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Original Research Article

Biological screening and docking studies of unique hybrids synthesized by conventional versus microwave-assisted techniques

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Abstract

Purpose: *To carry out the synthesis of various hybrids of 1,2,4-triazole in search of potential therapeutic enzyme inhibitory agents, and carry out docking and bovine serum albumin (BSA) binding studies on docking and bovine serum albumin (BSA) binding studies on the hybrids.*

Methods: *The target compounds were synthesized by following a multistep protocol. Compound 1 was synthesized from 4-methoxybenzenesulfonyl chloride (a) and ethyl isonipecotate (b). Compound 1 was refluxed with hydrazine to synthesize compound 2, which was converted to compound 3 through two consecutive steps. Compound 4 and different amines (5a-5i), were utilized to synthesize an array of electrophiles (6a-6i). A series of 1,2,4-triazole hybrids (7a-7i) were synthesized at room temperature by stirring together 3 and 6a-6i. The final structures of 7a-7i were elucidated through ^{1}H-NMR, ^{13}C-NMR and EI-MS spectroscopy. The BSA binding studies were performed by fluorometric titration. Furthermore, antioxidant and enzyme inhibition activities were determined colorimetrically.*

Results: *Compound 7d was the most active antioxidant agent, compared to butylated hydroxyanisole (BHA), while compounds 7d, 7e, 7f, 7g and 7i proved to be potent urease inhibitors with half-maximal inhibitory concentration (IC_{50}) values of 19.5 ± 0.12, 21.1 ± 0.68, 18.2 ± 0.78, 19.9 ± 0.77 and 17.9 ± 0.10 µM, respectively, compared to thiourea with an IC_{50} of 24.3 ± 0.24 µM. Compounds 7a, 7b, 7d, and 7e exhibited high butyrylcholinesterase inhibition potential, compared to eserine.*

Conclusion: *The synthesized compounds require studies further as potential therapeutic enzyme inhibitory agents in view of their urease inhibition as well as antioxidant activity.*

Keywords: *Ethyl isonipecotate, 1,2,4-Triazole, Urease inhibition, Butyrylcholinesterase inhibition*

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INTRODUCTION

Nitrogen-based heterocyclic compounds are pharmacologically active agents [1]. These moieties include piperidine [2-5] and 1,2,4-triazole [4,6-9], which show different biological activities. On the basis of their biological potential, we designed the current study to

combine piperidine and 1,2,4-triazole in a single compound to explore their biological potential. Both conventional and microwave-assisted synthetic protocols were utilized. Microwave-assisted synthesis is found to be more suitable in term of purity, time of synthesis and yield [10]. The designed series of compounds were screened for antioxidant and enzyme-inhibitory activity [13], followed by docking studies [11,12]. The docking studies helped elucidate the interaction of the most active synthesized compounds with a specific protein. The BSA binding studies provided more information about the protein interaction of the synthesized compounds.

EXPERIMENTAL

General

The ^{1}H - NMR and ^{13}C - NMR spectra were obtained with a Bruker spectrometer operating at 600 and 150 MHz, respectively. A Jasco - 320 spectrophotometer was used to collect the IR spectra. Thin layer chromatography helped to determine the completion of reactions and purity of the synthesized compounds. Both Alfa Aeser and Sigma Aldrich provided analytical grade chemicals.

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

Compound **1** was synthesized by stirring together 4-methoxybenzenesulfonyl chloride (**a**: 0.04 mol) and ethyl isonipecotate (**b**: 0.04 mol) in aqueous medium, maintained at pH 9 - 10 with 15 % Na_2CO_3, for 4 h at room temperature. Chilled distilled water was then added, and the resulting precipitate was filtered out, washed with distilled water and dried at room temperature.

Synthesis of 1-[(4-methoxyphenyl)sulfonyl] piperidine-4-carbohydrazide (2)

Compound **2** was synthesized by refluxing an equimolar mixture of compound **1** and hydrazine hydrate for 3.5 h in EtOH. **2** was precipitated by the addition of chilled distilled water, filtered out, washed with distilled water and dried at room temperature.

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

Equimolar amounts (0.5 mol) of compound **2** and phenyl isothiocyanate were refluxed for 3 h in EtOH to form an un-cyclized intermediate. The intermediate was refluxed with equimolar KOH for another 3 h to obtain the title compound. Compound **3** was precipitated at pH 4 - 5 by the addition of dil. HCl to the reaction mixture. The precipitate was filtered out, washed with distilled water and dried at room temperature.

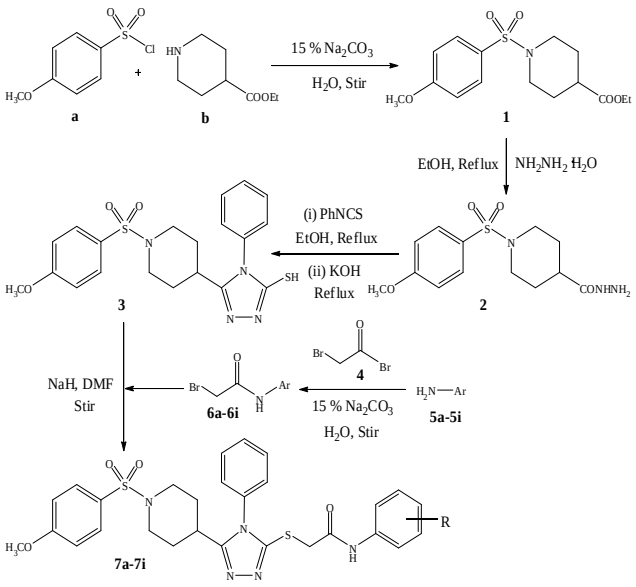
Synthesis of *N*-(substituted)-2-bromoacetamides (6a-6i)

Equimolar aryl amines (**5a-5i**) were stirred with 2-bromoacetyl bromide (**4**) for 2 h in distilled water at pH 9 – 10, maintained with 15 % Na_2CO_3. The precipitate was filtered out, washed with distilled water and dried at room temperature.

General procedure for synthesis of *N*-(substituted)-2-[(5-{1-[(4-methoxyphenyl) sulfonyl]-4-piperidinyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]acetamide (7a-7i)

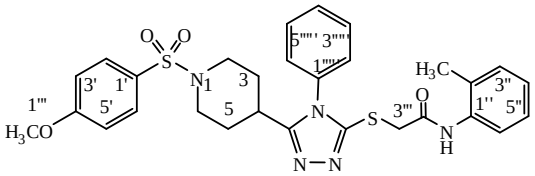
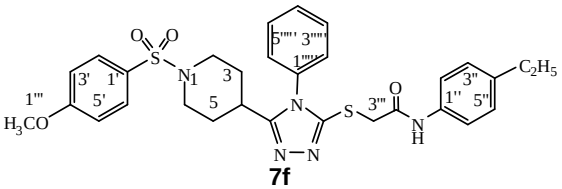
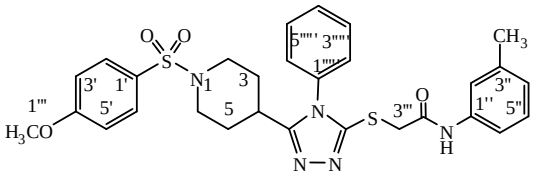
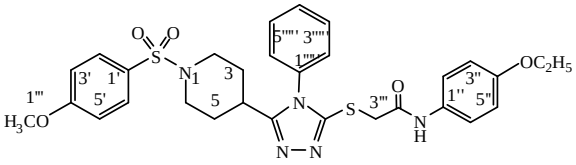
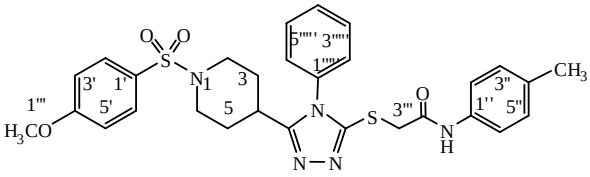
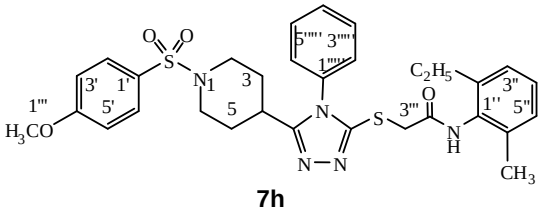
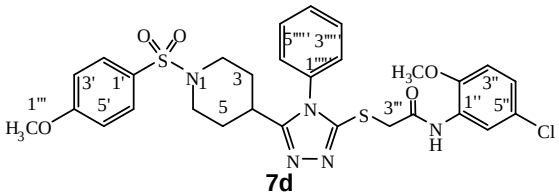
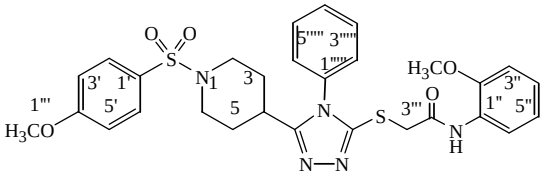
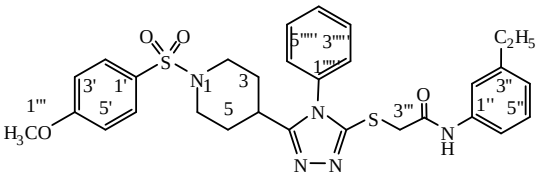
Conventional method: Compound **3** was stirred with an equimolar amount of NaH in DMF for 30 min. Equimolar electrophile (**6a-6i**) was added and the reaction mixture stirred for 12 - 20 h. The precipitate of **7a-7i** appeared upon addition of chilled distilled water and was filtered out, washed with distilled water and dried at room temperature.

Microwave-assisted method: Compound **3** was stirred with an equimolar amount of NaH as a catalyst in DMF for 5 s. Equimolar electrophile (**6a-6i**) was added and the reaction mixture stirred for 34 - 73 s. The precipitate of **7a-7i** appeared upon addition of chilled distilled water and was filtered out, washed with distilled water and dried at room temperature.



Scheme 1: Synthesis of heterocyclic acetamide derivatives of 1,2,4-triazole.

Table 1: Library of 1,2,4-triazole hybrids

 **7a**	 **7f**
 **7b**	 **7g**
 **7c**	 **7h**
 **7d**	 **7i**
 **7e**	

Antioxidant assay (DPPH assay)

The protocol described by Koleva and coworkers was used with some modifications to measure antioxidant activity [14]. Butylated hydroxyanisole (BHA) was the reference standard. The change in absorbance in the presence of test compound was noted and compared to that of control.

Butyrylcholinesterase (BChE) inhibition assay

The protocol described by Ellman *et al* was used with modifications to measure butyrylcholinesterase (BChE) inhibitory activity [15]. Eserine was the reference standard. The change in absorbance in the presence of test compound was noted and compared to that of control.

Urease inhibition assay

Mobley and coworkers reported a method to measure urease inhibition activity [16] which we used with some modifications. Thiourea was used as the reference standard. The change in absorbance in the presence of test compound was noted and compared to that of control.

Docking studies

The urease protein structure was obtained from the Protein Data Bank (PDB) ID: 3LA4 with a resolution of 2.05 Å [17]. The calculations of docked molecules were obtained using OpenEye Scientific Software. The different conformations were obtained through OMEGA 3.0.0 (OMEGA, version 2.4.6, 2013), and the binding interactions

were visualized using Discovery Studio Client v16.1.0 (BIOVIA, 2017).

BSA binding studies

BSA binding interactions were determined using the method used by Chmara and coworkers [18], with some modifications. Buffer and BSA were purchased from Sigma-Aldrich. Fluorescence was measured with an LS 55 Perkin Elmer fluorescence spectrophotometer [19]. Fluorescence of BSA in the presence of synthesized compounds was recorded and compared with that of BSA alone.

Statistical analysis

Each experiment was performed in triplicate, and the results are presented as mean ± SEM. MS Excel 2010 was used to perform statistical analysis with 85 %CI. The results of enzyme inhibition assays are expressed as IC_{50} (concentration for 50 % inhibition) using EZ-Fitz software (Perrella Scientific Inc, USA).

RESULTS

Hybrids bearing 1,2,4-triazole and piperidine were synthesized according to Scheme 1. The complete structures of the synthesized compounds are listed in Table 1. Comparison of conventional and microwave-assisted methods is given in Table 2 with regard to time and yield.

Spectral characteristics of synthesized molecules (7a-7i)

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

White amorphous solid; M.P: 108.0 ^{o}C; M.F.: $C_{29}H_{31}N_5O_4S_2$; M.M.: 577.71 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1500, 1300, 1250, 1150, 750; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.88 (s, 1H, NH), 7.98 (d, *J* = 10.8 Hz, H-6''''), 7.58 (d, *J* = 9.4 Hz, 2H, H-2', H-6'), 7.56-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.26-7.21 (m, 4H, H-2'', H-6'', H-4'''', H-5''''), 7.20-7.19 (m, 1H, H-3''''), 6.99 (d, *J* = 9.7 Hz, 2H, H-3', H-5'), 3.97 (s, 2H, H-2'''), 3.88 (s, 3H, H-1'''), 3.74-3.71 (m, 2H, H_e-2, H_e-6), 2.41-2.38 (m, 1H, H-4), 2.35-2.33 (m, 2H, H_a-2, H_a-6), 2.31 (s, 3H, H-3'''), 2.03-2.00 (m, 2H, H_e-3, H_e-5), 1.88-1.86 (m, 2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 165.58 (C-4'), 163.22 (C-5''''), 157.90 (C-3''''), 151.88 (C-6''''), 136.22 (C-1''''), 131.39 (C-1''), 130.75 (C-4''), 130.43 (C-3'', C-5''), 129.64 (C-2'', C-6''), 128.74 (C-2', C-6'), 127.45 (C-1'), 126.11 (C-2''''), 125.78 (C-6''''), 125.22 (C-3''''), 125.11 (C-5''''), 124.21 (C-4''''), 114.20 (C-3', C-5'), 55.66 (C-1'''), 45.49 (C-2, C-6), 36.34 (C-2'''), 32.01 (C-4), 29.30 (C-3, C-5), 17.43 (C-3'''); EIMS (*m/z*): 577 $[M]^+$, 371, 295, 255, 171, 108, 106, 52.

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

White amorphous solid; M.P: 105.3 ^{o}C; M.F.: $C_{29}H_{31}N_5O_4S_2$; M.M.: 577.71 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1500, 1325, 1250, 1100, 750; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.14 (s, 1H, NH), 7.69 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 7.63-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.45 (s, 1H, H-2''''), 7.42 (d, *J* = 7.9 Hz, H-6'''') ,7.26-7.21 (m, 3H, H-2'', H-6'', H-5''''), 6.99 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 6.94 (d, *J* = 7.3 Hz, 1H, H-4''''), 3.90 (s, 2H, H- 2'''), 3.88 (s, 3H, H-1'''), 3.78-3.74 (m, 2H, H_e -2, H_e-6), 2.52-2.49 (m, 1H, H-4), 2.36 (s, 3H, H-3'''), 2.34-2.28 (m, 2H, H_a-2, H_a-6), 2.06-2.00 (m, 2H, H_e-3, H_e-5), 1.90-1.86 (m, 2H, H_a-3, H_a- 5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.54 (C-4'), 163.00 (C-5''''), 157.96 (C-3''''), 154.88 (C-6''''), 138.82 (C-1''''), 138.37 (C-1''), 132.40 (C-3''''), 130.79 (C-6''''), 130.44 (C-3'', C-5''), 130.19 (C-4''), 129.77 (C-2'', C-6''), 128.72 (C-2''''), 126.96 (C-2', C-6'), 125.90 (C-1'), 120.36 (C-5''''), 116.93 (C-4''''), 114.21 (C-3', C-5'), 55.60 (C-1'''), 45.45 (C-2, C-6), 36.11 (C-2'''), 32.08 (C-4), 29.49 (C-3, C-5), 21.47 (C-3'''); EIMS (*m/z*): 577 $[M]^+$, 371, 295, 255, 171, 108, 106, 66.

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

White amorphous solid; M.P: 114.7 ^{o}C; M.F.: $C_{29}H_{31}N_5O_4S_2$; M.M.: 577.71 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1525, 1350, 1250, 1100, 725; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.12 (s, 1H, NH), 7.69 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.59-7.55 (m, 2H, H-3'', H-4''), 7.50 (d, *J* = 8.2 Hz, 2H, H-2'''', H-6''''), 7.23 (d, *J* = 6.7 Hz, 2H, H-2'', H-6''), 7.14 (d, *J* = 8.1 Hz, 2H, H-3'''', H-5''''), 6.99 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 3.88 (s, 5H, H-1''', H-2'''), 3.77- 3.75 (m, 2H, H_e-2, H_e-6), 2.52-2.49 (m,1H, H-4), 2.31-2.28 (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), 1.71 (s, 3H, H-3'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.39 (C-4'), 163.00 (C-5''''), 157.95 (C-3''''), 152.88 (C-6''''), 135.66 (C-1''''), 133.81 (C-1''), 132.40 (C-4''''), 130.78 (C-4''), 130.43 (C-3'', C-5''), 129.77 (C-2'', C-6''), 129.37 (C-3'''', 5''''), 127.80 (C-1'), 126.96 (C-2', C-6'), 119.79 (C-6'''', C-2''''), 114.21 (C-3', C-5'), 55.60 (C-1'''), 45.49 (C-2, C-6), 36.05 (C-2'''), 32.04 (C-4), 29.40 (C-3, C-5), 20.88 (C-3'''); EIMS (*m/z*): 577 $[M]^+$, 371, 295, 255, 171, 108, 106, 66.

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

Light-pink amorphous solid; M.P: 205.3 ^{o}C; M.F.: $C_{29}H_{30}N_5O_4S_2$; M.M.: 628.16 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1550, 1350, 1250, 1150, 725; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.22 (s, 1H, NH), 8.42 (s, 1H, H-6''''), 7.69 (d, *J* = 8.1 Hz, 2H, H-2', H-6'), 7.59-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.24 (d, *J* = 7.0 Hz, 2H, H-2'', H-6''), 7.01 (d, *J* = 8.7 Hz, 1H, H-3''''), 6.99 (d, *J* = 8.0 Hz, 2H, H-3', H-5'), 6.78 (d, *J* = 8.6 Hz, 1H, H-4''''), 3.98 (s, 2H, H- 2'''), 3.90 (s, 3H, H- 3'''), 3.88 (s, 3H, H-1'''), 3.76-3.74 (m, 2H, H_e-2, H_e-6), 2.54-2.51 (m, 1H, H- 4), 2.37-2.33 (m, 2H, H_a-2, H_a-6), 2.07-2.01 (m, 2H, H_e-3, H_e-5), 1.89-1.73 (m,2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.71 (C-4'), 163.00 C-5''''), 157.94 (C-3''''), 152.02 (C -6''''), 147.25 (C-2''''), 132.46 (C-1''''), 130.67 (C-1''), 132.40 (C-4''''), 130.41 (C-3'', C-5''), 129.75 (C-2'', C-6''), 128.96 (C-4''), 127.77 (C-5''''), 126.92 (C-2', C-6'), 125.76 (C-1'), 123.35 (C-6''''), 119.91 (C-3''''), 114.22 (C-3', C-5'), 110.84 (C-4''''), 56.05 (C -3'''), 55.61 (C-1'''), 45.45 (C-2, C-6), 35.88 (C-2'''), 31.76 (C-4), 29.51 (C-3, C-5); EIMS (*m/z*): 628 [M]$^{+}$, 371, 295, 255, 171, 108, 106, 52.

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

White amorphous solid; M.P: 157.3 ^{o}C; M.F.: $C_{30}H_{35}N_5O_4S_2$; M.M.: 593.76 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1550, 1325, 1250, 1150, 720; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.67 (s, 1H, NH), 7.90 (d, *J* = 7.9 Hz, 1H, H-6''''), 7.69 (d, *J* = 8.7 Hz, 2H, H-2', H-6'), 7.60-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.23-7.20 (m, 3H, H-2'', H-6'', H-5''''), 7.13 (t, *J* = 7.3 Hz, 1H, H-4''''), 6.99 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 3.98 (s, 2H, H-2'''), 3.88 (s, 3H, H- 1'''), 3.73-3.70 (m, 2H, H_e-2, H-6), 2.64 (q, *J* = 5.4 Hz, 2H, H-3'''), 2.59-2.53(m, 1H, H-4), 2.40-2.37 (m, 2H, H_a-2, H_a-6), 2.03-2.00 (m, 2H, H_e-3, H_e-5), 1.88-1.85 (m,2H, H_a-3,H_a-5), 1.14 (t, *J* = 3.2 Hz, 3H, H-4'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 167.08 (C-4'), 163.00 (C-5''''), 158.08 (C-3''''), 152.44 (C-6''''), 135.38 (C-1''''), 135.37 (C-1''), 130.77 (C-4''), 130.46 (C-3'', C-5''), 129.74 (C-2'', C-6''), 128.74 (C-2''''), 128.00 (C-1'), 126.91 (C-2', C-6'), 126.40 (C-5''''), 125.28 (C-6''''), 123.27 (C-4''''), 121.55 (C-3''''), 114.21 (C-3', C-5'), 55.57 (C-1'''), 45.30 (C-2, C-6), 35.59 (C-2'''), 31.77 (C-4), 29.38 (C-3, C-5), 24.60 (C-3'''), 14.20 (C-4'''); EIMS (*m/z*): 593 [M]$^{+}$, 371, 295, 255, 171, 108, 106, 66.

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

White amorphous solid; M.P: 130.0 ^{o}C; M.F.: $C_{30}H_{33}N_5O_4S_2$; M.M.: 591.74 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1550, 1350, 1250, 1150, 750; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.13 (s, 1H, NH), 7.68 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.60-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.53 (d, *J* = 8.4 Hz, 2H, H-2'''', H-6''''), 7.23 (dd, *J* = 7.8, 2.7 Hz, 2H, H-2'', H-6''), 7.16 (d, *J* = 8.3 Hz, 2H, H-3'''', H-5''''), 6.99 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 3.88 (s, 2H, H-2'''), 3.87 (s, 3H, H-1'''), 3.77-3.75 (m, 2H, H-2, H_e-6), 2.63 (q, *J* = 3.9 Hz, 2H, H-3'''), 2.52-2.49 (m, 1H, H-4), 2.32-2.26 (m, 2H, H_a-2, H_a-6), 2.06-2.00 (m, 2H, H_e-3, H_e-5), 1.99-1.85 (m, 2H, H_a-3, H_a-5), 1.22 (t, *J* = 3.5 Hz, 3H, H-4'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.41 (C-4'), 163.01 (C-5''''), 157.95 (C-3''''), 152.85 (C-6''''), 140.32 (C-1''''), 135.84 (C-1''), 132.38 (C-4''''), 130.78 (C-4''), 130.44 (C-3'', C-5''), 129.77 (C-2'', C-6''), 128.21 (C-2', C-6'), 127.74 (C-1'), 126.96 (C-3'''', C-5''''), 119.87 (C-2'''', C-6''''), 114.22 (C-3', C-5'), 55.61 (C-1'''), 45.51 (C-2, C-6), 36.02 (C-2'''), 32.02 (C-4), 29.40 (C-3, C-5), 28.34 (C-3'''), 15.75 (C-4'''); EIMS (*m/z*): 591 [M]$^{+}$, 371, 295, 255, 171, 108, 106, 52.

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

Light-pink amorphous solid; M.P: 117.0 ^{o}C; M.F.: $C_{30}H_{33}N_5O_5S_2$; M.M.: 607.74 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1500, 1320, 1250, 1150, 750; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 10.08 (s, 1H, NH), 7.69 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.60-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.52 (d, *J* = 9.0 Hz, 2H, H-2'''', H-6''''), 7.23 (dd, *J* = 7.8, 3.8 Hz, 2H, H-2'', H-6''), 6.99 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 6.87 (d, *J* = 9.0 Hz, 2H, H-3'''', H-5''''), 4.02 (q, *J* = 5.1 Hz, 2H, H-3'''), 3.88 (s, 3H, H-1'''), 3.87 (s, 2H, H-2'''), 3.77-3.74 (m, 2H, H_e-2, H_e-6), 2.53-2.49 (m, 1H, H-4), 2.32-2.29 (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), 1.41 (t, *J* = 2.7 Hz, 3H, H-4'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.25 (C-4'), 163.00 (C-5''''), 157.94 (C-3''''), 155.65 (C-4''''), 152.92 (C-6''''), 132.41 (C-1''''), 131.33 (C-1''), 130.78 (C-4''), 130.43 (C-3'', C-5''), 129.77 (C-2'', C-6''), 127.77 (C-1'), 126.96 (C-2', C-6'), 121.34 (C-3'''', C-5''''), 114.70 (C-2'''', C-6''''), 114.21 (C-3', C-5'), 63.70 (C-3'''), 55.60 (C-1'''), 45.49 (C-2, C-6), 36.00 (C-2'''), 32.04 (C-4),

29.40 (C-3, C-5), 14.84 (C-4'''); EIMS (*m/z*): 607 $[M]^+$, 371, 295, 255, 171, 108, 106, 52.

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

White amorphous solid; M.P: 110.0 ^oC; M.F.: $C_{31}H_{35}N_5O_4S_2$; M.M.: 605.77 g/mol; IR (KBr, cm^{-1}): 2850, 1700, 1600, 1500, 1350, 1250, 1150, 750; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.34 (s, 1H, NH), 7.68 (d, *J* = 8.6 Hz, 2H, H-2', H-6'), 7.63-7.55 (m, 3H, H-3'', H-4'', H-5''), 7.23 (br. s, 2H, H-2'', H-6''), 7.16-7.09 (m, 3H, H-3'''', H-4'''', H-5''''), 6.98 (d, *J* = 8.7 Hz, 2H, H-3', H-5'), 4.00 (s, 2H, H-2'''), 3.86 (s, 3H, H-1'''), 3.73-3.70 (m, 2H, H_e-2, H_e-6), 2.53 (q, *J* = 3.8 Hz, 2H, H-3'''), 2.39-2.35 (m, 3H, H-4, H_a-2, H_a-6), 2.19 (s, 3H, H-5'''), 2.03-1.97 (m, 2H, H_e-3, H_e-5), 1.87-1.85 (m,2H, H_a-3,H_a-5), 1.09 (t, *J* = 5.1 Hz, 3H, H-4'''); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.23 (C-4'), 163.01 (C-5''''), 157.99 (C-3''''), 152.90 (C-6''''), 141.06 (C-1''''), 133.00 (C-1''), 130.81(C-4''), 130.49 (C-3'', C-5''), 129.73(C-2'', C-6''), 128.18 (C-6''''), 127.57 (C-2''''), 126.90 (C-2', C-6'), 126.38 (C-1'), 123.89 (C-5''''), 121.90 (C-3''''), 118.23 (C-4''''), 114.22 (C-3', C-5'), 55.56 (C-1'''), 45.30 (C-2, C-6), 34.81 (C-2'''), 31.87 (C-4), 29.36 (C-3, C-5), 24.87 (C-3'''), 18.22 (C-5'''), 14.62 (C-4'''); EIMS (m/z): 605 $[M]^+$, 371, 295, 255, 171, 108, 106, 66.

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

White amorphous solid; yield: 87 %; M.P: 120.0 ^oC; M.F.: $C_{30}H_{33}N_5O_5S_2$; M.M.: 575.67 g/mol; IR (KBr , cm^{-1}): 2825, 1700, 1600, 1500, 1320, 1250, 1150, 750; ^{1}H-NMR ($CDCl_3$, 600 MHz, δ (ppm)): 9.97 (s, 1H, NH), 8.35 (d, *J* = 9.0 Hz, 1H, H-6''''), 7.69 (d, *J* = 8.8 Hz, 2H, H-2', H-6'), 7.57-7.54 (m, 3H, H-3'', H-4'', H-5''), 7.24 (dd, *J* =7.4, 2.2 Hz, 2H, H-2'', H-6''), 7.06 (t, *J* = 7.5 Hz, 1H, H-5''''), 6.98 (d, *J* = 8.8 Hz, 2H, H-3', H-5'), 6.94(t, *J* = 7.3 Hz,1H, H-4''''), 6.89 (d, *J* = 8.1 Hz, 1H, H-3''''), 4.01 (s, 2H, H-2'''), 3.88 (s, 3H, H-1'''), 3.76 (s, 3H, H-3'''), 3.74-3.72 (m, 2H, H_e-2, H_e-6), 2.54-2.52 (m, 1H, H-4), 2.39-2.36 (m, 2H, H_a-2, H_a-6), 2.05-2.02 (m, 2H, H_e-3, H_e-5), 1.89-1.86 (m,2H, H_a-3, H_a-5); ^{13}C-NMR ($CDCl_3$, 150 MHz, δ (ppm)): 166.22 (C-4'), 166.20 (C-2''''), 163.00 (C-5''''), 157.88 (C-3''''), 152.92 (C-6''''), 136.06 (C-1''''), 134.01 (C-1''), 131.11 (C-4''), 130.33 (C-3'', C-5''), 129.74 (C-2'', C-6''), 128.21 (C-6''''), 126.99 (C-2', C-6'), 126.37 (C-1'), 123.94 (C-5''''), 121.87 (C-3''''), 117.21 (C-4''''), 114.21 (C-3', C-5'), 55.57 (C-1'''), 55.23 (C-3'''), 45.39 (C-2, C-6), 34.80 (C-2'''), 31.86 (C-4), 29.49 (C-3, C-5); EIMS (*m/z*): 575 $[M]^+$, 371, 295, 255, 171, 108, 106, 52.

Table 2: Comparison of conventional and microwave-assisted methods

Compound	Reaction time		Reaction yield (%)	
	Conventional (h)	Microwave (s)	Conventional	Microwave
7a	13	39	73	90
7b	15	41	66	91
7c	16	63	78	90
7d	17	53	72	88
7e	13	38	48	81
7f	20	34	61	84
7g	12	47	77	92
7h	18	73	64	90
7i	16	69	58	87

Biological activities

The IC_{50} results for antioxidant and enzyme inhibition activities are given in Table 3 and the docking studies in Figures 1 to Figure 3. The results for BSA binding studies are given in Table 4 and Figures 4 and 5.

Table 3: Antioxidant and enzyme inhibition activities

Compound	IC_{50} (µM)		
	Antioxidant	Urease	BChE
7a	65.2±0.26	31.1±0.29	17.4±0.08
7b	56.2±0.45	45.5±0.45	25.2±0.12
7c	60.3±0.12	39.5±0.65	>500
7d	49.5±0.24	19.5±0.12	15.5±0.77
7e	62.2±0.45	21.1±0.68	22.2±0.15
7f	61.1±0.21	18.2±0.78	>500
7g	80.1±0.61	19.9±0.77	>500
7h	78.5±0.14	52.4±0.60	69.5±0.57
7i	76.2±0.21	17.9±0.10	68.2±0.22
BHA	**44.2±0.21**		
Thiourea		**24.3 ± 0.24**	
Eserine			**7.8 ± 0.24**

Table 4: Stern-Volmer quenching constants for the potent compounds warfarin and ibuprofen with BSA at 298K

Compound	$K_{SV} \times 10^2$ (M^{-1})	$k_q \times 10^{10}$ (M^{-1} s^{-1})	K_a (L/mol)	N
7a	4.45	4.45	2.52×10^1	0.6
7e	4.21	4.21	2.42×10^2	0.9
Warfarin			641000	1.07
Ibuprofen	3502	35	56870	1.26

DISCUSSION

Compound **7a** was selected for discussion. It was obtained as a white amorphous solid with a melting point of 108.0 ^oC. The IR peaks at 2850, 1700, 1600, 1500, 1300, 1250, 1150 and 750 cm^{-1} indicated different functional groups. In ^{1}H-NMR, two doublet peaks appearing at 7.58 and

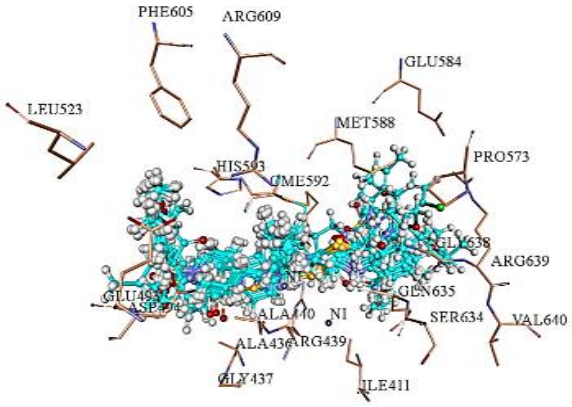


Figure 1: Binding orientation of all compounds in the active site of urease. Amino acid residues are shown in golden color and ligands in different colors

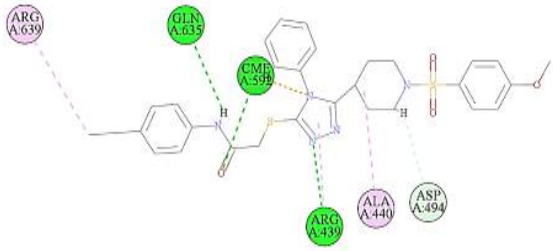


Figure 2: Binding interaction of compound 7f in the active site of urease

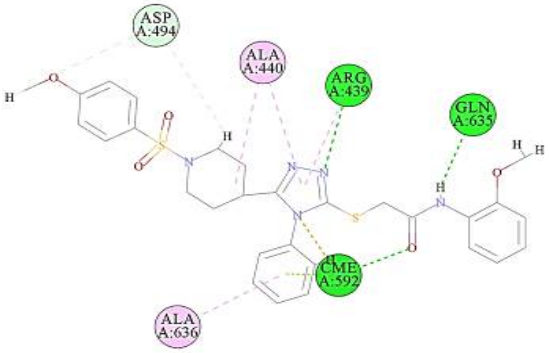


Figure 3: Binding interaction of compound 7i in the active site of urease

6.99 ppm were assigned to four protons of an aromatic ring with an attached sulfonyl group. The five protons of the phenyl ring attached to triazole were based on two multiplets at 7.56-7.55 and 7.26-7.21 ppm.

A doublet and two multiplets respectively appearing at 7.98, 7.26-7.21 and 7.20-7.19 ppm were assigned to the four protons of the phenyl ring attached to amidic group. The nine protons of the piperidine ring were explained by five multiplets at 3.74-3.71, 2.41-2.38, 2.35-2.33, 2.03-2.00 and 1.88-1.86 ppm. The methoxy, methylene and methyl groups were indicated by three singlets at 3.88, 3.97 and 2.31 ppm, respectively.

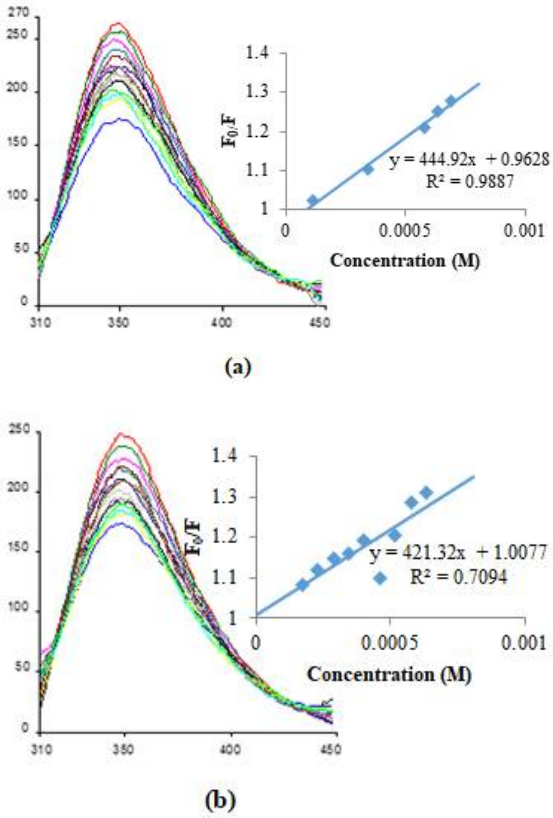


Figure 4: Fluorescence graph of BSA with (a) **7a** (b) **7e**. Insets show the Stern-Volmer plots.

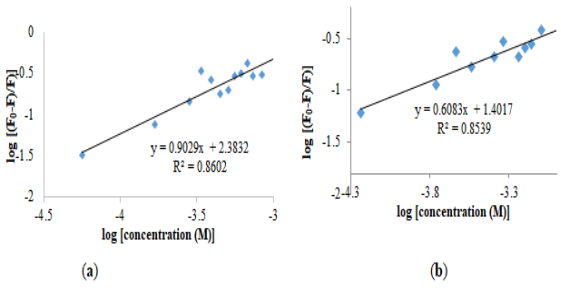


Figure 5: Fluorescence double reciprocal plots of binding of BSA with (a) **7a** (b) **7e**

The aromatic and aliphatic carbons were indicated by peaks appearing at 165.58 (C-4'), 163.22 (C-5""), 157.90 (C-3""), 151.88 (C-6""), 136.22 (C-1""), 131.39 (C-1"), 130.75 (C-4"), 130.43 (C-3", C-5"), 129.64 (C-2", C-6"), 128.74 (C-2', C-6'), 127.45 (C-1'), 126.11 (C-2""), 125.78 (C-6""), 125.22 (C-3""), 125.11 (C-5""), 124.21 (C - 4""), 114.20 (C-3', C-5'), 55.66 (C-1'"), 45.49 (C-2, C-6), 36.34 (C-2"'), 32.01 (C-4), 29.30 (C-3, C-5) and 17.43 (C-3'"). The spectral data supported the identification of **7a** as 2-[(5-{1-[(4-methoxyphenyl)sulfonyl]-4-piperidinyl}-4-phenyl-4*H*-1,2,4-triazol-3-yl)sulfanyl]-*N*-(2-methylphenyl)acetamide. Although both synthetic techniques achieved high yields, the microwave-

assisted technique was very effective in attaining high yield in less time of reaction.

Compounds **7a-7i** were shown to be effective antioxidant agents, where **7d** was the most active with an IC_{50} of 49.5 ± 0.24 µM compared to 44.2 ± 0.21 µM for the reference, BHA. This potential of **7d** might have been due to 2-chloro-5-methoxyphenyl as the amide aromatic ring.

All synthesized compounds displayed high urease inhibition activity as compared to the reference. Compounds **7d**, **7e**, **7f**, **7g** and **7i** were the most active with low IC_{50} values of 19.5 ± 0.12, 21.1 ± 0.68, 18.2 ± 0.78, 19.9 ± 0.77 and 17.9 ± 0.10 µM as compared to thiourea with an IC_{50} of 24.3 ± 0.24 µM.

BChE was inhibited by six of the nine synthesized compounds. The most active were **7a**, **7b**, **7d** and **7e** with low IC_{50} values. The highest potential was shown by **7d** with the lowest IC_{50} value, 15.5 ± 0.77 µM, which could have been due to 2-chloro-5-methoxyphenyl as the amide aromatic ring.

All the synthesized compounds were analyzed by molecular docking studies to determine the orientation in the active site of the target protein. Figure 1 shows the superimposition of all the docked compounds at the active site of urease. Binding interaction analysis of the most active compounds **7f** (Figure 2) and **7i** (Figure 3) indicated hydrogen bonding with Arg439 and CME592 through triazole and oxygen of acetamoyl moieties. Hydrophobic interactions with amino acid residues Ala440, Ala636 and Arg639 were also found. Hydrogen bonding is shown with green dotted line while π–alkyl interactions are shown with light pink dotted line.

The fluorescence emission spectral data (at an excitation of 295 nm) of BSA was recorded (Table 4). The strong emission by BSA appeared at λ_{max} = 336 nm. Fluorescence decreased on binding of the synthesized compounds with BSA. The Stern-Volmer equation (Equation 1) helped to determine the nature of this fluorescence quenching, that is, static or dynamic.

$$F_0/F = K_{SV}[Q] + 1 = k_q\tau_0[Q] + 1 \quad \text{..... (1)}$$

where F is fluorescence intensity of BSA in the presence of compounds, F_0 is fluorescence intensity of BSA in the absence of compounds, K_q is apparent bimolecular quenching rate constant, [Q] is concentration of synthesized compounds (quencher), K_{SV} is Stern-Volmer quenching constant, and τ_0 is the average life time of biomolecule without synthesized compound (10^{-8} s) [20]. The quenching of BSA was found to be static as indicated by the larger values of k_q than maximum scattering collision quenching rate constant (2×10^{10} M^{-1} s^{-1}).

CONCLUSION

Microwave-assisted protocol is superior to the conventional method in terms of high yield and short time. Biological screening along with docking and BSA binding studies indicate that compounds **7d**, **7e**, **7f**, **7g**, and **7i** are potent anti-urease agents. Thus, the current synthesis might be a good addition in the pharmaceutical industry after further biological evaluation.

DECLARATIONS

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Conflict of interest

No conflict of interest is associated with this work.

Contribution of authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors.

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