10.75
5e	91.07	-	5.26	-	-
5f	9.68	-	13.86	9.49	7.98
5g	-	-	5.4	-	-
5h	-	-	-	-	6.29
5i	-	-	-	4.76	-
5j	16.77	-	-	-	-
5k	37.01	2.6	20.73	-	25.31
5l	7.16	-	10.28	6.38	-
5m	-	-	-	-	-
5n	-	23.43	9.62	-	-
5o	-	5.61	3.75	29.39	-
5p	23.37	4.01	11.86	-	24.27
5q	8.83	16.3	10.75	2.54	1.58
Ofloxacin	95.60	95.43	93.98	94.28	92.75

Table 3: AChE inhibition and Anti-inflammatory activity

Compounds	AChE		Anti-inflammatory activity	
	Inhibition (%) at 0.5 mM	IC ₅₀ (μM)	% Inhibition at 25 μg/mL	IC ₅₀ ±SD
5a	87.73±0.25	51.45±0.16	47.3	36.4±.2
5b	89.92±0.19	21.26±0.14	36.1	54.7±.9
5c	87.39±0.19	35.74±0.15	96.7	2.5±0.3
5d	-	-	21.5	64.8
5e	88.32±0.27	37.69±0.15	34.9	58.7±8.5
5f	-	-	-	>100
5g	85.68±0.25	112.74±0.21	40.8	61.2±.4
5h	89.54±0.26	38.93±0.18	48.0	53.7±.7
5i	78.72±0.24	71.65±0.21	62.1	18.5±1.2
5j	26.13±0.23	-	42.6	39.8±.5
5k	91.74±0.27	16.25±0.16	-	>100
5l	90.42±0.26	15.25±0.17	97.2	2.3±0.1
5m	91.53±0.21	17.56±0.15	17.4	73.4±.9
5n	91.29±0.17	16.42±0.12	-	-
5o	91.26±0.25	17.16±0.18	59.4	17.9±2.4
5p	89.73±0.19	37.54±0.15	93.8	3.0±0.2
5q	29.51±0.15	-	98.5	1.5±0.1
Eserine	91.27±1.17	0.04±0.0001		
Ibuprofen			73.2	11.2±1.9

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} (L/mol) x 10 ⁴	K _q (L/mols ⁻¹) x 10 ¹²	K _a (L/mol)	N
5b	4.8123	4.8123	2.21 x 10 ⁴	0.93
5f	0.6246	0.6246	6.43 x 10 ²	0.77
5g	2.8721	2.8721	1.37 x 10 ³	0.68
5j	0.5782	0.5782	1.17 x 10 ³	0.83
Warfarin			641000	1.07
Ibuprofen	3502	35	56870	1.26

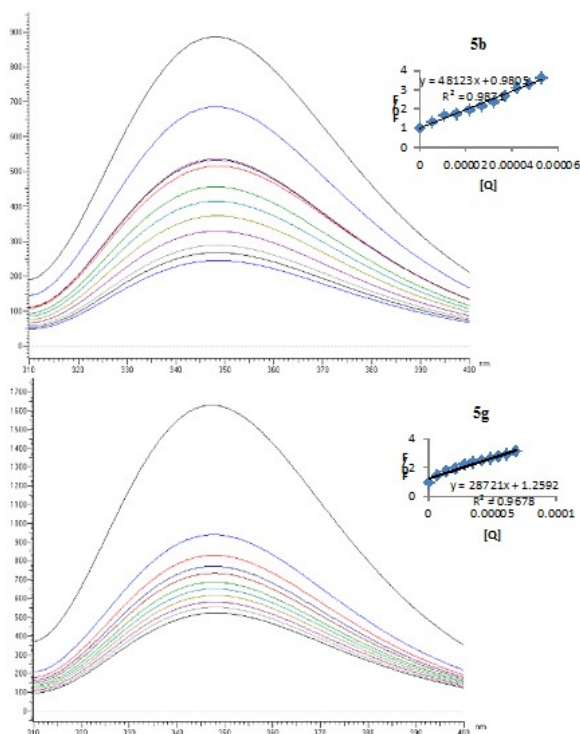


Fig. 1: Fluorescence spectra of BSA in presence of different amounts of selected compounds (5b and 5g) at 295 nm & 298 K and graph corresponding to the Stern-Volmer plot

(C-5), 130.2 (C-1'''), 130.1 (C-3'', C-5''), 129.8 (C-2'', C-6''), 127.2 (C-2''', C-6'''), 124.4 (C-4'''), 114.0 (C-3''', C-5'''), 45.4 (C-2', C-6'), 34.3 (C-1''), 31.9 (C-4'), 29.3 (C-3', C-5'), 22.7 (C-2''), 13.2 (C-3'''); EIMS (m/z): 487 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 51 [C₄H₄]⁺, 43 [C₃H₇]⁺.

4-(5-(Isopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5i)

Off white amorphous solid; Yield: 88%; M.P.: 96-97°C; Molecular formula: C₂₂H₂₅N₅O₄S₂; Molecular mass: 487.60 gmol⁻¹; IR (KBr, ν_{max} , cm⁻¹): 3196 (Ar C-H), 1668 (C=N), 1612 (Ar C=C), 1520 (N=O), 1399 (S=O), 1088 (C-N), 612 (C-S); ¹H-NMR (CDCl₃, 600 MHz, δ (ppm)): 7.54-7.51 (m, 3H, H-3''''-H-5'''''), 7.49 (d, J = 8.5 Hz, 2H, H-3'', H-5''), 7.16 (d, J = 9.5 Hz, 2H, H-2''''', H-6'''''), 6.66 (d, J = 9.4 Hz, 2H, H-2'', H-6''), 3.84 (sep, J = 6.8 Hz, 1H, H-1'''), 3.67-3.65 (m, 2H, H_{eq}-2', H_{eq}-6'), 2.52-2.48 (m, 1H, H-4'), 2.32 (dt, J = 2.8, 11.7 Hz, 2H, H_{ax}-2', H_{ax}-6'), 1.98 (dq, J = 3.5, 10.3 Hz, 2H, H_{eq}-3', H_{eq}-5'), 1.83-1.80 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.35 (d, J = 6.8 Hz, 6H, H-2''', H-3'''); ¹³C-NMR (CDCl₃, 150 MHz, δ (ppm)): δ 157.2 (C-3), 151.6 (C-4''), 150.5 (C-1''), 133.3 (C-5), 130.1 (C-1'''), 130.0 (C-3'', C-5''), 129.7 (C-2'', C-6''), 127.3 (C-2''', C-6'''), 124.5 (C-4'''), 114.0 (C-3''', C-5'''), 45.4 (C-2', C-6'),

38.5 (C-4'), 32.0 (C-1'''), 29.3 (C-3', C-5'), 23.4 (C-2''', C-3'''); EIMS (m/z): 487 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 51 [C₄H₄]⁺, 43 [C₃H₇]⁺.

4-(5-(n-Butylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5j)

Off white amorphous solid; Yield: 85 %; M.P.: 243-244 °C; Molecular formula: C₂₃H₂₇N₅O₄S₂; Molecular mass: 501.62 gmol⁻¹; IR (KBr, ν_{max} , cm⁻¹): 3106 (Ar C-H), 1688 (C=N), 1589 (ArC=C), 1500 (N=O), 1380 (S=O), 1006 (C-N), 613 (C-S); ¹H-NMR (CDCl₃, 600 MHz, δ (ppm)): 7.54-7.52 (m, 3H, H-3''''-H-5'''''), 7.49 (d, J = 8.6 Hz, 2H, H-3'', H-5''), 7.17 (d, J = 9.6 Hz, 2H, H-2''''', H-6'''''), 6.66 (d, J = 8.6 Hz, 2H, H-2'', H-6''), 3.66-3.64 (m, 2H, H_{eq}-2', H_{eq}-6'), 3.17 (t, J = 7.4 Hz, 2H, H-1'''), 2.54-2.50 (m, 1H, H-4'), 2.345-2.341 (m, 2H, H_{ax}-2', H_{ax}-6'), 1.98-1.96 (m, H_{eq}-3', H_{eq}-5'), 1.83-1.81 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.68 (qui, J = 5.2 Hz, 2H, H-2'''), 1.38 (sex, J = 7.4 Hz, 2H, H-3'''), 0.88 (t, J = 7.4 Hz, 3H, H-4'''); ¹³C-NMR (CDCl₃, 150 MHz, δ (ppm)): δ 157.3 (C-3), 152.3 (C-4''), 150.5 (C-1''), 133.3 (C-5), 130.2 (C-1'''), 130.1 (C-3'', C-5''), 129.8 (C-2'', C-6''), 127.2 (C-2''', C-6'''), 114.0 (C-3''', C-5'''), 45.4 (C-2', C-6'), 32.1 (C-4'), 31.9 (C-1'''), 31.3 (C-2'''), 29.3 (C-3', C-5'), 21.8 (C-3'''), 13.4 (C-4'''); EIMS (m/z): 501 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 57 [C₄H₉]⁺, 51 [C₄H₄]⁺.

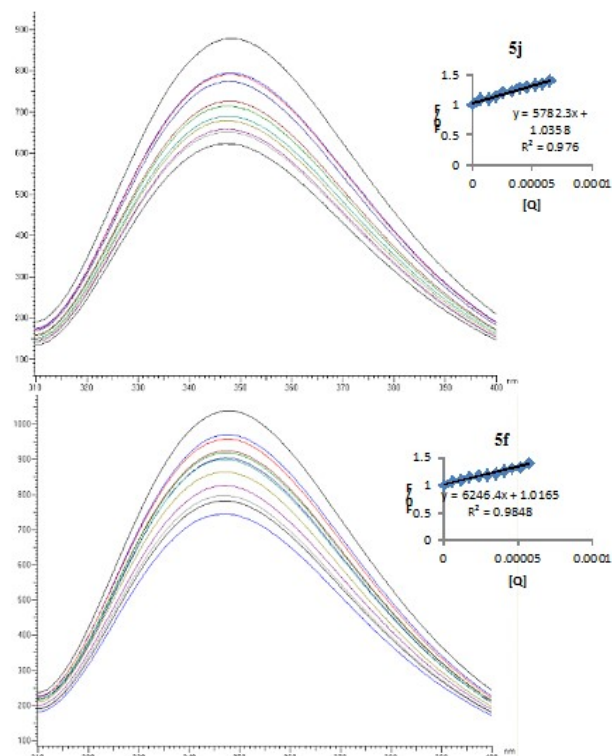


Fig. 2: Fluorescence spectra of BSA in presence of different amounts of selected compounds (5j and 5f) at 295 nm & 298 K and graph corresponding to the Stern-Volmer plot

1-(4-Nitrophenylsulfonyl)-4-(5-(n-pentylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)piperidine (5k)

Off white amorphous solid; Yield: 90%; M.P.: 155-156°C; Molecular formula: C₂₄H₂₉N₅O₄S₂; Molecular mass: 515.65 gmol⁻¹; IR (KBr, ν_{max} , cm⁻¹): 3066 (Ar C-H), 1640 (C=N), 1583 (Ar C=C), 1509 (N=O), 1389 (S=O), 1096 (C-N), 603 (C-S); ¹H-NMR (CDCl₃, 600 MHz, δ (ppm)): 7.53-7.52 (m, 3H, H-3'''-H-5'''), 7.50 (d, J = 9 Hz, 2H, H-3'', H-5''), 7.18 (d, J = 9.4 Hz, 2H, H-2''', H-6'''), 6.65 (d, J = 8.8 Hz, 2H, H-2'', H-6''), 3.67-3.64 (m, 2H, H_{eq}-2', H_{eq}-6'), 3.16 (t, J = 7.4 Hz, 2H, H-1'''), 2.52-2.48 (m, 1H, H-4'), 2.35-2.32 (m, 2H, H_{ax}-2', H_{ax}-6'), 1.99-1.96 (m, H_{eq}-3', H_{eq}-5'), 1.83-1.80 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.69 (qui, J = 7.5 Hz, 2H, H-2'''), 1.35-1.32 (m, 2H, H-3'''), 1.27-1.20 (m, 2H, H-4'''), 0.85 (t, J = 7.0 Hz, 3H, H-5'''); ¹³C-NMR (CDCl₃, 150 MHz, δ (ppm)): δ 157.3 (C-3), 152.3 (C-4''), 150.5 (C-1'''), 133.3 (C-5), 130.2 (C-1'''), 130.1 (C-3'''), 129.8 (C-2'', C-6''), 127.2 (C-2'''), C-6'''), 124.5 (C-4'''), 114.0 (C-3'''), C-5'''), 45.4 (C-2', C-6'), 32.4 (C-4'), 31.9 (C-1'''), 31.6 (C-2'''), 29.3 (C-3', C-5'), 28.7 (C-3'''), 22.5 (C-4'''), 14.0 (C-5'''); EIMS (m/z): 515 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 71 [C₅H₁₁]⁺, 51 [C₄H₄]⁺.

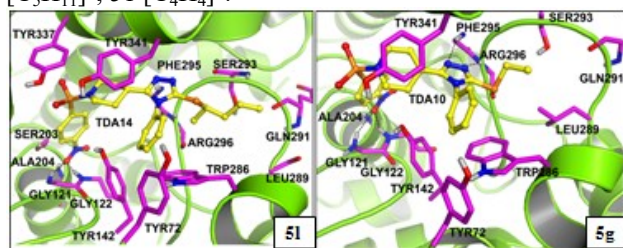


Fig. 3: Superimposition of the docked ligand 5l and 5g and experimental confirmation of donepezil (Magenta) in AChE main active site.

1-(4-Nitrophenylsulfonyl)-4-(5-(pentan-2-ylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)piperidine (5l)

Dark brown amorphous solid; Yield: 86%; M.P.: 124-125°C; Molecular formula: C₂₉H₂₉N₅O₄S₂; Molecular mass: 515.65 gmol⁻¹; IR (KBr, ν_{max} , cm⁻¹): 3096 (Ar C-H), 1688 (C=N), 1603 (Ar C=C), 1546 (N=O), 1385 (S=O), 1046 (C-N), 643 (C-S); ¹H-NMR (CDCl₃, 600 MHz, δ (ppm)): 7.66 (d, J = 8.4 Hz, 2H, H-2''', H-6'''), 7.57 (d, J = 5.6 Hz, 2H, H-3'', H-5''), 7.48 (t, J = 8.4 Hz, 2H, H-3''', H-5'''), 7.19 (t, J = 8.4 Hz, 1H, H-4'''), 6.70 (d, J = 8 Hz, 2H, H-2'', H-6''), 3.94-3.93 (m, 1H, H-1'''), 3.70-3.62 (m, 2H, H_{eq}-2', H_{eq}-6'), 2.71 (br.s, 1H, H-4'), 2.42-2.39 (m, 2H, H_{ax}-2', H_{ax}-6'), 2.29-2.27 (m, 2H, H_{eq}-3', H_{eq}-5'), 1.96-1.87 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.59 (q, J = 7.6 Hz, 2H, H-2'''), 1.35-1.32 (m, 2H, H-3'''), 1.27-1.20 (m, 3H, H-5'''), 0.86 (t, J = 7.0 Hz, 3H, H-4'''); ¹³C-NMR (CDCl₃, 150 MHz, δ (ppm)): δ 158.0 (C-3), 154.3 (C-4''), 152.3 (C-1'''), 136.9 (C-5), 132.4 (C-1'''), 131.3 (C-3'', C-5''), 130.6 (C-2'', C-6''), 129.1 (C-2''', C-6'''), 127.2 (C-4'''), 113.9 (C-3''', C-5'''), 45.8 (C-2', C-6'), 34.2 (C-4'), 32.7 (C-1'''), 31.6 (C-2'''), 30.1 (C-3', C-5'), 28.2 (C-3'''), 25.4 (C-4'''),

17.1 (C-5'''); EIMS (m/z): 515 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 71 [C₅H₁₁]⁺.

4-(5-(n-Heptylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5m)

Off white amorphous solid; Yield: 98%; M.P.: 201-202°C; Molecular formula: C₂₆H₃₃N₅O₄S₂; Molecular mass: 543.70 gmol⁻¹ IR (KBr, ν_{max} , cm⁻¹): 3128 (Ar C-H), 1666 (C=N), 1587 (Ar C=C), 1545 (N=O), 1407 (S=O), 1137 (C-N), 611 (C-S); ¹H-NMR (CDCl₃, 600 MHz, δ (ppm)): 7.54-7.51 (m, 3H, H-3'''-H-5'''), 7.49 (d, J = 8.5 Hz, 2H, H-3'', H-5''), 7.17 (d, J = 9.6 Hz, 2H, H-2''', H-6'''), 6.66 (d, J = 8.6 Hz, 2H, H-2'', H-6''), 3.67-3.63 (m, 2H, H_{eq}-2', H_{eq}-6'), 3.16 (t, J = 7.4 Hz, 2H, H-1'''), 2.49-2.48 (m, 1H, H-4'), 2.34 (dt, J = 2.8, 11.4 Hz, 2H, H_{ax}-2', H_{ax}-6'), 1.99-1.94 (m, H_{eq}-3', H_{eq}-5'), 1.83-1.80 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.69 (qui, J = 7.5 Hz, 2H, H-2'''), 1.35-1.25 (m, 8H, H-3'''-H-6'''), 0.86 (t, J = 7.0 Hz, 3H, H-7'''); ¹³C-NMR (CDCl₃, 150 MHz, δ (ppm)): δ 157.3 (C-3), 152.3 (C-4''), 150.5 (C-1'''), 133.3 (C-5), 130.2 (C-1'''), 130.1 (C-3'''), 129.7 (C-2'', C-6''), 127.2 (C-2''', C-6'''), 124.5 (C-4'''), 114.0 (C-3'''), C-5'''), 45.4 (C-2', C-6'), 32.4 (C-4'), 30.8 (C-1'''), 29.3 (C-3', C-5'), 29.0 (C-2''', C-3'''), 22.1 (C-4''', C-5''', C-6'''), 13.8 (C-7'''); EIMS (m/z): 543 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 99 [C₇H₁₃]⁺, 51 [C₄H₄]⁺.

1-(4-Nitrophenylsulfonyl)-4-(5-(n-octylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)piperidine (5n)

Light green amorphous solid; Yield: 87%; M.P.: 112-113°C; Molecular formula: C₂₇H₃₃N₅O₄S₂; Molecular mass: 557.73 gmol⁻¹; IR (KBr, ν_{max} , cm⁻¹): 3084 (Ar C-H), 1676 (C=N), 1613 (Ar C=C), 1513 (N=O), 1435 (S=O), 1156 (C-N), 603 (C-S); ¹H-NMR (CDCl₃, 600 MHz, δ (ppm)): 7.53-7.51 (m, 3H, H-3'''-H-5'''), 7.49 (d, J = 8.9 Hz, 2H, H-3'', H-5''), 7.17 (d, J = 9.5 Hz, 2H, H-2''', H-6'''), 6.66 (d, J = 8.5 Hz, 2H, H-2'', H-6''), 3.67-3.63 (m, 2H, H_{eq}-2', H_{eq}-6'), 3.15 (t, J = 7.4 Hz, 2H, H-1'''), 2.49-2.46 (m, 1H, H-4'), 2.34 (dt, J = 2.9, 11.4 Hz, 2H, H_{ax}-2', H_{ax}-6'), 2.00-1.94 (m, H_{eq}-3', H_{eq}-5'), 1.83-1.79 (m, 2H, H_{ax}-3', H_{ax}-5'), 1.69 (qui, J = 7.4 Hz, 2H, H-2'''), 1.37-1.32 (m, 2H, H-3'''-H-4'''), 1.29-1.21 (m, 2H, H-5'''-H-7'''), 0.88 (t, J = 7.2 Hz, 3H, H-8'''); ¹³C-NMR (CDCl₃, 150 MHz, δ (ppm)): δ 157.3 (C-3), 152.3 (C-4''), 150.5 (C-1'''), 133.3 (C-5), 130.2 (C-1'''), 130.1 (C-3'''), 129.7 (C-2'', C-6''), 127.2 (C-2''', C-6'''), 124.5 (C-4'''), 114.0 (C-3'''), C-5'''), 45.4 (C-2', C-6'), 32.5 (C-4'), 32.5 (C-1'''), 31.7 (C-2'''), 29.3 (C-3', C-5'), 29.1 (C-3'''), 29.0 (C-4'''), 28.7 (C-5'''), 22.6 (C-6''', C-7'''), 14.0 (C-8'''); EIMS (m/z): 557 [M]⁺, 386 [C₁₈H₁₈N₄O₄S]⁺, 295 [C₁₂H₁₃N₃O₄S]⁺, 270 [C₁₁H₁₄N₂O₄S]⁺, 186 [C₆H₄NO₄S]⁺, 123 [C₆H₅NO₂]⁺, 113 [C₈H₁₇]⁺, 51 [C₄H₄]⁺.

4-(5-(2-Chloroethylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5o)

Off white amorphous solid; Yield: 90%; M.P.: 184-185 °C; Molecular formula: $C_{21}H_{22}ClN_5O_4S_2$; Molecular mass: 508.01 $g\text{mol}^{-1}$; IR (KBr, ν_{max} , cm^{-1}): 3183 (Ar C-H), 1677 (C=N), 1609 (Ar C=C), 1525 (N=O), 1467 (S=O), 1175 (C-N), 633 (C-S); 1H -NMR ($CDCl_3$, 600 MHz, δ (ppm)): 7.55-7.52 (m, 3H, H-3''''-H-5''''), 7.50 (d, $J = 8.6$ Hz, 2H, H-3'', H-5''), 7.18 (d, $J = 10.9$ Hz, 2H, H-2''''', H-6'''''), 6.66 (d, $J = 8.6$ Hz, 2H, H-2'', H-6''), 3.87 (t, $J = 7$ Hz, 2H, H-1''''), 3.67-3.65 (m, 2H, H_{eq} -2', H_{eq} -6'), 3.50 (t, $J = 7$ Hz, 2H, H-2''''), 2.51-2.47 (m, 1H, H-4'), 2.34 (dt, $J = 11.2, 11.6$ Hz, 2H, H_{ax} -2', H_{ax} -6'), 2.01-1.94 (m, 2H, H_{eq} -3', H_{eq} -5'), 1.83-1.81 (m, 2H, H_{ax} -3', H_{ax} -5'); ^{13}C -NMR ($CDCl_3$, 150 MHz, δ (ppm)): δ 158.2 (C-3), 152.7 (C-4''), 151.0 (C-1''), 136.6 (C-5), 132.4 (C-1''''), 130.8 (C-3''), C-5''), 129.5 (C-2'', C-6''), 128.1 (C-2''''', C-6'''''), 127.1 (C-4''''), 114.2 (C-3''''', C-5'''''), 45.8 (C-2', C-6'), 39.2 (C-2''''), 34.2 (C-4'), 31.4 (C-1''''), 29.3 (C-3', C-5'); EIMS (m/z): 510 $[M+2]^+$, 508 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 63 $[C_2H_4Cl]^+$, 51 $[C_4H_4]^+$.

4-(5-(3-Bromopropylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5p)

Light green amorphous solid; Yield: 87%; M.P.: 138-140 °C; Molecular formula: $C_{22}H_{24}BrN_5O_4S_2$; Molecular mass: 566.49 $g\text{mol}^{-1}$; IR (KBr, ν_{max} , cm^{-1}): 3067 (Ar C-H), 1674 (C=N), 1620 (Ar C=C), 1528 (N=O), 1399 (S=O), 1092 (C-N), 633 (C-S); 1H -NMR ($CDCl_3$, 600 MHz, δ (ppm)): 7.51-7.48 (m, 3H, H-3''''-H-5''''), 7.47 (d, $J = 10.3$ Hz, 2H, H-3'', H-5''), 7.24 (d, $J = 6.5$ Hz, 2H, H-2''''', H-6'''''), 6.66 (d, $J = 8.5$ Hz, 2H, H-2'', H-6''), 3.65-3.64 (m, 2H, H_{eq} -2', H_{eq} -6'), 3.29 (t, $J = 7.3$ Hz, 2H, H-1''''), 2.52 (t, $J = 7.4$ Hz, 2H, H-3''''), 2.40 (br.s, 1H, H-4'), 2.27-2.26 (m, 2H, H_{ax} -2', H_{ax} -6'), 2.06 (qui, $J = 6.8$ Hz, 2H, H-2''''), 1.90-1.89 (m, 2H, H_{eq} -3', H_{eq} -5'), 1.80-1.76 (m, 2H, H_{ax} -3', H_{ax} -5'); ^{13}C -NMR ($CDCl_3$, 150 MHz, δ (ppm)): δ 158.3 (C-3), 152.4 (C-4''), 151.6 (C-1''), 136.2 (C-5), 132.7 (C-1''''), 130.5 (C-3''), C-5''), 129.2 (C-2'', C-6''), 128.4 (C-2''''', C-6'''''), 127.7 (C-4''''), 114.1 (C-3''''', C-5'''''), 45.2 (C-2', C-6'), 40.1 (C-3''''), 34.3 (C-4'), 32.3 (C-1''''), 30.4 (C-1'''), 29.2 (C-3', C-5'); EIMS (m/z): 568 $[M+2]^+$, 566 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 120 $[C_3H_6Br]^+$, 51 $[C_4H_4]^+$.

4-(5-((1,3-Dioxolan-2-yl)methylthio)-4-phenyl-4H-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine (5q)

Light yellow amorphous solid; Yield: 88%; M.P.: 176-177 °C; Molecular formula: $C_{23}H_{25}N_5O_6S_2$; Molecular mass: 531.60 $g\text{mol}^{-1}$; IR (KBr, ν_{max} , cm^{-1}): 3056 (Ar C-H), 1656 (C=N), 1596 (Ar C=C), 1539 (N=O), 1380 (S=O), 1089 (C-N), 650 (C-S); 1H -NMR ($CDCl_3$, 600 MHz, δ (ppm)): 7.55-7.51 (m, 3H, H-3''''-H-5''''), 7.49 (d, $J = 8.7$ Hz, 2H, H-3'', H-5''), 7.20 (d, $J = 9.7$ Hz, 2H, H-2''''', H-6'''''), 6.67 (t, $J = 6.9$ Hz, 1H, H-1''''), 6.66 (d, $J = 8.8$ Hz, 2H, H-2'', H-6''), 3.95 (t, $J = 3.7$ Hz, 2H, H_{eq} -3''''', H_{eq} -4'''''), 3.86 (t, $J = 5.1$ Hz, 2H, H_{ax} -3''''', H_{ax} -4'''''), 3.65-3.64 (m, 2H, H_{eq} -

2', H_{eq} -6'), 3.43 (d, $J = 4.3$ Hz, 2H, H-6''''), 2.51-2.49 (m, 1H, H-4'), 2.34-2.32 (m, 2H, H_{ax} -2', H_{ax} -6'), 1.95 (br.s, 2H, H_{eq} -3', H_{eq} -5'), 1.82-1.80 (m, 2H, H_{ax} -3', H_{ax} -5'); ^{13}C -NMR ($CDCl_3$, 150 MHz, δ (ppm)): δ 158.1 (C-3), 152.8 (C-4''), 151.2 (C-1''), 136.7 (C-5), 132.8 (C-1''''), 130.6 (C-3''), C-5''), 129.3 (C-2'', C-6''), 128.8 (C-2''''', C-6'''''), 126.2 (C-4''''), 114.0 (C-3''''', C-5'''''), 62.3 (C-1'''), 45.2 (C-2', C-6'), 40.1 (C-2''''), 38.2 (C-3''''), 36.1 (C-4'), 30.2 (C-4''''), 29.3 (C-3', C-5'); EIMS (m/z): 531 $[M]^+$, 386 $[C_{18}H_{18}N_4O_4S]^+$, 295 $[C_{12}H_{13}N_3O_4S]^+$, 270 $[C_{11}H_{14}N_2O_4S]^+$, 186 $[C_6H_4NO_4S]^+$, 123 $[C_6H_5NO_2]^+$, 87 $[C_4H_7O_2]^+$, 51 $[C_4H_4]^+$.

RESULTS

A series of unique 3,5-disubstituted-1,2,4-triazole based compounds bearing piperidine were synthesized in quest of highly biologically active agents. Protocol for the synthesis of all derived compounds (5a-q) is discussed in the experimental section and sketched in Scheme-1. The different aralkyl/alkyl groups are listed in table 1. All the synthesized compounds have been obtained in a notable yield. The purity and structures of synthesized compounds were confirmed through characterization by IR, 1H -NMR, ^{13}C -NMR and EIMS spectroscopic techniques. All the synthesized compounds were evaluated for antibacterial, anti-inflammatory and enzyme inhibition potential. The antibacterial potential was evaluated against Gram-positive and Gram-negative bacterial strains. The results for % inhibition of antibacterial screening are expressed in table 2. The results of % inhibition and IC_{50} values for anti-inflammatory and AChE enzyme inhibition potential are expressed in table 3. The IC_{50} values for anti-inflammatory potential have been expressed as mean \pm SD for varying concentration of 100, 10 and $1\mu g/mL$. The most of the compounds were found to be potent against the considered activities.

DISCUSSION

Light brown sticky compound 5a with yield 80% was selected for single compound discussion. The molecular formula ($C_{26}H_{24}BrN_5O_4S_2$) and molecular mass (614.53 $g\text{mol}^{-1}$) were confirmed through molecular ion peak at (m/z): 614 $[M]^+$ and number of protons in the 1H -NMR spectrum. The fragmentation peaks at 386, 295, 270, 186, 170, 123, 66 and 51 obtained by EIMS spectrum confirmed the structure of compound under consideration. With the help of IR data obtained at 3085 (Ar C-H), 2567 (S-H), 2127 (C-N-C), 1669 (C=N), 1715 (C=O), 1607 (Ar C=C), 1535 (N=O), 1387 (S=O), 1057 (C-N) and 617 (C-S); the presence of different functional groups was justified. The 1H -NMR spectrum corroborated the presence of 4-nitrophenyl sulfonyl by the peaks at δ 7.47 (d, $J = 9.6$ Hz, 2H, H-3'', H-5''), and 6.66 (d, $J = 8.7$ Hz, 2H, H-2'', H-6''). The peaks at δ 3.67-3.64 (m, 2H, H_{eq} -2', H_{eq} -6'), 2.46-2.42 (m, 1H, H-4'), 2.30 (dt, $J = 2.8, 11.6$

Hz, 2H, $H_{ax-2'}$, $H_{ax-6'}$), 1.96 (dq, $J = 3.9, 10.8$ Hz, 2H, $H_{eq-3'}$, $H_{eq-5'}$) and 1.81-1.69 (m, 2H, $H_{ax-3'}$, $H_{ax-5'}$) assured the presence of piperidine. The phenyl ring was confirmed by the signals appearing at δ 7.53-7.48 (m, 3H, H-3''', H-5''') and 7.36 (d, $J = 7.9$ Hz, 2H, H-2''', H-6'''). 3-Bromobenzylthio was confirmed by the presence of one singlet at 7.37 (s, 1H, H-2'''), two doublets at 7.21 (d, $J = 10$ Hz, 1H, H-4'''), 7.97 (d, $J = 8$ Hz, 1H, H-6''') and one triplet at 7.12 (t, $J = 7.9$ Hz, 1H, H-5'''). The peak at 4.14 (s, 2H, H-7''') confirmed the two protons present in the vicinity of heteroatom of sulphur. The broad band mode of ^{13}C -NMR confirmed the structure. Two quaternary carbons of triazole ring appeared at δ 162.5 (C-5''') and 157.7 (C-3'''). The 4-nitrophenylsulfonyl ring was justified by four signals appearing at δ 150.9 (C-4''), 150.5 (C-1''), 132.0 (C-3'', C-5'') and 130.2 (C-2'', C-6''). The phenyl ring directly attached to triazole ring showed four signals at δ 143.2 (C-1'''), 130.5 (C-4'''), 129.7 (C-2''', C-6''') and 114.0 (C-3''', C-5'''). The *S*-substituted 3-bromobenzyl group was confirmed through seven signals at δ 139.0 (C-3'''), 132.9 (C-1'''), 131.9 (C-2'''), 130.0 (C-6'''), 127.5 (C-4'''), 127.1 (C-5''') and 18.4 (C-7'''). Three signals appearing at δ 45.4 (C-2', C-6'), 36.7 (C-4') and 29.3 (C-3', C-5') were allotted to piperidine ring. Through these spectroscopic evidences, the compound 5a was confirmed as 4-(5-(3-bromobenzylthio)-4-phenyl-4*H*-1,2,4-triazol-3-yl)-1-(4-nitrophenylsulfonyl)piperidine. Likewise other compounds were corroborated structurally.

Antibacterial (in vitro) activity

Ofloxacin was used as reference standard. The series of compounds exhibited moderately less activity against the selected bacterial strains. The compounds, 5a (bearing 3-bromobenzyl group) and 5m (bearing *n*-heptyl group) remained inactive at all against all the bacterial strains. Compound 5q (bearing 1,3-dioxalan-2-ylmethyl group) remained active against all the strains but with too low percent inhibition. The compound, 5e bearing 4-chlorobenzyl group remained the most active one against *B. subtilis* (+) with inhibition of 91.07% comparative to ofloxacin with inhibition 95.60%. *B. subtilis* (+) and *E. coli* (-) were potentially inhibited by the majority of compounds. *S. aureus* (+), *P. aeruginosa* (-) and *S. typhi* (-) were potentially inhibited by least number of compounds. The compounds bearing chlorobenzyl groups (5d and 5e) remained more active than the compounds bearing bromobenzyl groups (5a and 5b). Furthermore, *para*-substituted compounds remained more active than the *ortho*-substituted ones. But among aliphatic ones, compound bearing 3-bromopropyl group (5p) remained more active than the compound bearing 2-chloroethyl group (5o). The compound bearing *n*-pentyl group (5k) remained the most active one among all the compounds bearing aliphatic groups as substituents. The compound bearing 2-pentyl group (5l) remained less active against the bacterial strains taken into account.

Anti-inflammatory (in vitro) activity

All the compounds showed anti-inflammatory activity except a few ones with variable values of $\text{IC}_{50} \pm \text{SD}$ comparative to Ibuprofen taken as reference. Three compounds 5e, 5i and 5o showed the least activity with IC_{50} values of 58.7 ± 8.5 , 18.5 ± 1.2 and $17.9 \pm 2.4 \mu\text{g/mL}$, respectively, as compared to IC_{50} value of Ibuprofen as $11.2 \pm 1.9 \mu\text{g/mL}$. The compounds 5c (bearing 2-methylbenzyl group), 5l (bearing 2-pentyl group), 5p (bearing 3-bromopropyl group) and 5q (bearing 1,3-dioxalan-2-ylmethyl group) remained the most active ones. The compounds bearing halobenzyl groups (5a, 5b, 5d and 5e) remained least but the compound bearing 2-methylbenzyl group (5c) remained much active in its anti-inflammatory action. The compound bearing 2-pentyl group (5l) remained the most active one among all the compounds bearing aliphatic groups as substituents.

AChE inhibition (in vitro) studies

The variable potential was observed by the whole series of compounds for the inhibition of acetyl cholinesterase enzyme. All the compounds were found very active against this enzyme except one compound 5g having IC_{50} value 112.74 ± 0.21 which might be due to the presence of ethyl group. All the compounds such as 5a, 5b, 5c, 5e, 5f, 5h, 5i, 5j, 5l, 5m, 5n, 5o, 5p were found very active against acetyl cholinesterase enzyme to inhibit its negative effects, having the IC_{50} values of 51.45 ± 0.16 , 21.26 ± 0.14 , 35.74 ± 0.15 , 37.69 ± 0.15 , 31.45 ± 0.14 , 38.93 ± 0.18 , 71.65 ± 0.21 , 16.25 ± 0.16 , 15.25 ± 0.17 , 17.56 ± 0.15 , 16.42 ± 0.12 , 17.16 ± 0.18 and $37.54 \pm 0.15 \mu\text{M}$. The IC_{50} values of the synthesized compounds were compared with the IC_{50} value of eserine used as the reference. Out of this whole array of active compounds 5l, 5m, 5n and 5o were highly active compounds possessing higher potential. The activity of these compounds might be due to the presence of 2-pentyl, *n*-heptyl, *n*-octyl and 2-chloroethyl groups in the structures.

Fluorescence quenching studies (BSA binding)

The tryptophan, phenylalanine and tyrosine are responsible for fluorescence of bovine serum albumin. At 295 nm the quenching fluorescence of excited BSA was measured. The fluorescence emission of the bovine serum albumin was gradually decreased by the increasing concentration of quencher upon titration (fig. 1 and fig. 2). The equation represented below describes the equilibrium between the free and bound molecules.

$$\text{Log} [(F_0 - F) / F] = \text{log } K_a + n \text{ log } [Q]$$

F_0 and F represent the fluorescence intensities in the absence and presence of quencher, respectively. K_a is the binding constant while 'n' is the number of binding sites. The emission intensities of BSA quenched by quencher were observed by the Stern-Volmer equation to understand the amount of complex compounds.

$$\frac{F_0}{F} = 1 + K_{sv}[Q] = K_q \tau_0 [Q] + 1$$

F_0 and F represent the fluorescence intensities in the presence and absence of quencher respectively. K_{sv} is the Stern-Volmer quenching constant, Q is the concentration of quencher. K_q is the bimolecular quenching rate constant and τ_0 is the half-life time of the fluorophore in the absence of quencher. Generally, all the employed compounds showed the binding in variable range measured in term of binding constant. Compounds 5b, 5f, 5g and 5j have values of binding constant as 2.21×10^4 , 6.43×10^2 , 1.37×10^3 and 1.17×10^3 respectively. The high binding constant value of compound 5b was due to the electron withdrawing group present at *para* position enhancing the protein-drug binding.

Binding of 5l and 5g with AChE Enzyme (Molecular docking)

Surflex-Dock module of SybylX-13 was used to dock either ligand 5l or 5g into the AChE main active site (fig. 3). Both inhibitors adopted the almost similar binding conformation in the AChE binding cavity as observed in experimentally determined conformation in a 4EY7 complex. Thus, our docking method was considered to be reliable. The graphical analysis of surflex-docking result showed that both inhibitors 5l and 5g incorporated deeply into the two sub-sites (CAS and PAS) of AChE main active site. Interestingly both ligands were able to acquire such a binding conformation in AChE active site, so that the nitrogen of triazole ring and oxygen of 4-nitrophenyl sulfonyl were able to make H-bond with the backbone NH_2 of Phe295 and GLY121 and GLY122 respectively. The phenyl ring attached with the nitrogen of triazole core is highly stabilized in the deep binding pocket where it may establish a weak interaction with ARG296 and TRP286. The NO_2 present at *para* position of phenyl sulfonyl group extends deeply into the CAS region where it may develop H-bond interaction with GLY121 and GLY122. The difference in the activity observed in both compounds most probably appeared at solvent-exposed region where the 2-pentyl group of the compound 5l was more extended as compared to the ethyl group of compound 5g. The deep extended portion of compound 5l in the solvent-exposed region is in the close vicinity of SER293, ARG296 and GLN291 and may develop Vander Waal's interaction with them. On the other hand as the ethyl group of compound 5g is not much extended and not able to any sort of interaction with residue, so 5l showed higher potential as anti-acetyl cholinesterase agent as compared to the compound 5g.

CONCLUSION

The heterocyclic compounds were synthesized because of their pharmacological importance and enhanced binding of compounds with bovine serum albumin, a plasma protein. All the compounds were analyzed by IR, ^1H -

NMR, ^{13}C -NMR and EIM Stechniques. The active binding sites were identified by molecular docking studies. Most of the compounds were found active as antibacterial and anti-inflammatory agents. Compound 5e presented potential antibacterial activity while compounds 5c, 5l, 5p and 5q showed the best anti-inflammatory activity. In addition to this, all the compounds were found highly active for acetyl cholinesterase enzyme inhibition. The highly active behaviour of the presented compounds recommends the *in vivo* analysis for deep in view of these compounds.

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