



Eco-Friendly Synthesis Of 4-Aminoantipyrene Derivatives And Biological Potential Evaluation Of Some Novel Derivatives With Their Computational Study

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Abstract: The current study deals with the synthesis of derivatives of 4-aminoantipyrene by condensation of it with aryl aldehydes along with synthesis of some novel derivatives by further condensation of these derivatives obtained from 4-aminoantipyrene and aryl aldehydes with hydrazides by green method using ecofriendly deep eutectic solvent (DES). The DES is cost-effective and biodegradable and plays the dual role of catalyst as well as green solvent. The DES could be recycled multiple times without appreciable loss of its catalytic activity. The present methodology offers advantages in terms of operational simplicity, environmental sustainability, shorter reaction time and produces excellent yield of the desired products. The synthesized derivatives were characterized by different spectroscopic techniques such as IR, UV-Visible, NMR and Mass spectrometry. The novel derivatives (5a-5g) were studied for their biological activity including antibacterial potential against *E. coli*, *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and antifungal activity against *Candida albicans* and *Aspergillus niger* by broth dilution method. Molecular docking, molecular dynamics simulations and DFT studies were performed to evaluate the antimicrobial potential of synthesized 4-aminoantipyrene derivatives. The compound 5g showed the highest binding affinity against both targets, *E. coli* DNA Gyrase B (−6.64 kcal/mol) and *C. albicans* CYP51 (−8.94 kcal/mol), comparable to reference ligands. MD simulations confirmed stable binding, with acceptable RMSD, RMSF and consistent hydrogen bond interactions throughout the simulation period. DFT analysis revealed that compound 5g possesses a balanced electronic profile with moderate HOMO–LUMO gap and favorable reactivity descriptors. These findings suggest that the compound 5g as a potential promising dual antibacterial and antifungal candidate.

Index Terms- 4-aminoantipyrene, Benzoic hydrazide, Antibacterial activity, Antifungal activity, Computational study

I. INTRODUCTION

Green chemistry supports sustainable development by minimizing the environmental impact of chemical processes and products. Over the past years, it has made significant progress across various sectors, emphasizing sustainable practices and the advancement of eco-friendly technologies[1]. In recent times, a new generation of ionic liquid, namely Deep Eutectic Solvent (DES) [2] emerged as an ecofriendly reaction media alternative to traditionally used hazardous organic solvents in various chemical processes[3]. It is a low-cost alternative to ionic liquids. It has some advantages like unique physicochemical properties, use of cost-effective initial compounds, ease of preparation, low flammability, minimal toxicity, biodegradability, non-flammability and renewability[4]. The various deep eutectic solvents were used as catalyst and or solvent for synthesis of schiff bases, Knoevenagel condensation, Symmetric azines, Chitin isolation, Biofuel production, Diels Alder reaction, Cross Coupling reaction, Henry reaction, Fridal Craft alkylation etc [5]. Now a days there is increasing microbial resistance to various available antibiotics hence there is need to search for distinctive compounds with biological potential against various bacteria and fungi. The most remarkable advances in the field of medicinal chemistry have been achieved by distinctive heterocyclic derivatives with better biological potential[6–9]. Heterocyclic compounds are abundantly distributed in nature and certain classes of these compounds are of interest due to many reasons, among which their biological values towards variety of strains. Many of the heterocycles act as drug with sulfur, oxygen, nitrogen, azomethine nitrogen and alcoholic or phenolic oxygen as donor atoms. Heterocyclic compounds exhibit variety of physiological properties viz., analgesic[10], anti-inflammatory[10], antioxidant[11] and anti-cancer activity[12]. N-heterocyclic compounds, serve as an active moiety in pharmaceutical formulations, particularly in non-steroidal anti-inflammatory drugs (NSAIDs) used to treat conditions such as arthritis and various musculoskeletal and joint disorders. Heterocycles like 4-aminoantipyrene is one of the

Pyrazolone compound converted into variety of flexible heterocyclic derivatives by condensation with various aldehydes and ketones and they were reported as superior reagents in biological[13], pharmacological, clinical and analytical applications[14]. 4-Aminoantipyrine (4-amino-1,5-dimethyl-2-phenylpyrazole-3-one, 4-AAP) features a pyrazolone moiety with a five-membered nitrogen ring and a free amino group, structurally similar to N-substituted amides. Chemistry of pyrazolone has garnered significant attention due to their biological potential including antioxidant[15], antibacterial[16–19], antifungal [20–23]. Their ligational behavior arises due to the availability of sp^2 hybridized orbital of the nitrogen atom of the azomethine group. The denticity of 4-aminoantipyrine derivatives enhances due to the presence of one or more donor atoms in proximity to the azomethine group. These derivatives are active and stable under a variety of oxidative and reductive conditions. Derivatives derived from 4-aminoantipyrine with a pyrazolone skeleton and its complexes have a variety of applications including biological, clinical, analytical and pharmacological applications[25]. Antimicrobial resistance represents a global obstacle as current antimicrobial agents are ineffective to treat many microbial infections. This is a critical issue as infection may kill or spread to others and increase healthcare cost. Due to this reason, the development of novel antimicrobial drugs against resistant microbes is essential. The present study deals with the synthesis of derivatives of 4-aminoantipyrine 3a-3k by using DES of ethylene glycol, urea and ammonium chloride (5:4:1) as a catalyst as well as solvent and novel derivatives 5a-5g by using DES of ethylene glycol, urea and ammonium chloride (5:4:1) of as a catalyst and ethanol as a co-solvent. The reaction takes place at room temperature, proceeds in shorter period, observed excellent yield of the desired products while conventional method takes several hours with more energy consumption. The DES used in the first step could be recycled nine times without losing its significant activity. The derivatives were characterized by IR spectroscopy, NMR spectroscopy, Mass spectrometry and UV-Visible Spectroscopy, thermal analysis(TGA). We also studied biological potential against various microorganisms by in-vitro study and MIC value which was determined by serial dilution method. Additionally, synthesized 4-aminoantipyrine derivatives were investigated to explore their potential as antimicrobial agents targeting E. coli DNA Gyrase B and C. albicans lanosterol 14α -demethylase. An integrated computational approach, including molecular docking, molecular dynamics simulations, and DFT analysis, was employed to evaluate their binding affinity, stability and electronic properties. This study aims to identify promising lead compounds with antibacterial potential and antifungal potential.

II. EXPERIMENTAL

MATERIALS AND MEASUREMENTS

All the chemicals, including 4-aminoantipyrine, aldehydes, benzoic hydrazide, phenyl hydrazine, 2-4-dinitrophenyl hydrazine, thiosemicarbazide and required solvents purchased from S. D. Fine Chem. Ltd. were used without further purification. To study the progress of the reaction TLC plates coated with silica gel were used as the stationary phase and n-hexane: ethyl acetate as mobile phase. Capillary method was used to determine the melting point of synthesized compounds. The FTIR spectra were recorded on ATR from Bruker, NMR spectra were recorded on Bruker spectrometer at 600MHz with DMSO- d_6 , $CDCl_3$, D_2O as solvents and chemical shift were recorded in ppm. We recorded mass spectra on Waters mass spectrometer 3100 mass detector, electronic spectra were obtained by Perkin Elmer Lambda 365 instrument and thermal analysis were done on Thermogravimetric analyzer TGA 4000(Perkin Elmer), antibacterial, antifungal study were done at Biocyte, research and development Pvt. Ltd, Sangli.

SYNTHESIS OF NOVEL DERIVATIVES:

The synthesis of novel derivatives of 4-aminoantipyrine were carried out in two steps as shown in **Scheme 1**.

SYNTHESIS OF DES

A deep eutectic solvent (DES) composed of ethylene glycol, urea and ammonium chloride in a 5:4:1 molar ratio was prepared using a reported method [26]. The components were mixed in the specified molar proportions and heated at 80°C until a clear liquid was obtained.

Step 1: Synthesis of 3a-3k

As represented in **Scheme 1**, the schiff bases 3a-3k were synthesized by mixing equimolar amount of 4-aminoantipyrine and aryl aldehydes in DES (2.0 g) and stirred at room temperature for about 9-11 minutes. After completion of reaction the product was extracted with ethyl acetate, the yellow colored precipitate obtained was filtered and dried. The progress of reactions was monitored by TLC using ethyl acetate: n-hexane (2:8) as a solvent system. The optimization of reaction condition and temperature was represented in **Table 1** and **Table 2** respectively. The results of 3a-3k were depicted in **Table 3**.

Table 1: Optimization of reaction condition for Step 1^(a)

Entry	Solvent	Time (min)	Yield ^(b) (%)
1.	-	120	10
2.	Ethanol	360	77[27]
3.	Glucose: Urea: NH_4Cl (3:7:1)	30	45
4.	Lactic acid: Urea: NH_4Cl (3:7:1)	30	35
5.	Choline Chloride: Glucose (6:4)	30	58
6.	Choline Chloride: Thiourea (1:2)	30	40
7.	Choline chloride: Oxalic acid (1:1)	30	38
8.	Choline Chloride: $ZnCl_2$ (1:2)	50	20
9.	ChCl: Glycerol (1:1)	40	50

10.	ChCl: FeCl ₃ (1:2)	30	20
11.	ChCl: Malic Acid (1:1)	30	30
12.	ChCl: Fumaric Acid (1:1)	30	35
13.	ChCl: Citric Acid (2:1)	30	40
14.	Choline Chloride: Urea (1:2)	30	45
15.	Alcohol +Choline Chloride: Urea (1:2)	25	78[23]
16.	Ethylene glycol: Urea: Ammonium Chloride (5:4:1)	09	98
^(a) Reaction conditions: a mixture of 1 mmol of 4-aminoantipyrine, 1 mmol of benzaldehyde, 2.0 g of DES, at room temperature ^(b) isolated yields			

Table 2: Optimization of Temperature

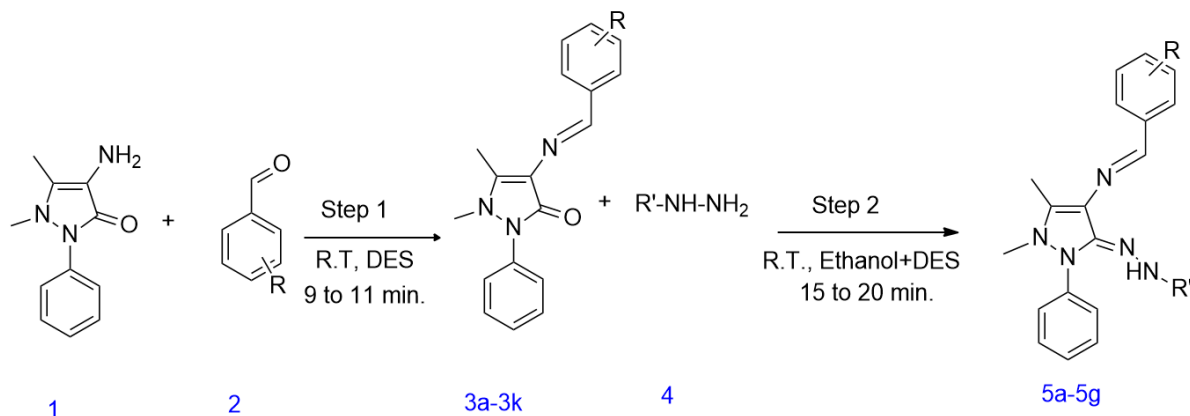
Entry	Temperature In °C	Yield %
3a	25	98
3a	35	98
3a	45	98
3a	55	98
3a	65	98
3a	75	98
3a	85	98

Table 3: Synthesis of 3a-3k

Entry	1	2	Product	Time (min)	Yield (%)	M. P. (°C) ^(b)
1	C ₁₃ H ₁₁ N ₃ O	Ph-CHO	3a	09	98	176[23,27]
2	C ₁₃ H ₁₁ N ₃ O	2-BrC ₆ H ₄ CHO	3b	10	98	190[23]
3	C ₁₃ H ₁₁ N ₃ O	3-BrC ₆ H ₄ CHO	3c	09	99	200[23]
4	C ₁₃ H ₁₁ N ₃ O	4-BrC ₆ H ₄ CHO	3d	10	99	223[23,28]
5	C ₁₃ H ₁₁ N ₃ O	2-FC ₆ H ₄ CHO	3e	09	97	177[29]
6	C ₁₃ H ₁₁ N ₃ O	4-FC ₆ H ₄ CHO	3f	09	98	231[29]
7	C ₁₃ H ₁₁ N ₃ O	4-NO ₂ C ₆ H ₄ CHO	3g	09	98	254[29]
8	C ₁₃ H ₁₁ N ₃ O	4-OCH ₃ C ₆ H ₄ CHO	3h	09	99	182[29]
9	C ₁₃ H ₁₁ N ₃ O	2-ClC ₆ H ₄ CHO	3i	10	98	192[29]
10	C ₁₃ H ₁₁ N ₃ O	4-ClC ₆ H ₄ CHO	3j	10	99	250[28]
11	C ₁₃ H ₁₁ N ₃ O	4-OHC ₆ H ₄ CHO	3k	11	98	226[29]
^(a) Reaction conditions: a mixture of 1 mmol of 4-aminoantipyrine, 1 mmol of aldehyde, 2.0 g of DES, at room temperature ^(b) isolated yields						

Step 2: Synthesis of 5a-5g from 3a-3d

As represented in **Step 2 of Scheme 1** the Schiff bases (3a-3d) obtained from 4-aminoantipyrine and aryl aldehyde (1 mmol) and benzoic hydrazide, phenyl hydrazine, 2,4-dinitrophenyl hydrazine, thiosemicarbazide (1 mmol) were dissolved separately in 3 cm³ ethanol (co-solvent) and mixed together then a catalytic amount of DES (2 g) was added and the reaction mixture was stirred at room temperature for about 20 minutes. After completion of reaction ice cold distilled water was added to it and the product obtained were filtered and dried. The progress of reaction was monitored by TLC using ethyl acetate: n-hexane (2:8) as a solvent system. The results are depicted in **Table 4**.



Scheme 1. Synthesis of Schiff bases of 4-amino antipyrine.

Table 4: Synthesis of 5a-5g

Entry	3	4	Product	Time (min)	Yield (%) ^(b)
1	3a	C ₇ H ₈ N ₂ O	5a	15	65
2	3b	C ₇ H ₈ N ₂ O	5b	15	67
3	3c	C ₇ H ₈ N ₂ O	5c	15	65
4	3d	C ₇ H ₈ N ₂ O	5d	15	65
5	3a	C ₆ H ₈ N ₂	5e	18	66
6	3a	C ₆ H ₄ N ₂ O ₅	5f	20	60
7	3a	CH ₄ N ₃ S	5g	18	66

^(a)Reaction conditions: a mixture of 1 mmol of 3a-3d, 1 mmol benzoic hydrazide, 5.0 mol % of DES, 3 cm³ alcohol at room temperature.
^(b)isolated yields

3a: 4-(benzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one

Pale yellow solid, m.p.176°C, Yield: (98 %), ¹H NMR (DMSO, 600MHz δ): 9.61(1H), 7.84 to 7.39 (10H), 3.20(3H), 2.48(3H), ¹³C NMR (DMSO,600 MHz δ in ppm); 10.22, 35.79, 116.74, 125.11,127.40,127.69, 129.21, 129.63 130.63, 135.02,137.39 152.66, 154.81, 160.16 , Mass Spectrum m/z 290.5(M-1).

3b: 4-((2-bromobenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one

Pale yellow solid, m.p.190°C, Yield: (98 %), ¹H NMR (DMSO, 600MHz δ): 9.93(1H), 8.17 to 7.36(9H), 3.24(3H), 2.50(3H), ¹³C NMR (DMSO,600 MHz δ in ppm); 10.18, 35.88, 116.60, 124.83, 125.40, 127.59, 128.40, 129.64, 132.14, 133.60, 134.83,136.23 152.70, 152.90, 159.78 , Mass Spectrum m/z 371.4(M+1).

3c: 4-((3-bromobenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one

Pale yellow solid, m.p.200°C, Yield: (99%), ¹H NMR (DMSO, 600MHz δ): 9.95(1H), 8.03 to 7.39 (9H), 3.23(3H), 2.49(3H), ¹³C NMR(DMSO,600MHzδinppm);10.23,35.56,122.76,125.33,127.14,127.64, 129.30,129.66,131.42,133.03,134.90,140.47,152.59, 152.79,159.86, Mass Spectrum m/z 369.3(M-1).

3d: 4-((4-bromobenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-one

Pale yellow solid, m.p.223°C, Yield: (99%), ¹H NMR (DMSO, 600MHz δ): 9.57(1H), 7.79 to 7.39(9H), 3.21(3H), 2.48(3H), ¹³C NMR(DMSO,600MHzδinppm);10.17,35.64,110.58,123.84,125.31,127.57,129.48, 129.66,132.22,134.89,137.21,152.63,153.22,159.90, Mass Spectrum m/z 371.4(M+1).

5a : (Z)-N'-(4-((Z)-benzylideneamino)-1,5-dimethyl-2-phenyl-1Hpyrazol-3(2H)-ylidene)benzohydrazide:

White solid, m.p.207°C, Yield: (65%), ¹H NMR (DMSO, 600MHz δ): 11.83(1H), 8.45(1H), 7.91 to 7.42(15H), ¹³C NMR (DMSO,600 MHz δ in ppm: 163.65,157.11, 149.05,148.30,134.80,133.88,132.25,132.09, 131.00,130.58,130.04,129.33,128.97,128.50, 128.08,127.58.105.94 Mass Spectrum m/z 408.3(M-1).

5b:N'-(4-((2-bromobenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)benzohydrazide:

White solid, m.p.:210°C, Yield:(67%), ¹H NMR (DMSO, 600MHz δ): 12.10(1H), 8.82(1H),8.01 to 7.35 (14H), ¹³C NMR (DMSO,600 MHz δ in ppm: 163.97,163.67,146.68,146.48,131.81,133.66,133.59,132.42, 132.23,129.26,129.23,128.98,128.73,128.60,128.17,127.76,124.06,121.93, 110.33, Mass Spectrum m/z 489.5(M+1).

5c : (Z)-N'-(4-((Z)-(3-bromobenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)benzohydrazide:
White solid, m.p.:170 °C, Yield:(65%), ¹H NMR (DMSO, 600MHz δ):11.97 (1H), 8.41(1H), 7.91 to 7.40 (14H),
¹³C NMR (DMSO,600 MHz δ in ppm): 163.79,163.25,146.58,146.46,
137.29, 133.68,133.07,132.49,132.38,131.50,129.73,129.69,129.58,129.18,128.99,128.14,126.73,122.66,108.93,
Mass Spectrum m/z 489.5 (M+1)

5d : (Z)-N'-(4-((Z)-(4-bromobenzylidene)amino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H)-ylidene)benzohydrazide:
White solid, m.p.179°C, Yield: (65%). ¹H NMR (DMSO, 600MHz δ): 11.90(1H), 8.42(1H), 7.90 to 7.50 (14H),
¹³C NMR (DMSO,600 MHz δ in ppm); 163.68,163.58,147.01,144.36,134.10,133.77,
132.50, 132.34,129.95,129.43,129.27,129.15,128.98,128.11,128.01,123.78,108.61 Mass Spectrum m/z 487.5 (M-1).

5e: (NZ)-N-benzylidene-1,5-dimethyl-2-phenyl-3-(2-phenylhydrazono)-2,3-dihydro-1H-pyrazol-4-amine :
White solid, m.p.154°C: Yield: (66%), ¹H NMR (DMSO, 600MHz δ): 10.30(1H), 7.84(1H), 7.63 to 6.71(15H),
¹³C NMR (DMSO,600 MHz δ in ppm);145.20, 136.41, 135.74, 129.33, 129.07, 128.90, 128.78, 128.60,128.01, 127.88, 125.70,
125.54, 118.72,118.65, 111.92,108.15, Mass Spectrum m/z 382.5 (M+1)

5f : (3Z,NZ)-N-benzylidene-3-((4-chlorophenyl)imino)-1,5-dimethyl-2-phenyl-2,3-dihydro-1H-pyrazol-4-amine:
Reddish solid, m.p.290°C, Yield: (60%), ¹H NMR (DMSO, 600MHz δ): 11.65(1H), 8.84(1H), 8.69 to 7.47(13H)
¹³C NMR (DMSO,600 MHz δ in ppm); 162.31, 149.38,144.45,137.04,133.76,
130.51,129.72,129.66,129.46,128.92,128.86,127.32,127.18,122.92,116.74, Mass Spectrum m/z 472.5 (M+1).

5g(E)-2-(4-((E)-benzylideneamino)-1,5-dimethyl-2-phenyl-1H-pyrazol-3(2H) ylidene)hydrazinecarbothioamide
Light yellow solid, m.p.166 °C, Yield: (66%), ¹H NMR (DMSO, 600MHz δ): 11.40(1H), 9.55(1H), 8.17 (2H), 7.95 to 7.33(10H),
3.30(3H), 2.42(3H) ¹³C NMR (DMSO,600 MHz δ in ppm); 178.00, 159.57, 154.32, 152.15, 142.22, 134.15, 130.09, 129.76, 129.09,
128.59, 128.68, 127.24, 122.17, 124.57, 116.29, 35.50, 9.71, Mass Spectrum m/z 365.4 (M+1).

ANTIBACTERIAL ACTIVITY

The newly synthesized derivatives of 4-aminoantipyrine (5a-5g) were screened against two gram-positive bacterial strains (*S. aureus* and *B. Subtilis*) and two gram-negative bacterial strains (*E. coli* and *P. aeruginosa*) using Streptomycin as the standard drug. The MIC values of the novel derivatives in comparison with standard drugs for correlation are presented in the Figure 3. Among the screened derivatives, 5a demonstrates significant inhibition activity against *P.aeruginosa* and *B. Subtilis* and 5c shows significant inhibition activity against *B. Subtilis* as shown in **Figure 1**. The MIC value of standard drug is 62.5 µg/ml.

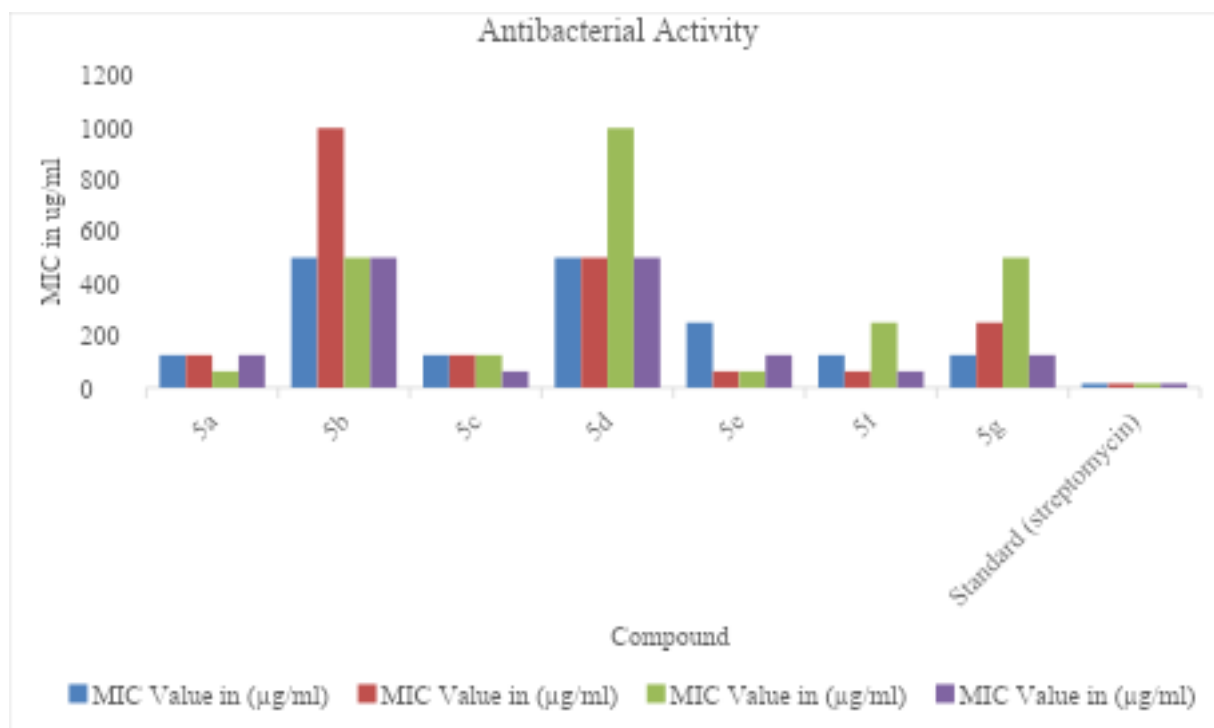


Figure 1: Graphical Representation against antibacterial activity

ANTIFUNGAL ACTIVITY

The in vitro antifungal effectiveness of the novel derivatives (5a-5g) was evaluated using the broth dilution method and the MIC values were measured in µg/ml. The derivatives were tested against the fungal strain *C. albicans* and *A. niger* with fluconazole as a standard. The MIC values of the new derivatives were reported in Figure 4. Among the screened derivatives 5a, 5c demonstrate notable inhibition activity against *C.albicans* and *A.niger* as represented in **Figure 2** The MIC value of standard drug is 15.62 µg/ml.

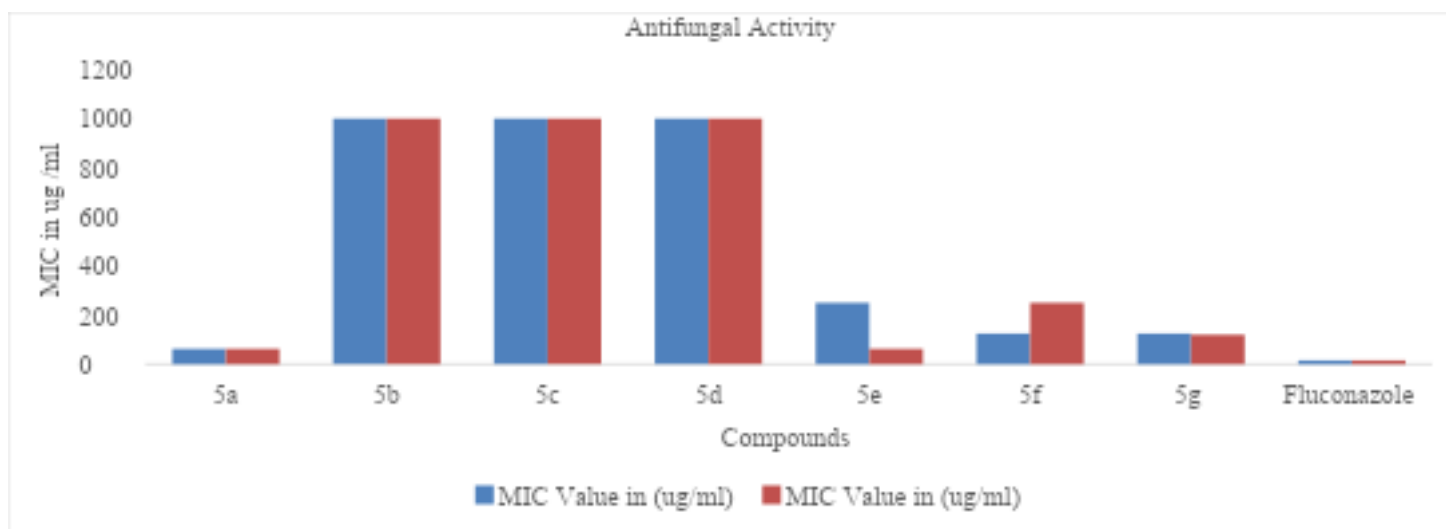


Figure 2: Graphical Representation against antifungal activity

COMPUTATIONAL STUDY

MOLECULAR DOCKING

Molecular docking studies were performed to evaluate the binding affinity and interaction patterns of the synthesized compounds against *E. coli* DNA Gyrase B (PDB ID: 6YD9) and *Candida albicans* lanosterol 14- α demethylase (PDB ID: 5TZ1). The docking calculations were carried out using the Glide module of the Schrödinger suite. The ligands were prepared using the LigPrep tool, where low-energy three-dimensional conformations were generated. Possible stereoisomers, ionization states and tautomeric forms were considered at physiological pH (7.0 ± 0.2). Energy minimization was performed using the OPLS force field to obtain stable ligand geometries. Protein structures were prepared using the Protein Preparation Wizard. This involved the addition of hydrogen atoms, assignment of bond orders and correction of missing side chains and loops wherever required. Protonation states of amino acid residues were predicted using PROPKA at pH 7.0. The hydrogen-bonding network was optimized followed by restrained energy minimization to remove steric clashes while preserving the overall protein structure. Subsequently, receptor grids were generated around the active site of each protein and docking was performed using Glide to predict binding modes and calculate docking scores for all synthesized compounds[30–32].

MOLECULAR DYNAMIC (MD) SIMULATION

Molecular dynamics (MD) simulations were carried out to evaluate the stability of the most promising compound, 5g, in complex with E6YD9 and 5TZ1 using the Desmond simulation package. Each protein–ligand complex was placed in an orthorhombic simulation box with a 10 Å buffer distance and solvated using the SPC water model. The systems were neutralized by adding appropriate counter ions and an ionic strength of 0.15 M NaCl was maintained to mimic physiological conditions. Energy minimization was performed to remove unfavorable contacts, followed by system equilibration using a short NVT ensemble and subsequent NPT equilibration. The production MD simulations were then conducted for 100 ns under NPT conditions at 300 K and 1 atm pressure. Temperature and pressure were maintained using the Nose–Hoover thermostat and Martyna–Tobias–Klein barostat, respectively, with a time step of 2 fs. Trajectory analyses were performed to assess root mean square deviation (RMSD), root mean square fluctuation (RMSF), hydrogen bond interactions and overall conformational stability of the complexes throughout the simulation period[33,34].

COMPUTATIONAL QUANTUM CHEMICAL ANALYSIS

Quantum chemical calculations for compounds 5a–5g were carried out using the Jaguar module. All structures were geometry-optimized employing the B3LYP functional with the 6-31G** basis set, integrating Becke's three-parameter exchange with the Lee–Yang–Parr correlation function. The optimized molecules were further analyzed for their frontier molecular orbital energies (HOMO and LUMO) to assess their electronic characteristics. In addition, global reactivity parameters were derived to provide insight into their chemical stability, charge transfer behavior, and overall reactivity profile [35,36].

III. RESULT AND DISCUSSION

The reaction of 1 mmol of 4-aminoantipyrine, 1 mmol of aryl aldehyde and 2.0 g of DES was chosen as a model reaction to optimize the reaction parameters as shown in **Scheme 1**. Initially, it was observed that the expected schiff base(derivative) product was not obtained in good yield when the reaction was conducted in the absence of DES (**Table 1, Entry 1**). Various deep eutectic solvents were employed at room temperature yielding the desired product in low amounts, as summarized in **Table 1**. Notably, ethylene glycol-based deep eutectic solvent composed of ammonium chloride, ethylene glycol and urea in a 1:5:4 ratio, proved to be an efficient solvent and reaction medium delivering a remarkably high product yield (**Table 1, Entry 16**). This deep eutectic solvent plays a crucial role in converting all reactants into the desired product. Fortunately, the reaction proceeds efficiently at room temperature yielding excellent results within just 11 minutes. The optimization of temperature is represented in **Table 2**. To determine the optimal concentration of deep eutectic solvent (DES), we investigated the model reaction using 0.5 g, 1.0 g, 1.5 g, 2.0 g, 2.5 g, and 3.0 g of ethylene glycol, urea ammonium chloride in a 5:4:1 ratio at room temperature. The corresponding product yields were 40%, 65%, 81%, 98%, 98%, and 98% respectively. These results indicate that 2.0 g of DES is sufficient to drive the reaction forward. In the absence of DES, the reaction did not produce a good yield. After optimizing these conditions, the methodology was applied to the synthesis of various benzylideneamino-1H-pyrazol-3(2H)-ones using different aryl aldehydes (1 mmol) and 4-aminoantipyrine (1 mmol). The results are summarized in **Table 3**. We also investigated the recyclability of DES for the model reaction involving 1 mmol of 4-aminoantipyrine and 1 mmol of aryl aldehyde under optimized reaction conditions, with the reaction progress monitored by TLC. Scale up and recycling studies were wonderful for the model reaction of 4-aminoantipyrine

(1 mmol) and benzaldehyde (1 mmol) in 2.0 g DES under the optimized reaction conditions (**Table 1, Entry 16**). The DES was recycled up to nine times without significant loss in activity or efficiency, consistently yielding high product outputs as shown in **Figure 3**.

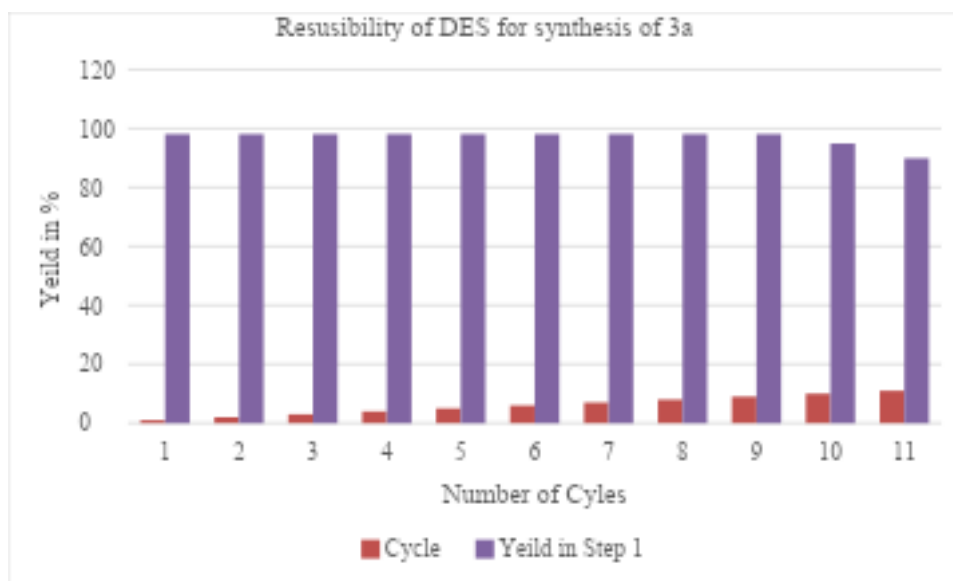


Figure 3: Reusability of DES in Step 1 of Scheme 1

We expanded our research to develop structurally diverse and biologically active heterocyclic compounds. However, under the optimized conditions, the reaction did not yield to the expected product in the **Step 2 of scheme 1**. By incorporating alcohol as a co-solvent (3 cm³) in the second step, we achieved an excellent yield of the desired product. The deep eutectic solvent (DES) (2 g), composed ethylene glycol, urea and ammonium chloride in a 5:4:1 ratio proved to be an efficient catalyst, with alcohol serving as a co-solvent for the second step. Using Schiff bases (1 mmol) (3a-3d) and benzoic hydrazide, phenyl hydrazine, 2,4-dinitrophenyl hydrazine, thiosemicarbazide (1 mmol) in the presence of DES and alcohol as a co-solvent, we successfully synthesized novel derivatives (5a-5g) as shown in **Table 4**. The reaction exhibited a clean profile with no detectable side products. This protocol presents several advantages, including the ease of preparation and handling of DES, cost-effective DES components and straightforward separation and purification processes making it an efficient approach for synthesizing structurally diverse and biologically active derivatives of 4-aminoantipyrene. Furthermore, DESs are biodegradable and non-toxic. The reaction was successfully scaled up to 5 g using a representative reaction. In the first step, The DES can be reused up to nine times. The deep eutectic solvent (DES) employed in this protocol demonstrates excellent recyclability. The thermal and spectroscopic analyses confirm the successful preparation of structurally diverse 4-aminoantipyrene derivatives (3a-3d and 5a-5g). The UV-Vis, IR, and NMR studies provide strong evidence for the formation of desired Schiff bases and the condensation between 3a-3d and hydrazide derivatives. The thermal analysis reveals their stability and non-hygroscopic nature making them potential candidates for further applications in medicinal and material chemistry.

IR SPECTRA

The IR spectra was recorded on ATR from Bruker and are depicted in **Table 5** The Azomethine (N=C) stretching vibration of 5a-5d novel derivative ranging from 1537 to 1599 cm⁻¹. The disappears of peak at 3431 cm⁻¹ due to -NH₂ group of 4-aminoantipyrene and appears of peak in the range of 1552 to 1563 cm⁻¹ of schiff base 3a-3d clearly indicates the condensation of 4-aminaoantipyrene and aryl aldehyde. The stretching frequency due to -C=O of 4-aminoantipyrene in the range of 1636 to 1647 cm⁻¹ disappears and new stretching frequency appears from 1639 to 1649 cm⁻¹ in the derivatives 5a -5d which indicates the condensation of -C=O of 4-aminoantipyrene with -NH₂ group of hydrazides. Sharp peak in the range from 2901 to 2940 cm⁻¹ indicates the presence of C-CH₃ group of 4-aminoantipyrene moiety. The C-Br stretching frequency of 3b,3c,3d,5b,5c,5d ranging from 578 to 594 cm⁻¹. The peaks appear in the range of 3311 to 3285 cm⁻¹ due to N-H stretching vibration in all novel derivatives. The derivative 5g shows peak at 1533.75 and 1331.11 cm⁻¹ due to symmetric and asymmetric stretching vibration of nitro group of 2,4-dnitrophenyl hydrazine derivatives respectively. The C=S frequencies of 5f includes 13338.13,1305.73,1128.51,1068.89,832.20,767.08 cm⁻¹ due to stretching, bending and symmetric stretching vibrations respectively.

Table 5: FTIR spectra of 3a-5g

Derivative	$\nu(\text{N-H})$ cm ⁻¹	$\nu(\text{Ar-CH})$ cm ⁻¹	$\nu(\text{Al-CH})$ cm ⁻¹	$\nu_{\text{py}}(\text{C=O})$ cm ⁻¹	$\nu_{\text{hydrazide}}(\text{C=O})$ cm ⁻¹	$\nu(\text{C=N})$ cm ⁻¹	(Ar-C=C) cm ⁻¹	C-Br cm ⁻¹	-NO ₂ As/sy	C=S,C+N/ C=S(sy)/ C=S(ben)
4-AAP	3431.69	3311.82	2989.45	1636.84	-	-	1494.33	-	-	-
3a	-	3037.50	2940.41	1647.77	-	1563.10	1479.47	-	-	-
3b	-	3063.56	2921.86	1647.10	-	1561.92	1483.17	594.18	-	-

3c	-	2965.05	2934.12	1641.89	-	1552.68	1469.14	593.4 5	-	-
3d	-	3056.45	2939.42	1644.89	-	1568.29	1480.95	584.8 2	-	-
5a	3195.21	3058.04	2902.48	-	1639.67	1576.20	1482.56	-	-	-
5b	3187.44	3056.66	2902.31	-	1644.02	1597.79	1467.05	578.0 8	-	-
5c	3196.24	3057.35	2902.34	-	1646.75	1599.85	1472.77	578.3 8	-	-
5d	3273.04	3056.34	2901.51	-	1649.75	1537.00	1470.65	580.0 6	-	-
5e	3311.81	3026.52	-	-	-	1591.47	1492.75	-	-	-
5f	3285.05	3101.84	-	-	-	1585.82	1487.10	-	1533.75 / 1331.11	-
5g	-	3038.82	-	-	-	1594.99	1481.79	-	-	1338.13,1305.73 / 832.20,767.08/ 1128.51,1068.89

NMR SPECTRA

¹H NMR spectra was recorded on Bruker spectrometer at 600 MHz in DMSO as solvent and chemical shift recorded in ppm. Aromatic protons of 5a (m,15 H) show peaks in the range of 7.42 to 7.91 ppm, 5b (m,14 H) shows peak in the range of 7.35 to 8.01 ppm, 5c (m,14 H) shows peak in the range of 7.40 to 7.91 ppm, 5d (m, 14H) peak in the range of 7.50 to 7.90 ppm, 5e (m, 15H) peak in the range of 6.63 to 6.71 ppm, 5f (m, 13H) peak in the range of 7.47 to 8.69 ppm, 5g (m, 10H) peak in the range of 7.33 to 7.95 ppm. Imine proton of 5a-5g derivatives in shows peak in the range of 8.84 to 9.55 and N-H proton of hydrazide shows peak in the range of 10.30 to 12.30 ppm. The conclusions drawn from these studies further support the mode of bonding discussed in their FTIR spectra of novel derivatives. ¹³C NMR spectra were recorded on Bruker spectrometer at 600MHz in DMSO as solvent and chemical shift recorded in ppm. ¹³C NMR spectra of 5a-5d shows disappears of peak of C due to C=O of carbonyl of 4-aminoantipyrene peak of C due to -C=O of hydrazide in the range of 162 to 163 ppm indicates the condensation of carbonyl group of 4-aminoantipyrene with benzoic hydrazide. ¹³C NMR of 5g shows peak at 178.00 ppm due to C of C=S. The disappearance of N-CH₃ and C-CH₃ peaks in the novel derivatives of 5a to 5f as compare to 3a-3d may be due to electronic effect, intramolecular H-bonding, dynamic conformation and structural rigidity. To confirm intramolecular hydrogen bonding in the derivatives 5a-5d confirmed by the peak in the IR spectra in the region of 3195 to 3196 cm⁻¹. D₂O exchange was also done for molecule 5a in which N-H peaks disappear which confirm its lability. In the derivatives 5e-5g in which absence of intramolecular H-bonding which was due to absence of carbonyl group of hydrazide, hence H-bonding is not major factor but dynamic conformation and structural rigidity is the major factor. To confirm dynamic conformation and structural rigidity the NMR spectra was run in CDCl₃ from -25°C to +25 °C where the same peaks N-CH₃ and C-CH₃ did not observed. To study further we had synthesized the derivative 5g, whose NMR shows the appearance of peaks N-CH₃ and C-CH₃ which confirms the H-bonding is not the major factor because its contain less electronegative Sulphur atom as compared to Oxygen atom, which is less H acceptor. So from all evidences we conclude that the derivatives 3a-3d shows sharp peaks in NMR due to limited conjugation on one side, absence of intramolecular hydrogen bonding and less sterically hindered conformation and lack of planarity. The novel derivatives 5a to 5f due to the bulky nature of aromatic hydrazides which lock the conformation and structure becomes rigid, planar so the molecule tumble slowly due to which the signal becomes weak or even broad which cannot be detected by NMR instrument. The rigidity also explained by hypsochromic shift of 5a than 3a.

UV-VISIBLE SPECTRA

The electronic absorption spectra of derivatives of 4-aminoantipyrene were recorded on Perkin Elmer Lambda 365 instrument from 200 nm to 700 nm at 298 K in dimethyl sulphoxide as solvent at concentration of 10 ppm in quartz cell of 1 cm path length. The electronic spectra of novel derivatives show broad band due to Π to Π^* transition of azomethine Chromophore and C=C present in derivative. 3a(325.17nm), 3b(305.77nm), 3c (304.74), 3d(305.73nm), 5a (273.30nm), 5b (276.43 nm) 5c (273.25 nm), 5d (276.60 nm), 5e (352.50 nm), 5f (394.00nm), 5g (340.50 nm).

THERMAL ANALYSIS

Thermal analysis of novel derivatives was carried out on Thermogravimetric analyzer TGA 4000 under the atmosphere of nitrogen (20 ml per minute) from 30°C to 800 °C with heating rate of 10°C per minute. Thermal data reveals that the novel derivatives of 4-aminoantipyrene were thermally stable and non hygroscopic in nature[23,37]. All the novel derivatives 5a-5d shows sharp endothermic peak. All the novel derivatives do not lose any weight loss below 200°C which indicates that there is no water of

crystallisation or no physical impurities. The decomposition temperature of novel derivatives are as follows: 5a, 307.10°C, 5b, 292.18°C, 5c, 339.36°C, 5d, 335.47°C, 5e 258.70°C, 5f 313.07°C, 5g 310.00 °C.

MOLECULAR DOCKING ANALYSIS

Docking analysis were conducted against *E. coli* DNA Gyrase B (PDB ID: 6YD9) and *C. albicans* lanosterol 14 α -demethylase (CYP51, PDB ID: 5TZ1) to comprehend the binding affinity and molecular interactions of the 4-aminoantipyrene derivatives. **Table 6** displays the docking score. Because of their established roles in bacterial DNA replication and fungal ergosterol biosynthesis, respectively, *E. coli* DNA Gyrase B (PDB ID: 6YD9) and *C. albicans* lanosterol 14 α -demethylase (CYP51, PDB ID: 5TZ1) were chosen as molecular targets to explain the antimicrobial potential of the synthesized 4-aminoantipyrene derivatives. Both enzymes are desirable and proven targets for the development of antimicrobial drugs because inhibition of DNA Gyrase B disturbs DNA supercoiling, resulting in bacterial cell death, while inhibition of CYP51 compromises fungal membrane integrity[38,39]. In contrast to the co-crystal ligand score of -7.982 kcal/mol, molecular docking investigations against DNA Gyrase B showed binding energies ranging from -3.175 to -6.64 kcal/mol. The compound **5g** had the greatest binding affinity (-6.64 kcal/mol), which was close to that of the reference ligand. Compounds **5e** (-5.066 kcal/mol) and **5f** (-5.083 kcal/mol) had modest scoring. While compounds **5b** and **5d** showed poor affinity, compounds **5a** and **5c** showed intermediate binding. Correlation with antibacterial activity against *E. coli* (MIC range: 125–500 g/mL) showed a satisfactory agreement with docking findings, while compound **5g** demonstrated excellent binding (MIC = 125 g/mL), consistent with its strong binding. While weakly docked compounds **5b** and **5d** showed decreased activity (MIC = 500 μ g/mL), compounds with intermediate docking scores, such **5a**, **5c**, and **5f**, also shown comparable activity. Despite excellent docking, compound **5e** showed relatively lesser activity (MIC = 250 g/mL), indicating the effect of other variables.

Table 6: In silico binding energies (kcal/mol) of synthesized 4-aminoantipyrene derivatives with *E. coli* DNA Gyrase B (6YD9) and *C. albicans* lanosterol 14 α -demethylase (5TZ1).

Compounds	<i>E. coli</i> DNA Gyrase B (PDB ID: 6YD9)	<i>C. albicans</i> CYP51 (PDB ID: 5TZ1)
5a	-4.024	-7.247
5b	-3.283	-8.648
5c	-4.037	-6.147
5d	-3.175	-6.532
5e	-5.066	-7.279
5f	-5.083	-5.022
5g	-6.64	-8.94
Co-crystal ligand	-7.982	-8.978

Azole antifungal medicines primarily target sterol 14-demethylase (CYP51), a cytochrome P450 enzyme necessary for the synthesis of sterol in eukaryotic cells. Fungal infections, especially those caused by *Candida albicans*, are often treated with azole inhibitors that are selective for the fungal enzyme over the human counterpart. The co-crystal ligand score 8.978 kcal/mol in docking experiments against CYP51, which showed binding energies ranging from -5.022 to -8.94 kcal/mol. Once again, compound **5g** showed the highest binding affinity (-8.94 kcal/mol), which was almost exactly the same as the reference ligand.

Compound **5b** came in second (-8.648 kcal/mol). While compounds **5c**, **5d**, and **5f** had lesser interactions, compounds **5a** and **5e** showed strong binding. Docking studies showed a limited connection with antifungal activity (MIC range: 62.5–1000 g/mL). Compound **5a** showed the greatest activity (MIC = 62.5 μ g/mL) despite considerable docking, whereas compound **5g** had excellent antifungal activity (MIC = 125 μ g/mL), suggesting its significant binding affinity. Additional information about the most promising compound **5g**'s inhibitory ability against both targets was revealed by binding interaction analysis. Compound **5g** showed significant hydrogen bonding interactions in *E. coli* DNA Gyrase B, where the terminal nitrogen of the thiourea moiety formed hydrogen bonds with residues Asp49 and Asn46, which are proximal to the ATP-binding region necessary for enzyme catalysis. Interestingly, the co-crystallized ligand is known to interact with important residues including Asp73, Arg76, Gly77, Gly101, and Arg136, which are essential for maintaining the ATPase pocket via electrostatic and hydrogen bonding interactions. Compound **5g** engages with residues close to the catalytic area, suggesting a similar binding orientation, which might interfere with ATP hydrolysis and prevent DNA supercoiling and bacterial replication (**Figure 4A**). The compound **5g** showed promising hydrophobic interactions inside the active region of the fungus lanosterol 14-demethylase (CYP51), which is its target. While the phenyl moiety showed π -cation interactions with the heme iron present in the catalytic core, the pyrazole ring engaged in π -stacking interactions with the adjacent hydrophobic residue Tyr132, which contributed to binding stability. Since coordination or interaction with the heme group is a well-known mechanism of CYP51 inhibition, this interaction is very important (**Figure 4B**). These combined interactions demonstrate compound **5g**'s ability to interfere with ergosterol synthesis in fungal cells and support the compound's considerable docking affinity.

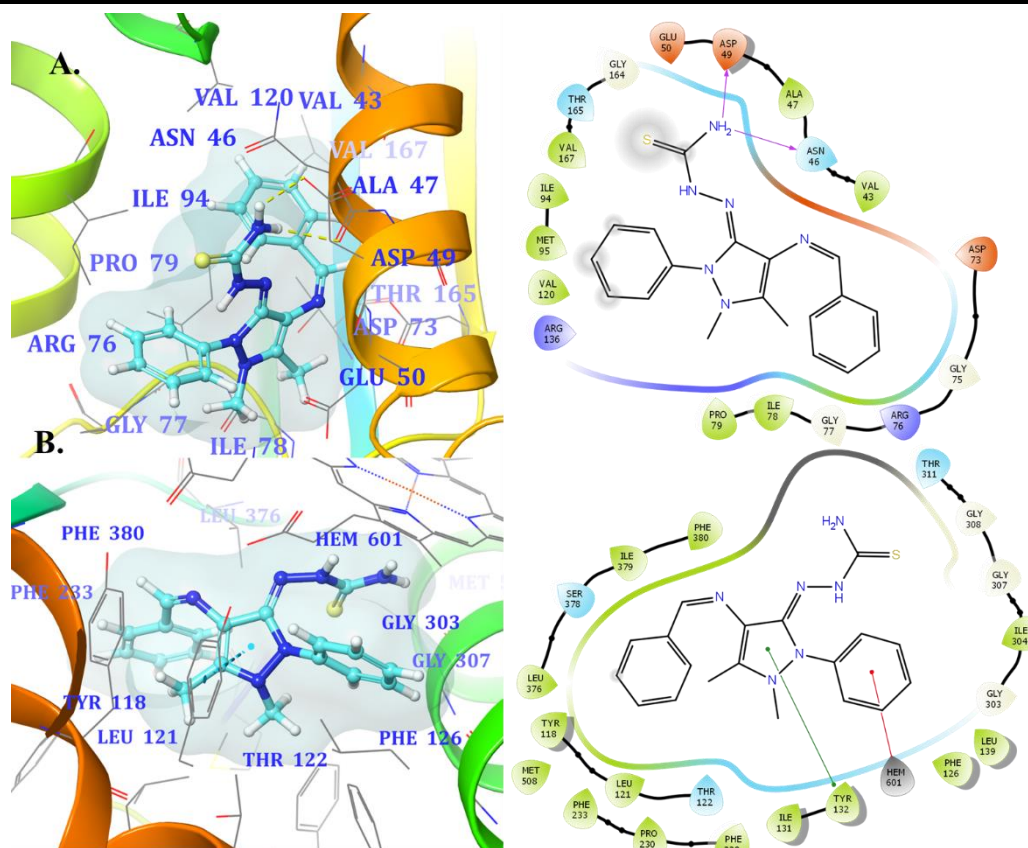


Figure 4: 2D and 3D interaction diagrams of Compound **5g** within the active site of **A. E. coli** DNA Gyrase B (PDB ID: 6YD9) and **B. C. albicans** Lanosterol 14 α -demethylase (PDB ID: 5TZ1)

MOLECULAR DYNAMICS SIMULATION STUDY

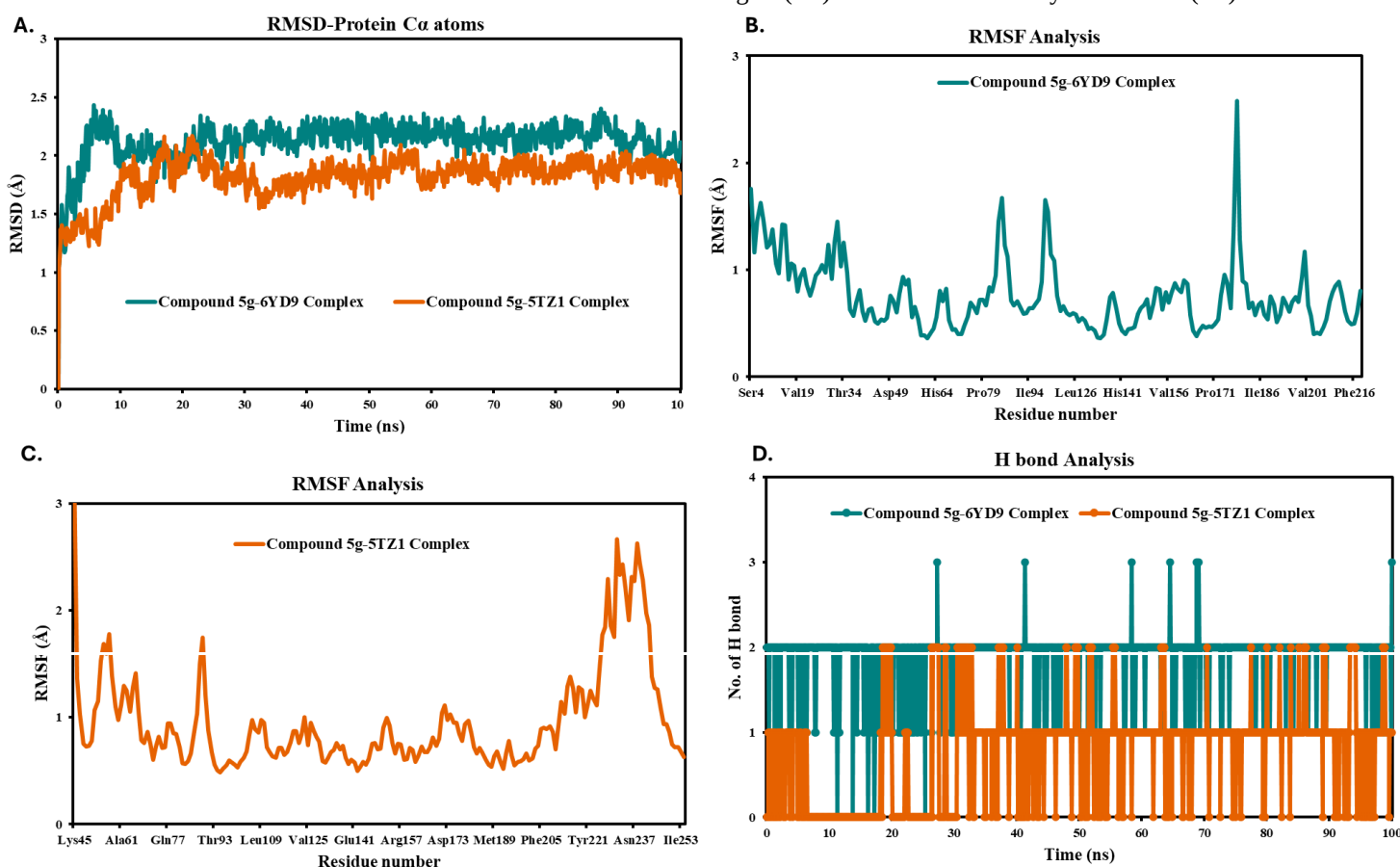
Molecular dynamics simulations were performed to investigate the stability and dynamic behavior of compound **5g** in complex with *E. coli* DNA Gyrase B (6YD9) and *C. albicans* lanosterol 14 α -demethylase (5TZ1) under physiological circumstances throughout a 100 ns simulation period. Beyond the static perspective from molecular docking, this method enables evaluation of how well the ligand stays accommodated inside the binding pocket throughout time. The complexes structural stability was monitored using the root mean square deviation (RMSD) of C α atoms. RMSD measures protein backbone variations from its original shape and is often used to assess equilibration and conformational consistency. The **5g**-6YD9 complex had an average RMSD of 2.12 Å and a maximum deviation of 2.43 Å, indicating substantial but tolerable oscillations throughout the simulation. The **5g**-5TZ1 complex had a lower average RMSD of 1.82 Å and maximum of 2.17 Å, indicating stronger structural stability. In both systems, the trajectories stabilized after the first equilibration phase and remained consistent with no sudden departures, indicating that compound **5g** possesses a stable binding mode inside the active site. The 5TZ1 complex has lower RMSD values, indicating a more stable structural arrangement than the 6YD9 system. To assess residue-level flexibility throughout the simulation, the root mean square fluctuation (RMSF) analysis was performed. RMSF offers information on the mobility of individual amino acid residues, with lower values indicating structural stiffness and greater values corresponding to flexible areas like loops or terminal segments. The **5g**-6YD9 complex had an average RMSF of 0.77 Å and a maximum variation of 2.58 Å, indicating that most residues were stable with little localized mobility. The **5g**-5TZ1 complex had a slightly higher average RMSF of 0.87 Å and a maximum of 3.21 Å, indicating marginally enhanced flexibility in certain locations. Importantly, residues inside the active site in both complexes showed very modest variations, allowing for sustained ligand binding throughout the simulation period. Interaction analysis revealed that compound **5g** had long-term interactions with critical residues in both targets throughout the simulation. The 6YD9 complex exhibited interactions with Val43, Asp45, Asn46, Ala47, Asp49, Glu50, Val71, Asp73, Arg76, Gly77, Ile78, Pro79, Ile94, Met95, His99, Gly117, Gly119, Val120, Thr165, Val167, and Arg209. Several of these residues, particularly Asp45, Asp49, and Asp73, are linked to the ATP-binding domain, indicating that the catalytic pocket is highly stable. Tyr118, Leu121, Val125, Phe126, Ile131, Tyr132, Phe228, Phe233, Met306, Gly307, Gly308, His310, Thr311, Leu376, His377, Ser378, Phe380, Cys470, Met508, and Val509 all interact with compound **5g** in the 5TZ1 complex. The persistence of these connections throughout the simulation indicates strong ligand accommodation and binding. Overall, the RMSD and RMSF profiles indicate that both complexes are stable under simulated physiological conditions, with no significant conformational changes. The slightly improved stability of the 5TZ1 complex, combined with consistent residue interactions in both systems, supports compound **5g**'s potential as a promising lead with dual antibacterial and antifungal activity, thereby increasing the dependability of the docking data through dynamic validation. Hydrogen bond analysis was performed to understand better the strength and persistence of polar interactions between compound **5g** and the target proteins. Hydrogen bonds are important contributors to ligand binding specificity and stability because they anchor the ligand to the active site and reduce conformational drift during simulation. The number of hydrogen bonds in the **5g**-6YD9 complex ranged from a maximum of 3.00 to an average of 1.87, indicating frequent and stable polar interactions throughout the simulation. This higher average indicates that compound **5g** forms consistent hydrogen bonds with key active site residues. According to the interaction profile, residues such as Asn46 and Asp49 make significant contributions, with high interaction fractions indicating persistent hydrogen bond formation. These residues are located in or near the ATP-binding region, demonstrating compound **5g**'s ability to effectively stabilize itself within the catalytic pocket. The presence of multiple hydrogen bonds along the trajectory promotes strong ligand retention and adds to the RMSD stability observed for this complex. In contrast,

the **5g**-5TZ1 complex had a maximum of 2.00 hydrogen bonds and a lower average of 0.79, indicating fewer and less persistent hydrogen bonding interactions. Although hydrogen bonding is present, it occurs frequently rather than consistently. Notably, THR 311 appears to make a significant contribution to hydrogen bond formation, as evidenced by its higher interaction fraction. However, the overall lower average indicates that compound **5g**'s binding in this complex is influenced by other interaction types rather than hydrogen bonding. The interaction profile supports this observation, with hydrophobic residues such as Tyr118, Phe126, Tyr132, Phe228, and Phe380 making significant contributions, indicating that hydrophobic interactions dominate the 5TZ1 complex's stability. Furthermore, the presence of ionic interaction with Cys470 suggests alternative stabilization mechanisms that compensate for the lower hydrogen bond count. Overall, the hydrogen bond analysis shows that the **5g**-6YD9 complex is stabilized through consistent and multiple hydrogen bonding interactions, which contribute to strong and specific binding within the active site. In contrast, the **5g**-5TZ1 complex is more dependent on hydrophobic and auxiliary interactions, with hydrogen bonds playing a supporting but less dominant role. These differences highlight compound **5g**'s distinct binding mechanisms across the two targets while maintaining overall complex stability, as evidenced by the RMSD and RMSF results as shown in **Figure 5**.

QUANTUM CHEMICAL ANALYSIS

To better understand the electronic properties and reactivity profile of the synthesized compounds (5a-5g), quantum chemical calculations were performed using density functional theory (DFT). Frontier molecular orbital (FMO) analysis, combined with global reactivity descriptors, provides information about chemical stability, charge transfer ability, and potential interactions with biological targets as shown in **Table 7**.

Table 7: DFT-Based Frontier Molecular Orbital Energies (eV) and Global Reactivity Parameters (eV)



$$\Delta E = E_{LUMO} - E_{HOMO}; IP = -HOMO; EA = -LUMO; \eta = (IP - EA) / 2; \mu = -(IP + EA) / 2; \chi = (IP + EA) / 2; S = 1 / 2\eta; \omega = \mu^2 / 2\eta$$

Code	5a	5b	5c	5d	5e	5f	5g
HOMO	-0.171	-0.175	-0.176	-0.175	-0.149	-0.185	-0.170
LUMO	0.037	0.073	0.054	0.043	-0.064	0.078	0.056
ΔE	0.133	0.102	0.122	0.132	0.086	0.107	0.114
Ionization potential (IP)	0.171	0.175	0.176	0.175	0.149	0.185	0.170
Electron affinity (EA)	0.037	0.073	0.054	0.043	0.064	0.078	0.056
electronegativity (X)	0.104	0.124	0.115	0.109	0.106	0.131	0.113
Chemical hardness (η)	0.067	0.051	0.061	0.066	0.043	0.054	0.057

Chemical softness (S)	7.496	9.818	8.182	7.599	11.684	9.305	8.760
Chemical potential (μ)	-	-	-	-	-0.106	-	-
	0.104	0.124	0.115	0.109		0.131	0.113
Electrophilic index (ω)	0.081	0.150	0.109	0.091	0.132	0.161	0.112

The highest occupied molecular orbital (HOMO) of a molecule represents its ability to donate electrons, while the lowest unoccupied molecular orbital (LUMO) represents its ability to accept electrons. The energy gap ($\Delta E = E_{\text{LUMO}} - E_{\text{HOMO}}$) reflects chemical reactivity and kinetic stability. Lower ΔE values indicate higher reactivity and easier charge transfer. Ionization potential (IP) represents the energy required to remove an electron and is directly proportional to HOMO energy, whereas electron affinity (EA) reflects the ability to accept an electron and is proportional to LUMO energy. Electronegativity (χ) refers to a molecule's ability to attract electrons, whereas chemical hardness (η) indicates resistance to charge transfer. Softer molecules (higher softness, S) are typically more reactive. The chemical potential (μ) reflects electron escaping tendency, while the electrophilicity index (ω) measures stabilization energy when a system acquires additional electronic charge, indicating electrophilic character. Variations in HOMO and LUMO energies between the studied compounds indicate differences in electronic distribution and reactivity. Compound **5e** had the highest HOMO energy (0.149 eV), indicating a strong electron-donating ability, while compound **5f** had the lowest HOMO energy (0.185 eV), indicating greater energy but a lower electron donation tendency. Compound **5a** (0.037 eV) had the highest LUMO energy, indicating a lower ability to accept electrons, while compound **5f** (0.078 eV) had the lowest LUMO energy, indicating a higher capability. The HOMO-LUMO energy gap provides additional support for these findings. Compound **5e** had the lowest ΔE value (0.086 eV), indicating high chemical reactivity and low kinetic stability in the series. Compounds **5a** and **5d** had higher eV values (0.133 and 0.132 eV, respectively), indicating greater stability. Compound **5g** has a moderate ΔE of 0.114 eV,

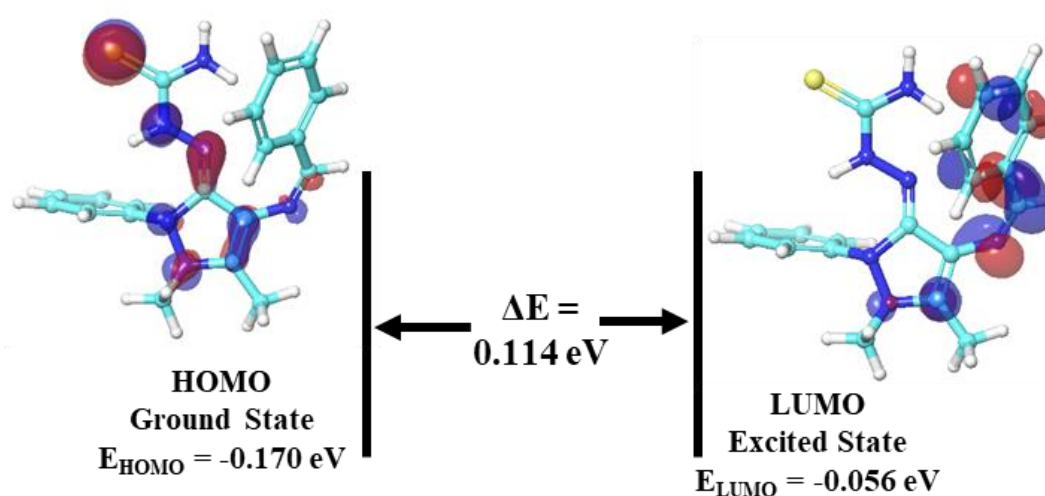


Figure 6: Frontier molecular orbital (HOMO–LUMO) distribution of representative compound **5g**.

indicating a balanced profile of stability and reactivity, which is ideal for biological activity. Ionization potential and electron affinity values followed a pattern consistent with HOMO and LUMO energies. Compound **5f** had the highest IP (0.185 eV), indicating greater resistance to electron removal, whereas compounds **5b** and **5f** had relatively higher EA values, indicating better electron-accepting capacity. The highest electronegativity values were found in compound **5f** (0.131 eV), indicating a stronger tendency to attract electrons. Chemical hardness and softness emphasize reactivity differences. Compound **5e** had the lowest hardness (0.043 eV) and the highest softness (11.684), indicating a highly reactive nature. In contrast, compounds such as **5a** and **5d** had higher hardness values, indicating greater resistance to electron density deformation. Compound **5g** demonstrated moderate hardness (0.057 eV) and softness (8.760), indicating a balanced reactivity profile. Compound **5f** had the most electron value (-0.131 eV), indicating a stronger tendency to retain electrons. The chemical potential values for all compounds were negative, indicating thermodynamic stability. The electrophilicity index was highest for compound **5f** (0.161 eV), followed by **5b** and **5e**, indicating a strong electrophilic character, whereas compound **5g** had a moderate value (0.112 eV), indicating its potential to participate effectively in intermolecular interactions without excessive reactivity. For the representative compound **5g**, the HOMO is primarily localized over the pyrazole ring and the sulfur atom of the thiourea moiety, indicating that these are important electron-donating sites. In contrast, the LUMO is distributed over the phenylmethanimine fragment, indicating that it is the primary electron-accepting region (**Figure 6**). This spatial separation of HOMO and LUMO promotes intramolecular charge transfer and improves interaction with biological targets. Overall, the quantum chemical analysis indicates that compound **5g** has an optimal balance of stability, reactivity, and charge transfer properties when compared to other derivatives. This balanced electronic profile could contribute to the positive binding interactions and biological activity seen in docking and molecular dynamics studies.

IV. CONCLUSION

Structurally diverse and biologically active derivatives of 4-aminoantipyrine (3a-3k and 5a-5d) were synthesized by using deep eutectic solvent (DES). The DES formulated by combining ethylene glycol, urea, and ammonium chloride in a 5:4:1 molar ratio function dually as both solvent and catalyst. This composition facilitates efficient product formation under mild, room-temperature conditions, significantly reducing reaction times compared to traditional methods. The DES is inexpensive, has low viscosity, is easily prepared, biodegradable, and eco-friendly. It plays a dual role as both solvent and catalyst, eliminating the need for additional catalysts. The DES employed in this protocol demonstrates excellent recyclability. In the first step, it can be reused up to nine times. All the derivatives were characterized by ^1H NMR, ^{13}C NMR, and Mass, UV-Visible, and FTIR spectral analysis. The integrated computational approach identified compound **5g** as the most promising candidate among the synthesized derivatives. Strong docking

affinity, stable MD behavior, and consistent interactions with key active site residues support its effective binding. Hydrogen bonding and hydrophobic interactions collectively contributed to ligand stabilization in both targets. DFT results further confirmed its balanced stability and reactivity, favoring biological interactions. Overall, compound 5g emerges as a potential lead for further development as a dual antimicrobial agent.

V. ACKNOWLEDGEMENT

We are very thankful to the Management of Deccan Education Society, Kirti M. Doongursee College, Dadar (W), Mumbai, for providing necessary research facilities and guidance during research work.

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