SYNTHESIS, MOLECULAR DOCKING, ANTIOXIDANT AND ANTIBACTERIAL ASSESSMENT OF THYMOL AZO DERIVATIVES

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ABSTRACT

Thymol, a naturally occurring monoterpenoid phenol, has sparked attention for its potential therapeutic properties, including antioxidant and antibacterial activities. In this study, I emphasized synthesizing azo derivatives of thymol and evaluating their molecular docking, antioxidant, and antibacterial properties. The synthesis involved the chemical modification of thymol to create azo compounds, followed by computational docking studies to predict their potential binding affinity towards specific target proteins. A total of nine azo derivatives of thymol have been synthesized from different substituted aromatic amines and characterized by IR and proton NMR. The antioxidant activity of the synthesized azo derivatives was assessed using a DPPH assay, highlighting their potential as free radical scavengers. Furthermore, the antibacterial properties of the derivatives were evaluated against Staphylococcus aureus and Escherichia coli to assess their potential as antimicrobial agents. Additionally, drug-like properties of derivatives were evaluated using bioinformatic tools such as PASS prediction, molecular docking, and Lipinski rules of five, as well as toxic nature and LD 50 values. All nine compounds exhibited significant antibacterial action, with one showing strong antioxidant activity. Furthermore, the docking scores of derivatives fell between 124 to 106 (LibDock score) against two bacterial targets. The findings of this work provide important insights into potential applications of thymol azo derivatives as multifunctional molecules with antioxidant and antibacterial properties, necessitating additional research into their potential therapeutic usage.

Keywords : Thymol ; Molecular docking ; Azo derivatives ; Antioxidant ; Antibacterial

1. INTRODUCTION

Antibacterial drug resistance, an increasing worldwide health concern, poses a considerable challenge to successful bacterial illness therapy. Bacteria have evolved and adapted to antibiotics over time, rendering them less efficient or, in some circumstances, completely ineffective. Antibacterial medication resistance has far-reaching consequences, affecting not just individual patient outcomes but also public health and healthcare systems. Infections that were once curable with simple medications are now more complex and costly to treat. The emergence of multidrug-resistant bacteria, colloquially known as superbugs, significantly limits treatment options, putting patients at risk of severe disease, increased death, and prolonged hospital admissions. Chemical modification or molecular manipulation of naturally occurring compounds is a viable strategy for combating the rising problem of antimicrobial drug resistance. The worldwide health catastrophe of antimicrobial medication resistance develops when microbes evolve and acquire methods to withstand the effects of antimicrobial medicines, rendering previously successful therapies useless. To address this issue, scientists are investigating numerous techniques for improving the efficacy and broadening the spectrum of existing antimicrobial medicines. One such method is to alter natural chemicals in order to create novel derivatives with enhanced antibacterial action. This method capitalizes on the potency of natural chemicals and increases their medicinal potential. The logic and benefits of chemical modification of natural molecules in the battle against antimicrobial drug resistance are discussed here.

Thymol is a naturally occurring monoterpene derivative of cymene, extracted from several plants viz., *Thymus vulgaris* and *Trachyspermum ammi*, etc.; it is a chemically p-cymene derivative named, 5-Methyl-2-(1-methylethyl) phenol. It had been reported as a potent antibacterial, antifungal and antioxidative agent.

Derivatives of thymol have been shown to have a variety of biological activities, including antibacterial, antimalarial, anti-cancer, antioxidant, and anti-leishmania activity. According to the available literature, the thymol was structurally modified by the inclusion of the diazenyl group, amino methylated at C-4 and C-6 positions, and etherified on the phenolic thymol system, resulting in significant antibacterial effects. Recently, thymol-derived pyrazoline and chalcone scaffolds were identified as promising antimalarial options for controlling Plasmodium falciparum. The Schiff base of thymol derivatives was developed, and the compounds demonstrated strong antioxidant action.

A fascinating area of study in photochemistry and pharmaceuticals is the azo group chromophore (R-N = N-R′). Simple methods for producing azo molecules include diazotization and the aromatic coupling reaction. A fascinating area of study in photochemistry and pharmaceuticals is the azo group chromophore (R-N = N-R′). Simple methods for producing azo molecules include diazotization and the aromatic coupling reaction. Diazotization is the process of turning primary aromatic amines into their diazonium salt. Diazonium salts are significant synthetic intermediates that can go through electrophilic substitution reactions to add functional groups and coupling reactions to produce azo dyes and &the organic reaction

between a diazonium salt and an additional aromatic compound is known as azo coupling. In this electrophilic aromatic substitution reaction aryldiazonium cation is an electrophile whereas activated arene is nucleophile. These azo compounds are organic colorants, which are produced in the world at a rate of one million tons annually, have a variety of uses, including the coloring of various materials, biological and medical research, organic synthesis, and more sophisticated uses like corrosion inhibitors. Furthermore, azo dyes have antibacterial and antiprotozoal characteristics, as well as the ability to stimulate wound healing. When compared to anionic dyes, cationic dyes are more active in acidic medium and preferentially attack gramme +ve bacteria. Scarlet red and dima zone4 are the most prevalent azo dyes used as antiseptics.

2. EXPERIMENTAL

2.1 Material & Method

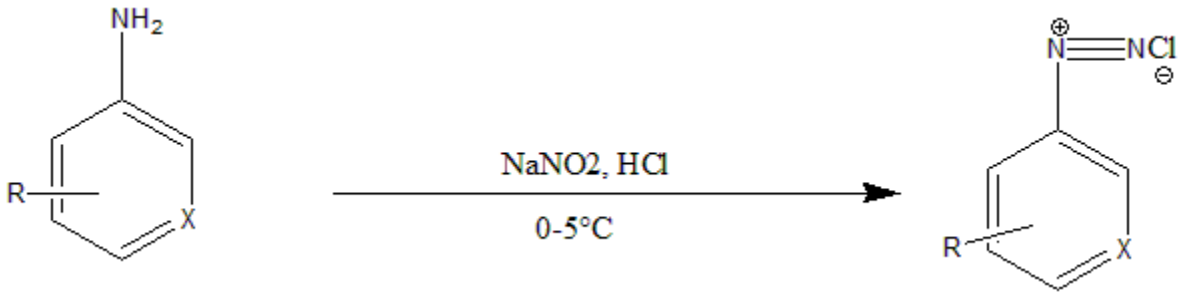
All the chemicals were purchased from SRL, Sigma Aldrich, Central drug house (CDH), HiMedia and Merck India. Most of the chemicals and solvents used were of LR grade.

Additionally, we utilized applications like Discovery Studio, Chemsketch, and Chemdraw as well as bioinformatics tools and biological databases like PDB (Protein Data Bank). The PDB (Protein Data Bank) was founded in Brookhaven National Laboratories (BNL) and is the only collection of structural data of biological macromolecules in existence. It includes macromolecule structural data derived from X-ray crystallography, NMR, and other techniques. Discovery Studio is an automated tool for docking. It's intended to forecast how tiny compounds, like substrates, would interact with a receptor with well-known 3D structures.

Melting point was determined by capillary tube method and digital melting point measuring apparatus. IR spectra of synthesized compound were recorded on Perkin-Elimer 4000-400cm-1 spectrophotometer by ATR scanning method. Wavelength maxima of compounds were measured by Shimadzu UV-1800 UV/Visible Scanning Spectrophotometer. 1H NMR (400MHZ) and 13C NMR (100MHZ) spectra were recorded in DMSO-d6 and CDCl3 solvent on Bruker instrument.

2.2 Scheme of the work

Step 1:



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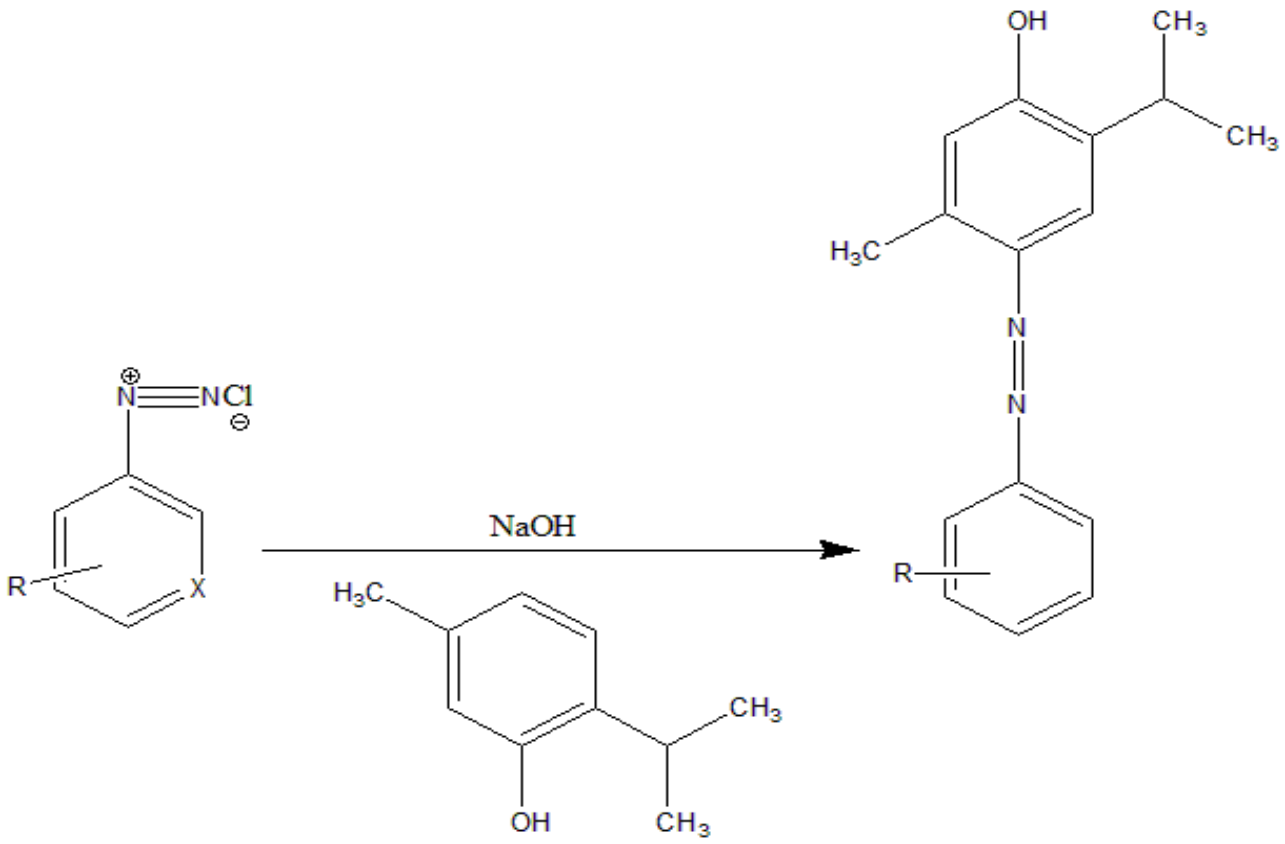


Table 1: Chemical structure of newly synthesized compounds

S. N	Compound code	R	X
1	3A	C6H6	N
2	8A	C6H5N	CH
3	3B	3-Br	CH
4	4B	4-Br	CH
5	2C	2-Cl	CH
6	2,6C	2,6-Cl	CH
7	3F	3-F	CH
8	4F	4-F	CH

Characterization of azo derivatives of thymol

1. 4-[(E)-(isoquinolin-4-yl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(3A)**

Melting point: 130°C, Appearance: Orange solid, %yield:80- 90%

1H NMR (400 MHz, DMSO) δ 10.40 (s, 1H), 9.37 (s, 1H), 8.75 (s, 1H), 8.14 (dd, J = 35.9, 8.4, 1.3 Hz, 2H), 7.72 (dd, 0H), 3.20 (hept, J = 6.8 Hz, 1H), 2.66 (s, 2H), 2.09 (s, 3H), 1.20 (dd, J = 6.9 Hz, 6H). IR (ATR, γ, cm−1) 3100(OH str.), 2850(CH2 str.), 2980(CH Ar.), 1610(C=C str.), 1490(N=N str.), 1400(C-O str.), 1380(OH bending).

2. 5-methyl-2-(propan-2-yl)-4-[(E)-(quinolin-8-yl) diazenyl] phenol**(8A)**

Melting point: 125°C, Appearance: Black color powder
%yield: 81%
1H NMR (400 MHz, DMSO) δ 9.24 (s, 1H), 9.09 – 8.56 (m, 1H), 8.27 (d, J = 8.1 Hz, 1H), 7.92 (td, 3H), 6.95 (s, 1H), 6.53 (d, J = 7.7 Hz, 1H), 3.17 (hept, 1H), 2.64 (s, 1H), 2.15 (s, 5H), 1.19 (dd, 7H).
IR (ATR, γ, cm−1) 3100(OH str.), 2950(CH Ar.), 2850(CH2 str.), 1600(C=C str.), 1450(N=N str.), 1380(C-O str.), 1250(OH bending).

3. 4-[(E)-(3-bromophenyl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(3B)**

Melting point: 140-145°C, Appearance: Brick yellowish powder, %yield: 58%
1H NMR (500 MHz, Chloroform-d) δ 7.93 (t, J = 1.6 Hz, 1H), 7.65 – 7.60 (m, 1H), 7.50 (d, J = 0.6 Hz, 1H), 7.48 – 7.42 (m, 2H), 6.95 (s, 1H), 6.76 (d, J = 0.8 Hz, 1H), 3.35 – 3.25 (m, 1H), 1.22 (d, J = 7.0 Hz, 6H).
IR (ATR, γ, cm−1) 3200(OH str.), 2900-3000(CH Ar. Str.), 2820(CH2 str.), 1600(C=C str.), 1580(N=N str.), 1230(C-O str.), 1150(OH bending), 790(C-Br)

4. 4-[(E)-(3-bromophenyl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(4B)**

Melting point: 150°CAppearance: Brick red color powder, %yield: 75%
1H NMR (500 MHz, Chloroform-d) δ 7.93 (t, J = 1.6 Hz, 1H), 7.65 – 7.60 (m, 1H), 7.50 (d, J = 0.6 Hz, 1H), 7.48 – 7.42 (m, 2H), 6.95 (s, 1H), 6.76 (d, J = 0.8 Hz, 1H), 3.35 – 3.25 (m, 1H), 1.22 (d, J = 7.0 Hz, 6H).
IR (ATR, γ, cm−1) 3243(OH str.), 2961(CH Ar. Str.), 2871(CH2 str.), 1609(C=C str.), 1577(N=N str.), 1252(C-O str.), 1155(OH bending), 758(C-Br).

5. 4-[(E)-(2-chlorophenyl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(2C)**

Melting point: 130°C, Appearance: Brick red color powder, %yield: 60%
1H NMR (500 MHz, Chloroform-d) δ 7.77 (dd, J = 7.4, 1.5 Hz, 1H), 7.52 – 7.45 (m, 3H), 7.31 – 7.24 (m, 1H), 6.95 (s, 1H), 6.76 (s, 1H), 3.37 – 3.25 (m, 1H), 1.22 (d, J = 7.0 Hz, 6H).
IR (ATR, γ, cm−1) 3294(OH str.), 3000(CH Ar. Str.), 2850(CH2str.), 1586(C=C str.), 1467(N=N str.), 1140(OH bending), 749(C-Cl str.).

6. 4-[(E)-(2,6-dichlorophenyl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(2,6C)**

Melting point: 140°C, Appearance: Red color powder, %yield: 59%
1H NMR (400 MHz, CDCl3) δ 7.63 (dd, 1H), 7.34 (dd, J = 8.1, 0.9 Hz, 2H), 7.16 (dd, J = 37.2 Hz, 1H), 6.68 (s, 1H), 3.14 (hept, 1H), 2.56 (s, 3H), 2.33 – 1.97 (m, 0H), 1.22 (dd, 6H).
IR (ATR, γ, cm−1) 3000(OH str.), 2900(CH Ar. Str.), 2800(CH2 Str.), 1600(C=C str.), 1480(N=N Str.), 1200(OH bending), 750(C-Cl str.)

7. 4-[(E)-(3-fluorophenyl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(3F)**

Melting point: 125-130°C, Appearance: Dark brown color powder, %yield: 47%
1H NMR (400 MHz, DMSO-d6) δ 8.17 (s, 1H), 7.58 (s, 0H), 7.51 (d, J = 2.2 Hz, 0H), 7.41 (d, J = 5.0 Hz, 0H), 7.21 (d, J = 0.9 Hz, 0H), 6.82 (s, 1H), 3.29 (hept, 1H), 2.55 (hept, J = 0.5 Hz, 3H), 1.21 (d, J = 6.9 Hz, 6H).
IR (ATR, γ, cm−1) 3200-3100(OH str.), 2990(CH Ar. Str.), 2880(CH2 str.), 1590(C=C str.), 1470(N=N str.), 1350(C-O str.), 1310(OH bending), 1100(C-F str.).

8. 4-[(E)-(4-fluorophenyl) diazenyl]-5-methyl-2-(propan-2-yl) phenol**(4F)**

Melting point: 120-130°C, Appearance: Brick red color powder, %yield: 92%

1H NMR (500 MHz, Chloroform-d) δ 7.72 – 7.66 (m, 2H), 7.49 (d, J = 0.6 Hz, 1H), 7.24 – 7.17 (m, 2H), 6.95 (s, 1H), 6.76 (d, J = 0.8 Hz, 1H), 3.37 – 3.25 (m, 1H), 1.22 (d, J = 7.0 Hz, 6H).

IR (ATR, γ, cm−1) 3200-3100(OH str.), 2990(CH Ar. Str.), 2850(CH2 str.), 1600(C=C str.), 1510(N=N str.), 1400(C-O str.), 1350(OH bending), 1120(C-F str.)

2.3 Molecular docking

To obtain greater insight into the binding mechanism of the compounds with target protein docking studies were performed using Discovery studio 2.0. For interaction investigations, the top scoring compounds from the biggest cluster were selected. The docking receptor is the crystallographic structure of the target protein, which is downloaded from the RCSB Protein Data Bank, and all proposed chemicals are chosen as ligand molecules. The protein was constructed before docking the screened ligands into the protein active site by removing the heteroatom as well as the crystallographic ally detected water molecules. Chemdraw Ultra 8.0 was used to draw the ligand molecules. Docking was performed using the LibDock module of DS v2.0 (Catalyst, Accelrys Software) to investigate the binding method of synthesized compounds into the active site of MurB. LibDock was chosen because of its capacity to dock onto the receptor's hot spots (polar and non-polar interaction sites) and its short computation time. The ligand was kept flexible throughout the process, whereas the target protein was remained rigid. MurB crystal structure was retrieved from the protein data bank (PDB ID: 1UXY, 1HSK). Water molecules were removed, and hydrogen atoms were introduced. In the active site, a radius of 9 was defined from the geometric centroid of the ligand. The designed structure was docked in the active site. The best poses of each docking study were selected, and on the basis of LibDock scores.

2.4 Antibacterial activity

All of the synthesized compounds have been investigated for antibacterial activity against gram-positive (*S. aureus)* and gram-negative (*E. coli)* bacterial strain through the agar well diffusion method. For comparison, the antibiotic ampicillin has been used as a reference. Bacterial culture strains were kept on nutritional agar slant for 24 hours at 370.5 °C. The antibacterial activity was determined using a nutrient agar plate seeded with 0.1 mL of 105 CFU/mL dilutions of the corresponding bacterial culture strain suspension produced in sterile saline (0.85%). For each bacterial strain, 3 mm diameter wells were filled with 0.1mL of chemical solution at preset concentrations of 10, 50, and 100 g/mL. All of the plates were incubated for 24 hours at 370.5 °C. Compound inhibition zones were measured in millimeters.

2.5 Antioxidant activity

All compounds' radical scavenging activity was determined using a DPPH assay method.

In brief, concentrations (10, 20, 30, 40, 50 µg/ml) of all chemicals and Ascorbic acid were transferred to separate test tubes, which were then mixed with 2ml of 50 µM methanolic solution of DPPH as a free radical reserve and thoroughly shaken. The test tubes were incubated at room temperature for 30 minutes. The control was made up of all chemicals without synthesized compounds. The absorbance variations of the substances were measured at 517 nm. The lower the absorbance of the reaction mixture, the greater the free radical scavenging activity. The absorbance obtained was converted into %inhibition (%radical scavengering activity), and the IC50 was calculated using linear regression.

%inhibition (%radical scavengering activity):

(Absorbance of control-Absorbance of sample/ Absorbance of control) *100

3. RESULT&DISCUSSION

In this present communication, synthesis, molecular docking antioxidant and antibacterial activity of some azo derivatives of thymol reported from different substituted aromatic amines. Initially, substituted diazonium salt were prepared by diazotization method. These, substituted diazonium salts were treated with alkaline thymol for coupling reaction that predicts 2-isopropyl-5-methyl-4-(Substituted azobenzyl) diazenyl phenol (1-8). The newly synthesized compounds were established on the basis of IR and 1H NMR spectroscopic method. The IR spectra of the compounds showed the presence of primary amine group in primary aromatic amines which were taken as starting material and absence of primary amine group as well presence of hydroxyl group at 3243 $cm^{-1,}$ N=N at 1577 cm^{-1} in **4B**, indicating the formation of product. In 1H NMR spectra, a broad peak due to presence of –OH is observed at 7.93 ppm, other shifts and number of protons proved the structure of the products.

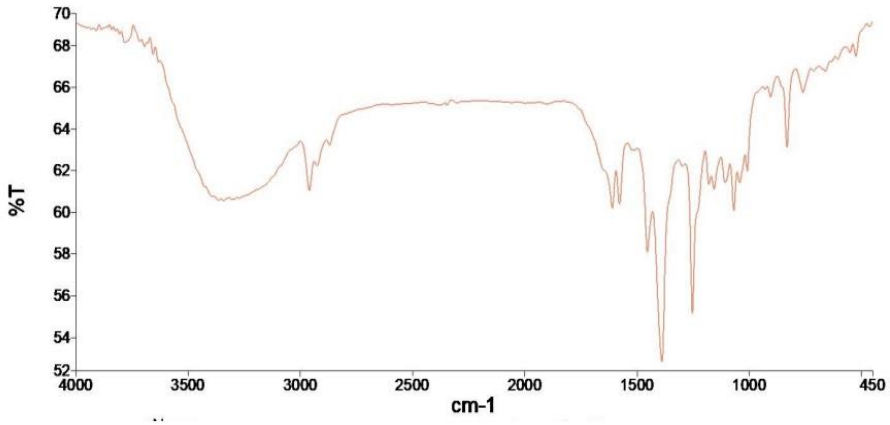


Figure 1: IR spectra of 4B

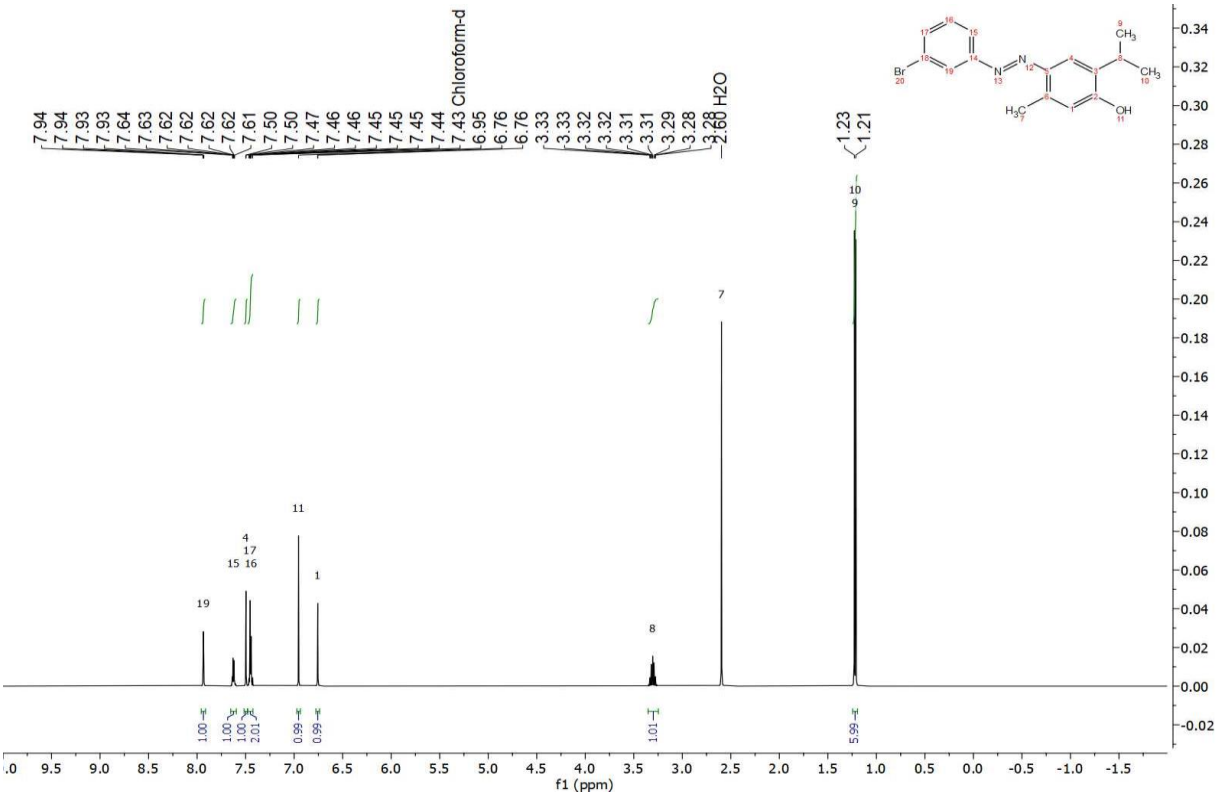


Figure 2: 1H NMR spectra of 4B

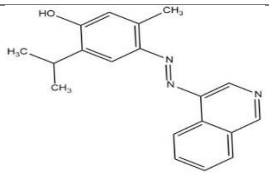
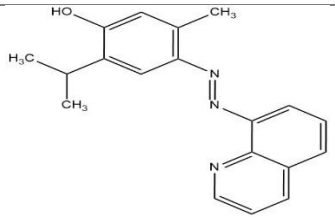
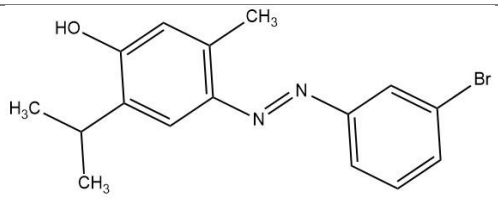
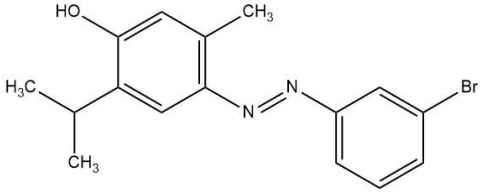
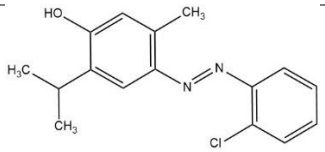
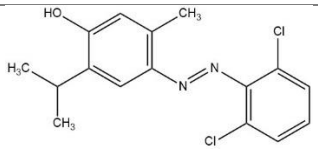
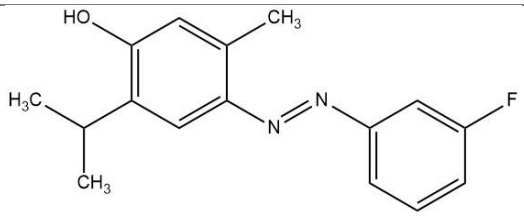
Docking results showed that all the synthesized compound has hydrogen bond interaction (HBs), hydrophobic interaction (HyP), Halogen bond interaction and vanderwaals interaction (VdW) with target enzyme MurB of respective bacteria. In this, hydrogen bond is of type; conventional hydrogen bond & carbon-hydrogen bond. Conventional hydrogen bond represented in dotted dark green line while carbon-hydrogen bond in dotted light green line. Dotted pink lines/purple line/orange line represents hydrophobic interaction that includes alkyl, π-alkyl, amide π stacked, π- π stacked, π-sigma, π-sulfur etc. Surrounding amino acids of target in green color shows hydrogen bond interaction with ligand. Dotted blue line represents halogen bond interaction (D...X-Y), which is interaction of a halogen and an electron donor species, where X is an electrophilic halogen atom, D is an electron donor, and Y is a carbon, nitrogen, or halogen atom. In 1896, Guthrie reported the synthesis of an ammoniac-iodine complex, and in 1970, Odd Hassel described the parallels in halogen and hydrogen bonding. The investigation of XB interaction has piqued the interest of researchers in a variety of domains, including rational medication design and theoretical chemistry calculations.

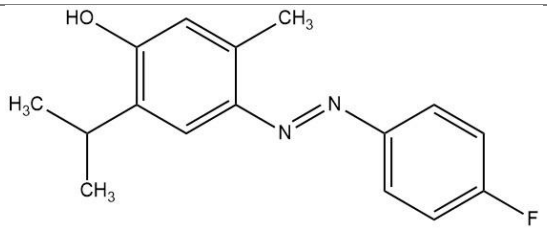
From the results of DPPH assay in table, it was found that all the compounds have IC50 less than 10. Compound 4F shows IC50 less than IC50 of standard ascorbic acid whereas the compound 3A showed higher IC50 value among all. According to literature the lower the IC50 value, the more potent the compound is at scavenging DPPH, implying more antioxidant activity.

From the results in table, it was found that all the compounds showed antibacterial activity. Among these compounds 3A, 8A & 3B showed greater zone of inhibition than standard ampicillin against gram negative (E. coli) bacteria at the concentration of 10,50 and 100 µg/ml. 2C is the least active among all the

synthesized compound. Synthesized compounds 3A,3B,2C,2_6C, 4F,4B showed excellent activity against gram positive bacteria (S. aureus) at concentrations 10,50 and 100 µg/ml Compound 3A,4B & 3B showed excellent activity against both gram negative as well as gram positive bacteria. It shows that -OH, halogen (Br), N=N, N in ring have greater role in antibacterial activity of synthesized compounds.

Table 2: Summary of synthesized compounds

Compound code	Structure	Molecular formula	Molecular weight(g/mol)
3A		$C_{19}H_{19}N_3O$	305.37
8A		$C_{19}H_{19}N_3O$	305.37
3B		$C_{16}H_{17}BrN_2O$	332.05
4B		$C_{16}H_{17}BrN_2O$	332.05
2C		$C_{16}H_{17}ClN_2O$	288.10
2,6C		$C_{16}H_{16}Cl_2N_2O$	322.06
3F		C16 H17 F N2 O	272.13

4F		C16 H17 F N2 O	272.13

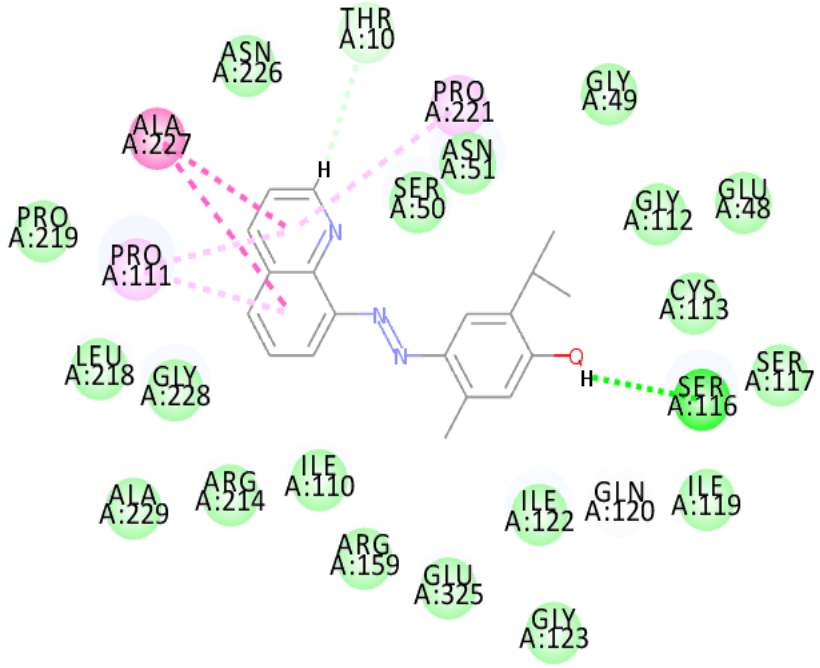


Figure 3: Docked 2D diagram of 8A against 1uxy

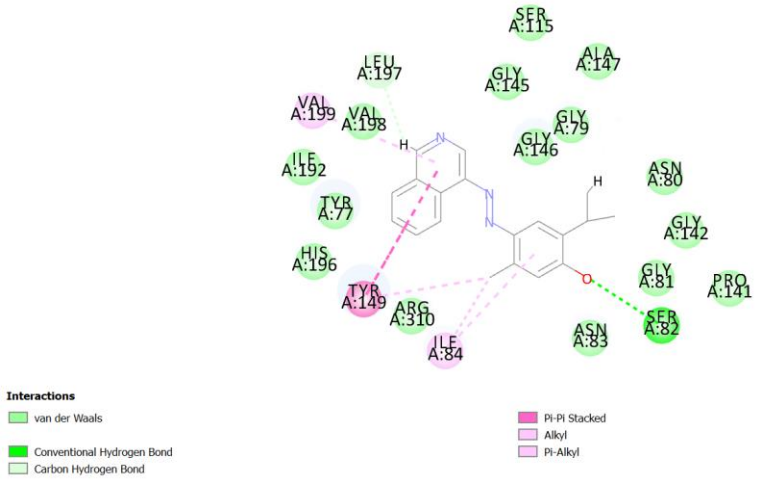


Figure 4: Docked 2D diagram of 3A against 1oye

Table 3: Lipinski rule of synthesized compounds

Compound code	Molecular weight	No. of hydrogen bond	No. of hydrogen bond acceptor	TPSA (A)	No. of rotable bond	Log P
3A	305.37	1	4	57.84	3	3.34
8A	305.37	1	4	57.84	3	3.34
3B	333.22	1	3	44.95	3	3.63
4B	333.22	1	3	44.95	3	3.93
2C	288.10	1	3	44.95	3	3.41
2,6C	322.06	1	3	88.65	3	3.47
3F	272.13	1	4	44.95	3	3.43
4F	272.13	1	4	44.95	3	3.49

Table 4: Predicted toxicity of synthesized compounds

S. N	Compound code	Predicted LD_{50} (mg/kg)	Predicted toxicity class
1	3A	5000	6
2	8A	4818	5
3	3B	4818	5
4	4B	4818	5
5	2C	1250	4
6	2,6C	1250	4
7	3F	4818	5
8	4F	4818	5

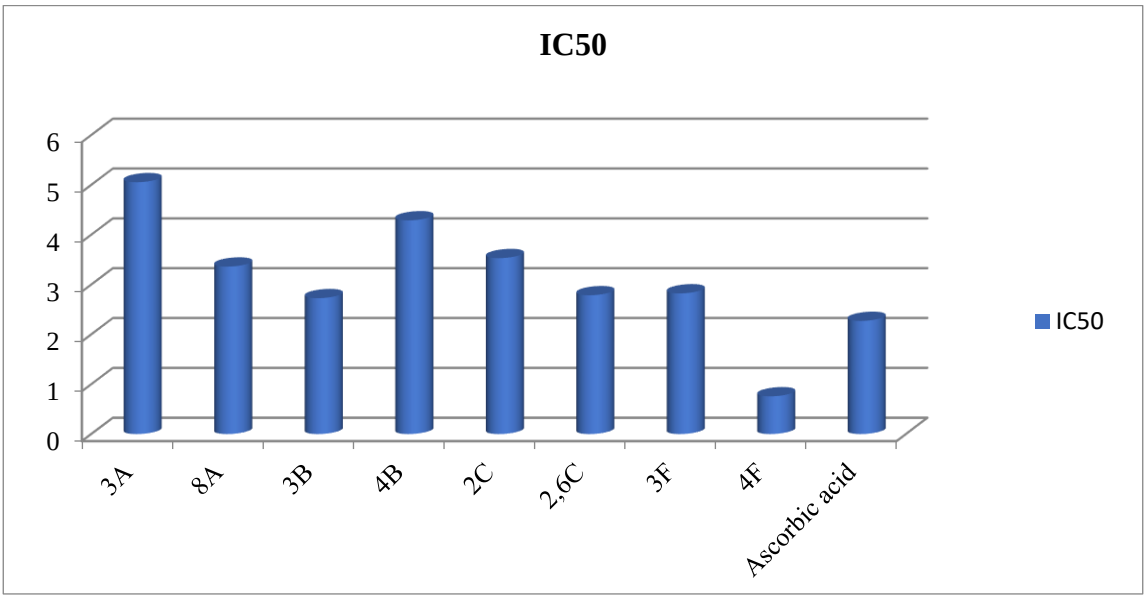


Table 5: IC50 of synthesized compounds

Serial No.	Synthesized compounds	IC_{50}
1	3A	5.0533
2	8A	3.3603
3	3B	2.7264
4	4B	4.2882
5	2C	3.53
6	2,6C	2.7852
7	3F	2.8229
8	4F	0.7569
9	Ascorbic acid	2.27

Table 6: ZOI of standard and test compound against E.coli

Serial No.	Compounds	Zone of inhibition (in mm)		
		10µg/ml	50 µg/ml	100 µg/ml
1	3A	35±0.22	36.6±0.33	38.3±0.33
2	8A	35.6±0.33	37.1±0.33	44±0.33
3	3B	33.6±0.33	35.3±0.33	43.6±0.33
4	4B	30±0.33	33.01±0.33	33.9±0.33
5	2C	11±0.60	14.6±0.33	17.1±0.33

6	2,6C	15.6±0.57	17.6±0.88	18.9±0.33
7	3F	31.6±0.33	38.6±0.33	38.3±0.33
8	4F	28.3±0.33	29.03±0.33	31.6±0.33
9	Ampicillin	29±0.44	29.88±0.33	31.09±0.33

Table 7: ZOI of standard and test compound against S. aureus

Serial No.	Compounds	Zone of inhibition (in mm)		
		10µg/ml	50 µg/ml	100 µg/ml
1	3A	42±0.57	44.3±0.33	46.03±0.33
2	8A	13.3±0.33	16.3±0.33	22±0.33
3	3B	33.8±0.33	36.6±0.33	38.6±0.33
4	4B	33±0.33	35.88±0.55	37.01±0.33
5	2C	37.6±0.33	39.3±0.33	41.01±0.33
6	2,6C	40±0.57	41.6±0.33	43±0.88
7	3F	32.9±0.33	35.06±0.33	36.7±0.33
8	4F	34.6±0.33	41.3±0.66	40.6±0.33
9	Ampicillin	32.3±0.55	38.71±0.33	38±0.33

4. CONCLUSION

In summary, we have developed an operationally simple, economic, efficient, environment benign protocol in synthesis of primary aromatic amines with thymol moiety by the simple diazotization and coupling method. Further these compounds were evaluated for their antibacterial antioxidant activity. all the compounds have antibacterial against gram positive strain *S. aureus* and gram-negative strain *E. coli*. Ampicillin was taken as standard drug which acts by inhibiting the bacterial cell wall synthesis. Compounds **3A, 3B, 2C, 2_6C** and **4F** showed excellent activity at concentrations 10,50 and 100µg/ml against gram positive bacteria. Compounds **3A, 8A, 3B, 4B, 3F, 4F** showed excellent activity against gram negative bacteria at concentrations 10,50 and 100µg/ml.

Based on the aforementioned, it may conclude that the synergistic interaction of hydrophilic and lipophilic properties of a molecule plays an important role in antibacterial activity. The presence of lipophilic aromatic rings capable of interacting with the hydrophobic spaces of bacterial enzymes. The azo moiety is also significant in bacteriostatic action. The presence of **-N=N-** is easily protonated under acidic conditions and reacted with the phosphate group on the peptidoglycan layer of bacteria, inhibiting cell wall synthesis or inhibiting bacterial growth via H-bond interaction of the compound with the active site of enzyme. The presence of a **-OH** group in the synthesized compounds contributes to the hydrophilic characteristics of the compounds, which improves antibacterial activity. The addition of halogens has also improved the lipophilic properties for easy penetration into the bacterial cell. Thus, the presence of **-OH, -N=N-, halogens,** and **nitrogen containing heterocyclic nucleus** played a significant effect on antibacterial activity of compound.