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Efficient Green Synthesis and Biological Evaluation of Thiadiazole Derivatives

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Abstract: Thiadiazole and its derivatives have been studied extensively because of their wide range of biological activity. Diverse biological activities, such as antibacterial, anti-inflammatory, and antiviral, have been associated with thiadiazole derivatives. These derivatives were explored by the *in-silico* method by using the docking model. Given research work aimed at improving thiadiazole yield and quality by using a green chemistry approach, and the synthesized compounds were screened for antibacterial activities. In the present work, some new thiadiazole ring containing compounds were synthesized, but the synthesis was carried out using green chemistry methods like microwave and ultrasonication techniques. Compounds were screened by spectral analysis, and further antimicrobial evaluation was also done. These derivatives were explored by the *in-silico* method by using the docking model. All the screened derivatives exhibited good results, especially the 3C compound, which showed the lowest binding energy with the receptor (-7.449 Kcal/mol). Research using the green chemistry approach gives the successful synthesis of thiadiazole derivatives with good yield in between 75- 90%. As compared to conventional synthesis methods, these methods require less time; the newly synthesized compounds also reflect moderate to promising antimicrobial activity against *E.coli* and *P.aureginosa.* All the selected derivatives were docked with the selected PDB code's active site and assessed for binding free energy and their interaction with the receptor. 3e compound showed maximum binding free energy -7.449 Kcal/mol.

Keywords: thiadiazole; green chemistry; conventional; microwave; ultrasonication; antimicrobial.

1. Introduction

Thiadiazoles belong to five-membered nitrogen–sulfur ring system with two nitrogen and one sulfur atom and are widely present as a unit in the structure of various biologically active molecules and act as a useful intermediate in the synthesis of various important heterocycles [1]. Chemically Thiadiazole exist in four isomeric forms viz-1, 2, 3-thiadiazole; 1, 2, 5-thiadiazole; 1, 2, 4-thiadiazole and 1, 3, 4-thiadiazole [2]. Thiadiazole possesses sulfide linkage, which gives retaliate characteristic to thiadiazole; also, oxygen, nitrogen, and the presence of a sulfur atom affect the physical and chemical properties of thiadiazole [3]. Different drug classes like anti-inflammatory, antimicrobial, antiviral, antiepileptic, antitubercular agents contain thiadiazole nucleus as a building block in their chemical structure, giving the conclusion that thiadiazole and its derivatives have diverse potent activities and can be regarded as pharmacologically significant scaffolds [4]. In recent years research shows wide

applications of thiadiazole and its derivatives such as antimicrobial [5] antituberculosis, anticonvulsants, anti-inflammatory, antihypertensive [6] antioxidant, human adenosine A3 antagonist[7], anticancer [8, 9], and antifungal activity [10]. Vit. B1 incorporates thiazolium ring showing the prominent role of thiadiazole in nature also [11].

While considering the synthesis, it is now challenging to find an approach that consists of environment-friendly chemical processes. Products require the development of novel and cost-effective methods that are pollution-free also.

Green chemistry is one of the significant and latest chemistry concepts that uses various principles, which causes a reduction in cost, time, use, or generation of hazardous substances in the process of chemical production [12]. In the present scenario, green chemistry techniques such as ultrasonic and microwave-assisted organic synthesis (MAOS) affect synthetic, medicinal chemistry widely and significantly by reducing time expense and enabling reproducible chemistry development with improvement in yield and quality of products[13-14]. Thus one can conclude that green chemistry synthesis is an enabling technology for accelerating drug design, discovery, and development processes. Hence, this field has-ever-growing importance resulting in the development scores of thiadiazoles [15-17]. In the present research work, new thiadiazole derivatives were synthesized from thiosemicarbazide using green chemistry, an eco-friendly, nonhazardous, reproducible, and economical approach; furthermore, the structures of synthesized compounds were confirmed based on spectral data. Further, the synthesized compounds were tested for their antimicrobial activity. The structures of synthesized compounds were confirmed based on spectral data. The compounds were also tested for their antimicrobial activities by standard methods.

2. Materials and Methods

2.1. Materials.

The reaction was carried out with analytical reagent grade chemicals, which were commercially procured from various chemical units, and glasswares used were made of pyrex glass. Used solvent and reagents were of LR grade and were purified before use. 120-160 mesh silica gel G was obtained from E.Merck India Ltd., which was used for TLC analytical chromatography. The solvent system used was ethyl acetoacetate: petroleum ether (7:3). For vacuum filtration, ashless Whatman number 1 filter paper was used. Melting points were determined in an open glass capillary using melting point apparatus and are uncorrected. IR Spectra KBr) was recorded on FTIR Spectrophotometer (Shimadzu FTIR 84005, 4000-400cm^{-1}). The electrospray mass spectra were recorded on a THERMO Finnigan LCQ Advantage max ion trap mass spectrometer. ^{1}H NMR was recorded on a Bruker DRX-300 MHz spectrometer in $CDCl_3$ using TMS as an internal standard, with ^{1}H resonance frequency of 300 MHz. Chemical shift values are expressed in δ ppm. For antimicrobial activities, Minimum Inhibitory concentration (MIC) was found by using the serial dilution method.

2.2. Synthesis of compounds.

2.2.1. General Method for Synthesis of thiosemicarbazide derivatives (1a-1e).

Substituted aniline derivative was taken and was dissolved in ethanol (95%, 50 ml) and ammonia solution (20 ml). Then 20 ml of carbon disulfide was added slowly to a solution of aniline derivatives with shaking. The resulting solution was allowed to stand for 1 hour. Then

0.1M sodium chloroacetate and 20 ml of 50% hydrazine hydrate were added to the solution. The reaction mixture was warmed gently, filtered, and evaporated to half of its volume, and kept overnight. The solid thus obtained was filtered and purified by recrystallization from ethanol. Their characterization is given in table 1.

Spectral characterization of thiosemicarbazide derivatives are as follows:

✓ **1-phenyl thiosemicarbazide (1a)IR (KBr) *v*/cm-1(2a):** $C_7H_9N_3S$, 3005 (C-H), 1625 (-C-C), 3234 (-N-H), 3230 (-N-H), 3222 (-N-H), 2563(-C-S); **^{1}H NMR (400 MHz, DMSO-d6, δ, ppm):** 2 (s, 2H), 2 (s, 1H), 4 (s, 1H), 6.66 (m, 5H); **MS (m/z):** 168(M+1).

✓ **1- (3-nitro phenyl) thiosemicarbazide (1b**): **IR (KBr) *v*/cm-1:** 3008 (C-H), 1622 (-C-C), 3214 (-N-H), 3250 (-N-H), 3123 (-N-H), 1496 (NO_2), 2632(-C-S) ;**^{1}H NMR(400 MHz, DMSO-d6, δ, ppm):** 1.97 (s, 2H), 2 (s, 1H), 3.96 (s, 1H), 7.54 (d, 2H), 7.59 (s, 1H); **MS (m/z):** 212 (M+1).

✓ **1- (3-methyl phenyl) thiosemicarbazide (1c): IR (KBr) *v*/cm-1:** 3000 (C-H), 1619 (-C-C), 3200 (-N-H), 3247 (-N-H), 3103 (-N-H) , 2632(-C-S); **^{1}H-NMR(400 MHz, DMSO-d6, δ, ppm):** 2.33 (s, 2H), 2.38 (s, 1H), 4.08 (s, 1H), 2.35 (s,3H), 6.46 (s, 2H) ; **MS (m/z):** 182 (M+1)

✓ **1- (3-methoxy phenyl) thiosemicarbazide (1d): IR (KBr) *v*/cm-1:** 3012(C-H), 1600 (-C-C), 3226 (-N-H), 3240 (-N-H), 3100 (-N-H) , 3000(-C-H), 2632(-C-S); **^{1}H NMR(400 MHz, DMSO-d6, δ, ppm):** 2.12 (s, 2H), 2 (s, 1H), 3.89 (s, 1H), 3.73 (s, 3H), 7.04 (d, 2H) , 6.22 (s, 1H); **MS (m/z):** 198 (M+1).

✓ **1- (3-chloro phenyl) thiosemicarbazide (1e): IR (KBr) *v*/cm-1**3022(C-H), 1622 (-C-C), 3232 (-N-H), 3220 (-N-H), 3100 (-N-H), 2632(-C-S), 740 (-Cl) ; **^{1}H NMR(400 MHz, DMSO-d6, δ, ppm):** 2.22 (s, 2H), 2.11 (s, 1H), 3.79 (s, 1H), 6.64 (d, 2H); **MS (m/z):** 202 (M+1).

2.2.2. Synthesis of Thiadiazole derivatives (2a-2e) and (3a-3e).

2.2.2.1. Microwave irradiation method (2a-2e).

Substituted thiosemicarbazide (0.10M), substituted benzoic acid (0.01M). Phosphorous oxychloride (25 ml) were taken into a beaker and dissolved in a minor quantity of dimethylformamide (10 ml) [18]. To this solution, concentrated sulphuric acid (10 drops) was added while stirring. A funnel was hanged in the beaker and covered with a watch glass. The reaction mixture was subjected to microwave irradiation at 300 W for 3 min, with a pulse rate of 30 sec in a laboratory microwave oven. Allow the flask's contents to attain room temperature, and pour it directly into a beaker having 30 ml of cold water and stirred vigorously [19]. The crude product was filtered onto a Buchner funnel using suction and washed with small portions of cold water, and dried. It was purified by recrystallization from hot alcohol. Their characterization data is given in Table 2.

2.2.2.2. Ultrasonic irradiation method (3a-3e).

Substituted thiosemicarbazide (0.05 M), substituted benzoic acid (0.01 M), and concentrated sulphuric acid (5 mL) was taken in a beaker (50 ml). The mixture was subjected to ultrasonic irradiation for 20 min at room temperature [20,21]. Furthermore, the reaction mixture was poured over ice-cooled water. The crude product was filtered and crystallized with DMF. The structure of synthesized compounds (3a-3e) is shown in fig 1. Their characterization data is given in Table 3.

Spectral characterization of thiadiazole derivatives are as follows:

✓ **4-(5-(phenylamino)-1,3,4-thiadiazol-2-yl) benzoic acid(3a): IR (KBr, cm^{-1})** 3379 (N-H Amines), 3073 (C-H Ar), 2225 (C=N Ar), 2952 (hydrogen bonded acids) ,1725 (C=O Acids), 2625 (O-H Acids), 1590 (C=C Ar); **^{1}H NMR**: **($CDCl_3$, δ, ppm):** 4 (s, 1H,NH of thiadiazole), 7.01 (s, 2H, Ar-(C-H)), 6.5 (s, 3H, Ar C-H), 11 (s, 1H,OH). **MS (m/z):** 298.06 (M+1).

✓ **4-(5-(3 nitro phenylamino)-1,3,4-thiadiazol-2-yl) benzoic acid(3b): IR (KBr, cm^{-1}):** 3408 (N-H Amines), 3342 (C-H Ar), 2300 (C=N Ar), 1665(C=C Ar), 1689 (C=O Acids), 1400 (C-N Ar), 1570 (NO_2); **^{1}H NMR:($CDCl_3$, δ, ppm):** 3.97 (s, 1HNH of thiadiazole), 7.39 (s, 2H, Ar-(C-H)), 7.66 (s, 3H, Ar C-H), 11.01 (s, 1H,OH) **MS (m/z):** 343 (M+1).

✓ **4-(5-(3 methyl phenylamino)-1,3,4-thiadiazol-2-yl) benzoic acid(3c): IR (KBr, cm^{-1}):** 3453 (N-H Amines), 3250 (C-H Ar), 2175 (C=N Ar),1566 (C=C Ar), 1427(NO_2 Nitro compound), 1295 (C-N Ar), **^{1}H NMR:($CDCl_3$, δ, ppm):** 2.35 (s, 3H of CH3),4.0 (s, 1H,NH of thiadiazole), 7.43 (s, 2H, Ar-(C-H)), 6.27 (s, 3H, Ar C-H), 10.94 (s, 1H,OH)**MS (m/z):** 312 (M+1).

✓ **4-(5-(3 methoxy phenylamino)-1,3,4-thiadiazol-2-yl) benzoic acid(3d): IR (KBr, cm^{-1}):** 3400 (N-H Amines), 3212 (C-H Ar), 2155 (C=N Ar),1560 (C=C Ar), 2850(C-H cmethoxy), 1740 (O-C str), 1288 (C-N Ar),**^{1}H NMR:($CDCl_3$, δ, ppm):** 5.97 (s, 3H of CH3),4.0 (s, 1H,NH of thiadiazole), 8.19 (s, 2H, Ar-(C-H)), 6.90 (s, 3H, Ar C-H), 11.93 (s, 1H,OH) **MS (m/z):** 328 (M+1).

✓ **4-(5-(3 chloro phenylamino)-1,3,4-thiadiazol-2-yl) benzoic acid(3e): IR (KBr, cm^{-1})** 3419 (N-H Amines), 3100 (C-H Ar), 2917 (C-H Ar), 2113 (C=NAr), 1595 (C=C Ar), 723 (C-Cl), 1339 (C-N Ar), **^{1}H NMR:($CDCl_3$, δ, ppm):** 4.0 (s, 1H,NH of thiadiazole), 7.43 (s, 3H, Ar-H)), 8.19 (s, 1H, Ar C-H), 11 (s, 1H,OH). 2.34 ; **MS (*m/z*)** :332 (M+1)

2.2.3. Antibacterial activity.

2.2.3.1. Material and methods.

The microbiological testing of the synthesized compounds was done by the agar well diffusion method. Ampicillin was used as standard drugs [22]. Nutrient agar media were used for the purpose, which contains the constituents, as presented in table 1.

Table 1. Constituents of agar medium.

S. No.	Constituents	Quantity required
1.	Peptic digest	5 gm/litre
2	Yeast Extract	1.5 gm/litre
3	Beef Extract	1.5 gm/litre
4	Sodium chloride	5 gm/litre
5	Agar	15 gm/litre
6	Distilled Water q.s.	1 litre

2.2.4. Experimental procedures.

The Agar-diffusion method was used for the determination of the preliminary antibacterial activities. The agar well diffusion test was performed using nutrient agar medium, and the medium was autoclaved at 15 lbs pressure (121 °C) for 15 min then was immediately cooled to 50-55 °C in a water bath after removing it from the autoclave [23-26]. The cooling medium was poured into sterile Petri plates to a uniform depth of 4 mm; this is equivalent to approximately 40 ml in a 90 mm plate. After solidification of the agar medium, culture was inoculated into the medium. All of the works were performed in a laminar flow; within 15

minutes of adjusting the inoculums' density, a sterile cotton swab was dipped into the standardized bacterial suspension or inoculated with 1mL organism suspension [27]. The sterile swab was used on the nutrient agar medium's surface to ensure an even distribution of the inoculums. The plates were undisturbed for 3 to 5 minutes to ensure excess moisture absorption [28]. A sterilized 7mm cork borer was used to make agar wells. The concentrations of the 500 and 600 µg/ml of the diluted test compound stock solutions were placed into each well and 100 % DMSO as a control.

3. Results and Discussion

Synthesis of Thiadiazole derivatives using a green chemistry approach proved to be a good technique for maximal yield with cost-effective and less time consumption. By following the microwave irradiation and ultrasonication technique, derivatives are obtained in good yield ranging from 60-90 %. While comparing the yield of two techniques for synthesis of compounds, it has been found that the microwave irradiation method gives a better yield ranging from 85- 90 % as by use of ultrasonic irradiation method yield ranges from 75-80%. Structures of the synthesized substituted thiadiazole were confirmed from their respective IR, ^{1}H- NMR studies.

Thiadiazole based heterocyclic derivatives were also found to have encouraging antimicrobial activity [29-33]. Some are promising and need to be further investigated to get better agents. The antimicrobial screening observed that all the compounds exhibited activity against all the organisms employed. Among all synthesized five compounds, 2c, 2d, 3c, 3d show good antibacterial activity against *E. coli* and *P. aeruginosa* gram-negative bacteria and shows mild to moderate activity compared with reference compound ampicillin. Rank score and physicochemical properties of synthesized ligands are shown in Fig 2. Docking study of all five compounds against *E. coli* is shown in Fig 3. Among all docked compounds, 3e was found to be the most potent pose of which is shown in Fig 4. Docking of compounds is also done on *P. aeruginosa*, as shown in Fig 5. As we consider all results obtained from antibacterial tests together, we can say that entire compounds tested are active antimicrobial in nature.

Table 2. Physicochemical data of substituted thiosemicarbazides.

S. No.	Code no.	R	Molecular Formula	M.W.	R_f value	(%) Yield	M.P. (°C)
1.	1a	H	$C_7H_9N_3S$	167.2	0.74	82	230
2.	1b	3-nitro	$C_7H_8N_4O_2S$	212	0.94	81.34	234
3.	1c	3-Methyl	$C_8H_{11}N_3S$	181	0.58	82.32	237
4.	1d	3-methoxy	$C_8H_{11}N_3OS$	197	0.73	86.50	238
5.	1e	3-chloro	$C_7H_8ClN_3S$	201	0.59	87.57	241

Table 3. Physicochemical data for the newly synthesized thiadiazole compounds (microwave irradiation).

S. No.	Code no.	R	R_1	Molecular formula	M.W.	R_f value	(%) yield	M.P (°C)
1.	2a	H	H	$C_{15}H_{11}N_3O_2S$	297	0.72	86.00	321
2.	2b	3-nitro	H	$C_{15}H_{10}N_4O_4S$	342	0.86	90.09	330
3.	2c	3-methyl	H	$C_{16}H_{13}N_3O_2S$	311	0.76	85.00	340
4.	2d	3-methoxy	H	$C_{16}H_{13}N_3O_3S$	327	0.82	91.90	348
5.	2e	3-chloro	H	$C_{15}H_{10}ClN_3O_2S$	331	0.81	86.00	346

Table 2 showed physicochemical characteristics of substituted thiosemicarbazides, while Table 3 showed physicochemical characteristics of substituted thiosemicarbazides.

Table 4 showed the physicochemical characteristics of the newly synthesized Thiadiazole compounds (ultrasonic irradiation).

Table 4. Physicochemical data for the newly synthesized Thiadiazole compounds (ultrasonic irradiation).

S. No.	Code no.	R	R_1	Molecular Formula	M.W.	R_f value	(%) yield	M.P (°C)
1.	3a	H	H	$C_{15}H_{11}N_3O_2S$	297	0.75	76.00	322
2.	3b	3-nitro	H	$C_{15}H_{10}N_4O_4S$	342	0.83	72.00	341
3.	3c	3-methyl	H	$C_{16}H_{13}N_3O_2S$	311	0.77	74.00	340
4.	3d	3-methoxy	H	$C_{16}H_{13}N_3O_3S$	327	0.84	75.90	348
5.	3e	3-chloro	H	$C_{15}H_{10}ClN_3O_2S$	331	0.83	76.00	347

Table 5 showed the data obtained from the antibacetrria activity of synthesized compounds.

Table 5. Antibacterial activity data of synthesized compounds.

S.No.	Compounds	Concentration (µg/ml)	*E. coli*	*P. aureginosa*
1.	2a	500	10.3±0.38	13.3±0.12
		600	12.8±0.15	13.9±0.82
2.	2b	500	12.7±0.33	9±0.99
		600	12.8±0.16	10.7±0.33
3.	2c	500	16.3±0.45	14.2±0.77
		600	17.2±0.33	14.9±00.33
4.	2d	500	17±0.55	16.7±0.22
		600	17.9±0.78	17.8±0.55
5.	2e	500	10.1±0.99	12.6±0.76
		600	11.4±0.33	12.9±0.90
6.	3a	500	11±0.45	11.7±0.12
		600	12.9±0.74	11.8±0.45
7.	3b	500	12±0.45	12.7±0.32
		600	12.9±0.65	12.8±0.55
8.	3c	500	16±0.50	17.7±0.12
		600	16.9±0.68	17.8±0.54
9.	3d	500	16±0.56	15.7±0.14
		600	16.9±0.63	16.8±0.35
10.	3e	500	10±0.45	10.7±0.26
		600	10.9±0.72	10.8±0.45
11.	Ampicillin	500	19.0±0.35	21.7±0.12
			17.9±0.42	17.8±0.45

3.1. Docking study result.

All the selected derivatives were docked with active sites of selected PDB code and assessed for binding free energy and their interaction with the receptor. Table 6 concluded the binding free energy in Kcal/mole. The synthesized derivatives showed good binding energy with the selected receptor (PDB ID: 1X7V). Among these, 3e compounds showed maximum binding free energy -7.449 Kcal/mol.

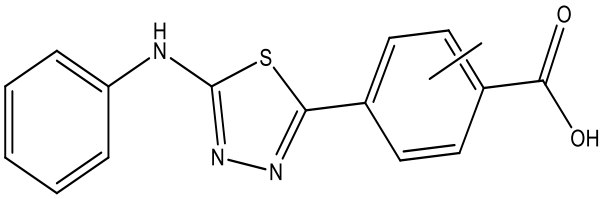
Table 6. Summarize the binding free energy in Kcal/mole and the synthesized derivatives with good binding energy with the selected receptor.

S. No.	Compound	slog P	TPSA	LF V score
1	3a	3.6	77.9	-6.968
2	3b	3.4	123.8	-6.848
3	3c	4	77.9	-6.334
4	3d	3.7	87.2	-7.23
5	3e	4.3	77.9	-7.449

3.2. Docking study on PDB ID 1J2R (E. coli) and Pseudomonas aeruginosa PDB ID (1X7V).

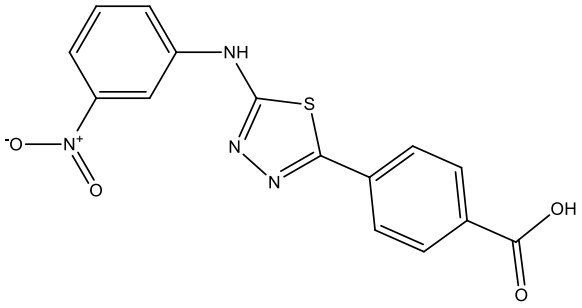
Figure 1 shows the structure of the compound prepared by the ultrasonication method.

3a)



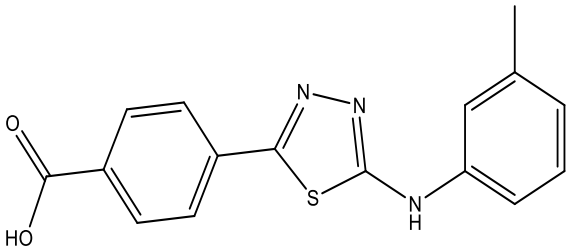
4-(5-(phenylamino)-1,3,4-thiadiazol-2-yl)benzoic acid compound with ethane (1:1)

3b)



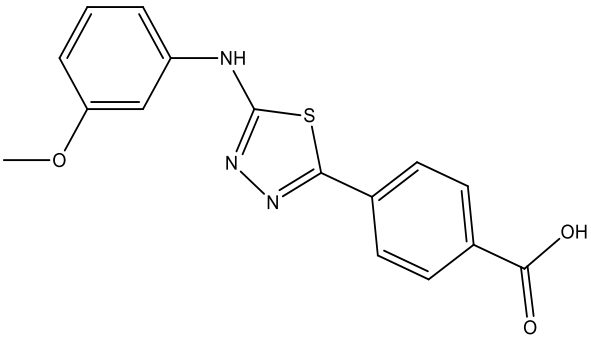
4-(5-((3-nitrophenyl)amino)-1,3,4-thiadiazol-2-yl)benzoic acid

3c)



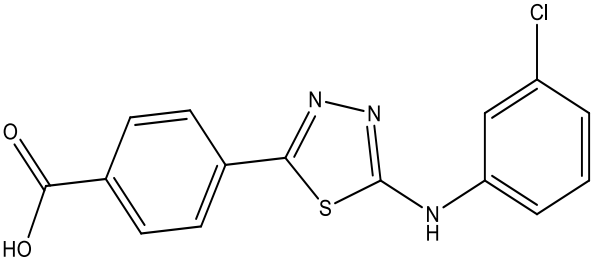
4-(5-(*m*-tolylamino)-1,3,4-thiadiazol-2-yl)benzoic acid

3d)



4-(5-((3-methoxyphenyl)amino)-1,3,4-thiadiazol-2-yl)benzoic acid

3e)



4-(5-((3-chlorophenyl)amino)-1,3,4-thiadiazol-2-yl)benzoic acid

Figure 1. Structure of synthesized compounds 3a-3e (by ultrasonication method).

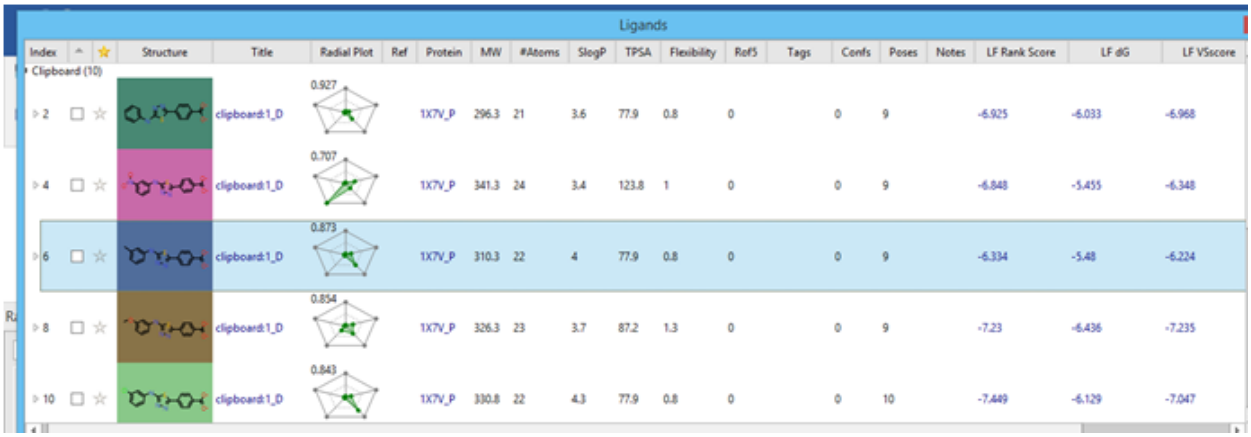


Figure 2. Showing rank score and physicochemical properties of synthesized ligands for docking study.

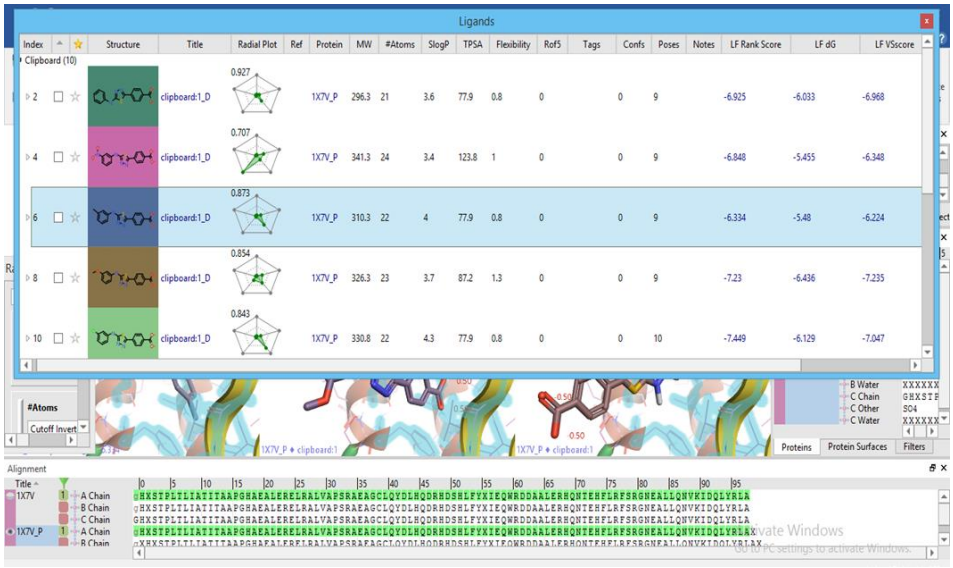


Figure 3. Showing synthesized drug-receptor interactions with PDB ID 1X7V with their amino acid sequences.

Figure 2 shows the rank score and physicochemical properties of synthesized ligands for docking study. Figure 3 shows synthesized drug-receptor interactions with PDB ID 1X7V with their amino acid sequences.

In this series, the compound 3e is the most active compound against *P. aeruginosa* based on docking score energies and antimicrobial evaluation.

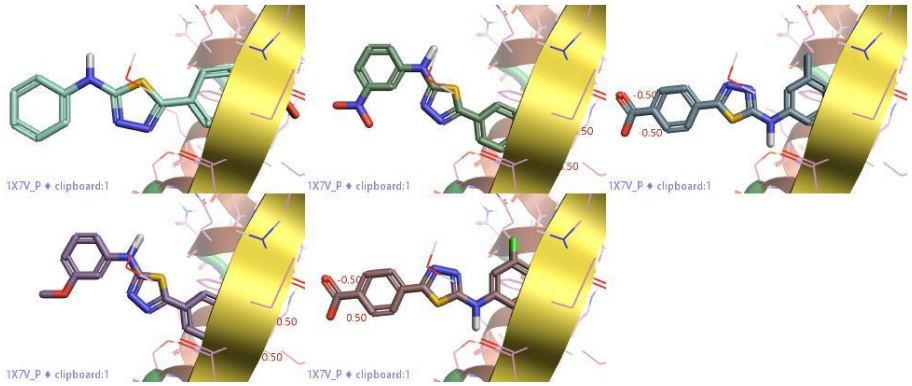


Figure 4. *In silico* screening (Docking) study of compounds (3a-3e) on *E. Coli (PDB ID -1X7V).*

Figure 4 shows *in silico* screening (Docking) study of compounds (3a-3e) on *E. coli* (PDB ID -1X7V).

Most active compound: Figure 5 shows the docking pose of compound 3e with *E. Coli* (PDB ID -1X7V), and figure 6 shows *in silico* screening (Docking) study of compounds (3a-3e) on *Pseudomonas aeruginosa* (PDB ID -1X7V).

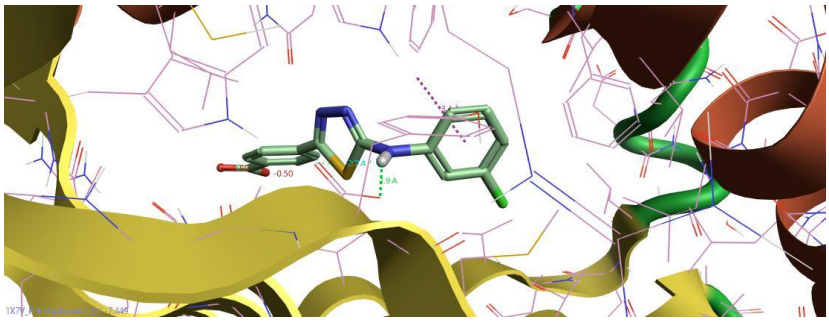


Figure 5. Most promising docking pose of compound 3e with *E. Coli (PDB ID -1X7V).*

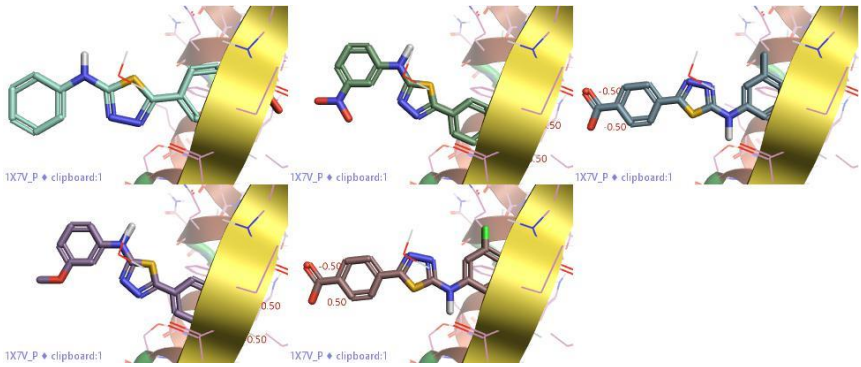


Figure 6. *In silico* screening (Docking) study of compounds (3a-3e) on *Pseudomonas aeruginosa (PDB ID -1X7V).*

4. Conclusions

Results and practical yield obtained using the green chemistry approach in the synthesis of thiadiazole derivatives conclude that conventional methods of synthesis can be substituted by ultrasonic and microwave irradiation methods (Green chemistry methods). By the methods of microwave and ultrasonic irradiation, thiadiazole derivatives are synthesized with good yield. Also, there is saving of time and chemicals. Thus Green chemistry methods can be used in the future in place of conventional synthetic methods. The compound 3e was the most active compound against *P. aeruginosa* based on docking score energies and antimicrobial evaluation.

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Conflicts of Interest

The authors declare no conflict of interest.

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