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Synthesis, Spectral Characterization, Antibacterial Evaluation and Pharmacophore Analysis of Novel 1,3,4-Thiadiazole-Indole Schiff Bases

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Abstract: A series of novel Schiff bases (1A–1I) was synthesized from 5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (ITA) through condensation with substituted aromatic aldehydes and acetophenones under reflux conditions. The synthesized compounds were characterized by melting point, FT-IR, ^1H NMR, and EI-MS analyses. Formation of the Schiff bases was confirmed by the characteristic C=N stretching absorption at 1611–1636 cm^{-1} . Among the synthesized derivatives, the 2-chlorophenyl analogue 1H exhibited the most promising antibacterial activity, particularly against *E. coli* and *S. typhi*, whereas compounds 1B and 1D showed comparatively better activity against Gram-positive bacteria. Compound 1E also demonstrated notable activity against Gram-negative strains. Pharmacophore modelling following MMFF94 minimization revealed important hydrophobic/lipophilic, hydrogen-bond donor, and hydrogen-bond acceptor features. Overall, structural modification of the Schiff-base aryl moiety influenced both antibacterial activity and pharmacophoric characteristics, with compound 1H identified as the most promising candidate for further optimization.

Keywords: 1,3,4-thiadiazole; Schiff base; indole; spectral characterization; hydrophobic/lipophilic; Pharmacophore; MMFF94.

I. INTRODUCTION

Five-membered heteroatomic systems containing nitrogen and sulphur are mostly studied in medicinal chemistry due to their useful electronic properties, hydrogen-bonding ability and substitutional flexibility of structure modification [1-3]. Among these 1,3,4-thiadiazole has emerged as a versatile pharmacophore, that has been reported to show antibacterial, antifungal, antiviral, anti-inflammatory and other biological activities [4-7]. Recent Indian studies and reviews have also highlighted the significance of this ring system in the development of antimicrobial compounds [8-10]. The present research study focuses on a small series of indole-containing Schiff base series (1A-1I) 1,3,4-thiadiazole framework. The work combines synthesis and spectral characterization with a limited antibacterial evaluation and pharmacophore modelling. This approach helps related the structural features of the compound to their observed activity and provides useful information for further modification and medicinal-chemistry optimization [11-15]. The goal of the current effort is to construct pharmacophore models for these compounds. Hydrogen donor, acceptor, and lipophilic centres in the molecules are revealed by the pharmacophore models. The pharmacological actions of the molecules differ as a result of structural variations in the molecules [4, 16,17].

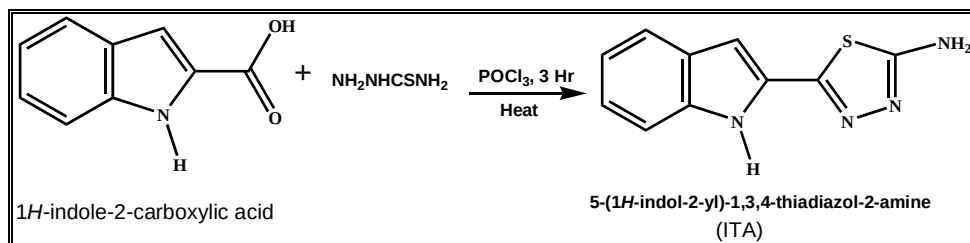
II. EXPERIMENTAL METHODOLOGY

A. Materials and instrumentation

All chemicals used in synthesis were of analytical reagent (AR) grade. The progress of reaction was monitor by using TLC in (9:1) Chloroform: Methanol solvent system. Melting points were measured in an open capillary and are uncorrected. ^1H NMR spectra were obtained on a Bruker 400 MHz spectrometer using DMSO- d_6 and TMS as internal std. at SIF, IIT Vellore. EI mass spectra were obtained by GC/MS at SIF, IIT Vellore. FT-IR spectra were recorded on Perkin-Elmer instrument.

B. Preparation of the 5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (ITA)

The starting 5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (ITA) was prepared by reacting 1H-Indole-2-carboxylic acid (0.06 mol), thiosemicarbazide (0.09 mol) and POCl_3 (0.18 mol) were refluxed at 60–65 $^\circ\text{C}$ for 3 hours with constant stirring.



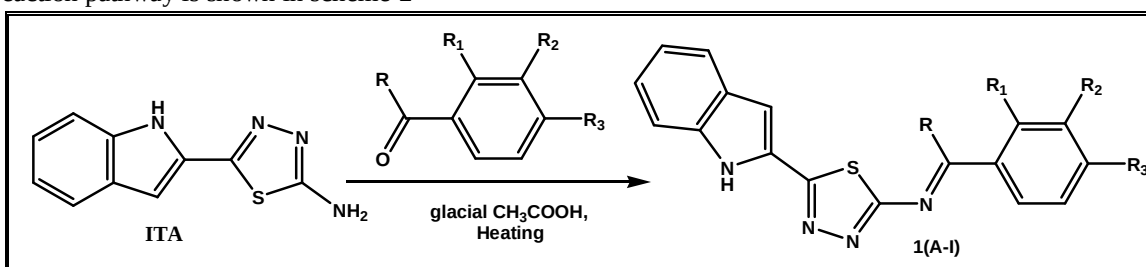
Scheme-1

Physical and spectral characterization of 5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (ITA) : Off-white solid, Yield: 64%, m.p:110–112 °C, ^1H NMR (DMSO,400 MHz) δ ppm: 5.85 (s, 2H, -NH₂), 6.61 (s,1H, Ar-H), 7.10- 7.82 (m,4H, Ar-H), 10.45 (s,1H, Indole -NH), GCMS (m/z): 216 (M⁺), IR (cm⁻¹): 3422,3250 (NH₂), 1615 (C=N),745 (C-S), 918 (N-N) Molecular Formula: C₁₀H₈N₄S

C. General Synthesis of Schiff Bases (1A-1I)

ITA (0.01 mol) and substituted aldehyde/acetophenone (0.01 mol) were refluxed with glacial acetic acid (10 ml) at 70–80 °C for 5

h. General reaction pathway is shown in scheme-2



Scheme-2

Here For;

- | | | |
|---|---|---|
| 1A: R=H, R ₁ =H, R ₂ =H, R ₃ =H | 1B: R=H, R ₁ =OH, R ₂ =H, R ₃ =H | 1C: R=H, R ₁ =OH, R ₂ =H, R ₃ =OH |
| 1D: R=H, R ₁ =H, R ₂ =OCH ₃ , R ₃ =OH | 1E: R=H, R ₁ =H, R ₂ =NO ₂ , R ₃ =H | 1F: R=CH ₃ , R ₁ =H, R ₂ =H, R ₃ =H |
| 1G: R= CH ₃ , R ₁ =H, R ₂ =H, R ₃ =OH | 1H: R= CH ₃ , R ₁ =Cl, R ₂ =H, R ₃ =H | 1I: R= CH ₃ , R ₁ =H, R ₂ =H, R ₃ = NO ₂ |

D. Physical and spectral characterization of Schiff Bases(1A-1I)

- (E)-N-benzylidene-5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (1A): Off-white solid, Yield: 64%, m.p:208-210 °C, IR (cm⁻¹): 3247 (NH Indole), 1643 (C=N),722 (C-S), 1267 (C-N), Molecular Formula: C₁₇H₁₂N₄S
- (E)-2-((5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-ylimino)methyl)phenol (1B): Off-white solid, Yield: 62%, m.p:215–217 °C, ^1H NMR (DMSO,400 MHz) δ ppm: 6.34 (s,1H,Ar-H); 6.39–6.95 (m,4H,Ar-H); 7.08–7.45 (m,3H,Ar-H); 7.69 (d,1H,Ar-H); 8.48 (s,1H-N=CH), 11.01(s,1H,-NH Indole), 11.74 (s,1H,-OH), GCMS (m/z): 320 (M⁺), IR (cm⁻¹): 3429 (OH), 3284 (NH), 1615 (C=N), 1297 (C–N), 1142 (C–O), 704 (C–S), Molecular Formula: C₁₇H₁₂N₄OS
- (E)-4-((5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-ylimino)methyl)benzene-1,3-diol (1C): Yellow-solid, Yield: 61%, m.p:238–240 °C, ^1H NMR (DMSO,400 MHz) δ ppm: 6.54(s,1H,Ar-H), 6.69(d,2H,Ar-H), 6.88(s,1H,Ar-H), 7.08-7.74(m,4H,Ar-H), 8.46 (s,1H-N=CH), 9.21(s,1H,-OH Phenol), 10.59 (s,1H,-NH Indole), 11.55 (s,1H, -OH Phenol), GCMS (m/z): 336 (M⁺), IR (cm⁻¹): 3440 (OH), 3280 (NH), 1619 (C=N), 1254 (C–N), 1152 (C–O), 704 (C–S), Molecular Formula: C₁₇H₁₂N₄O₂S
- ((E)-4-((5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-ylimino)methyl)-2-methoxyphenol (1D): Yellow-solid, Yield: 63%, m.p:229–231 °C, ^1H NMR (DMSO,400 MHz) δ ppm: 3.65(s,3H, CH₃), 6.50(s,1H,Ar-H), 6.52-6.80 (d,2H,Ar-H), 6.90-7.81(m,5H,Ar-H), 8.39 (s,1H-N=CH), 9.06(s,1H,-OH Phenol), 11.30 (s,1H,-NH Indole), IR (cm⁻¹): 3431 (OH); 3245 (NH); 1589 (C=N); 2843 (CH); 1121 (C–O); 1152 (C–O); 703 (C–S), Molecular Formula: C₁₇H₁₂N₄O₂S
- (E)-N-(3-nitrobenzylidene)-5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (1E): Light yellow-solid, Yield: 70%, m.p:229–231 °C, ^1H NMR (DMSO,400 MHz) δ ppm:6.64(s,1H,Ar-H), 6.82-7.30(m,3H,Ar-H), 7.40-7.91(d,3H,Ar-H), 8.12(d,1H,Ar-H), 8.39(s,1H,Ar-H), 8.80(s,1H-N=CH), 11.31 (s,1H,-NH Indole), GCMS (m/z): 349 (M⁺), IR (cm⁻¹): 3280 (NH), 1611 (C=N), 1505/1347 (NO₂); 1242 (C–N), 706 (C–S), Molecular Formula: C₁₇H₁₁N₅O₂S

- 6) (E)-5-(1H-indol-2-yl)-N-(1-phenylethylidene)-1,3,4-thiadiazol-2-amine (1F): Yellow-solid, Yield: 61%, m.p:207–209 °C, IR (cm⁻¹): 3284 (NH), 1639 (C=N), 1512 and 1441 (Aro. C=C)); 1319 (C–N), 716 (C–S), Molecular Formula: C₁₈H₁₄N₄S
- 7) (E)-4-(1-(5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-ylimino)ethyl)phenol (1G): Light yellow-solid, Yield: 57%, m.p:244–246 °C, ¹HNMR (DMSO,400 MHz) δppm: 2.38(s,1H,CH₃), 6.39(s,1H,Ar-H), 6.78 (d,2H,Ar-H), 7.00(d,2H,Ar-H), 7.20-7.89(m,4H,Ar-H), 9.69(s,1H, -OH Phenol), 11.14 (s,1H,-NH Indole), GCMS (m/z): 334 (M⁺), IR (cm⁻¹): 3466 OH; 3305 NH; 1612 C=N; 2917 CH; 1236 C–N; 1170 C–O; 686 C–S, Molecular Formula: C₁₈H₁₄N₄OS
- 8) (E)-N-(1-(2-chlorophenyl)ethylidene)-5-(1H-indol-2-yl)-1,3,4-thiadiazol-2-amine (1H): +Off white solid, Yield: 60%, m.p:252–254 °C, ¹HNMR (DMSO,400 MHz) δppm: 2.49(s,1H,CH₃), 6.40(s,1H,Ar-H), 6.81-7.21 (m,4H,Ar-H), 7.29-7.65(m,4H,Ar-H), 11.27 (s,1H,-NH Indole), GCMS (m/z): 351 (M⁺), IR (cm⁻¹): 3340 (NH), 1636 (C=N), 1564 and1469 (C=C), 1285 (C–N), 1176 (C–Cl), 714 (C–S), Molecular Formula: C₁₈H₁₄N₄OS
- 9) (E)-5-(1H-indol-2-yl)-N-(1-(4-nitrophenyl)ethylidene)-1,3,4-thiadiazol-2-amine (1I): Off white-solid, Yield: 57%, m.p:248–250 °C, ¹HNMR (DMSO,400 MHz) δppm: 2.81(s,1H, CH₃), 6.46(s,1H, Ar-H), 6.95-7.70 (m,4H,Ar-H), 8.00(d,2H, Ar-H), 8.39(d,2H, Ar-H) , 11.29 (s,1H,-NH Indole), GCMS (m/z): 334 (M⁺), IR (cm⁻¹): 3319 (NH), 1625 (C=N), 1486 and 1332 (NO₂), 723 (C–S), Molecular Formula: C₁₈H₁₃N₅O₂S.

III. DEVELOPMENT OF THE PHARMACOPHORE MODEL

Following steps are used for the development of pharmacophore models.

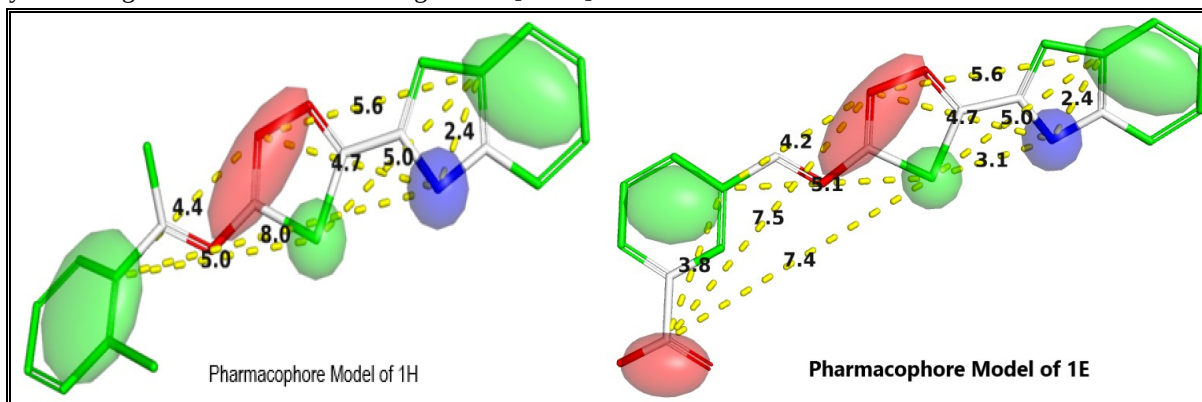
Structure Drawing: ChemSketch 15 (freeware) was used to draw structures of the molecules.

Structure Optimization: Avogadro 2 was employed to optimize the three-dimensional (3D) structures of the molecules.

Molecular Alignment: This step was accomplished using Open3Dalign.

Model Generation: all the molecules were introduced in PyMOL 2.0. Then, PyMOL plugin ‘LIQUID’ was employed to generate consensus model using default settings [18-20].

Pharmacophoric regions were interpreted as hydrophobic/lipophilic (green), hydrogen-bond acceptor (HBA; red) and hydrogen-bond donor (HBD; blue) features. The distances reported below are distances between the corresponding contour regions, not necessarily bond lengths or distances from a single atom [21-24].



IV. RESULTS AND DISCUSSION

A. Synthesis and structural confirmation

Synthesized Schiff bases (1A-1I) were obtained in moderate to good yields. ITA on condensation with aromatic aldehyde or ketone, converted the primary amino group into an imine linkage. A characteristic C=N absorption was observed at 1589-1636 cm⁻¹, while the indole N-H band appeared at 3245-3340 cm⁻¹. Phenolic derivatives (2B, 2C, 2D and 2G) showed additional O-H bands, whereas nitro derivatives (2E and 2I) exhibited characteristic NO₂ absorptions. In the ¹H NMR spectra, the imine proton appeared at δ 8.39-8.80 ppm, while indole NH signal appeared at δ 10.6-11.3 ppm. Schiff bases synthesized from acetophenone showed methyl singlet at δ 2.38-2.81ppm. These spectral values confirmed Schiff base formation and the presences of substituents on ring.

B. Antibacterial Screening

To correlate structural features with pharmacophore characteristics, a preliminary comparative biological screening was performed. Disc diffusion method was carried out at conc. of 40 µg/disc against *E. coli*, *S. typhi*, *S. aureus*, and *P.acnes*, with tetracycline as the reference drug. The measured inhibition zones are summarized below in Table1.

Sr. No.	Compound Code (Growth inhibition) mm	Zone of Inhibition in mm			
		Gram-negative bacteria		Gram-positive bacteria	
		E.Coli	S.Typhi	S.Aur	P.Acne
1	1A	13 ± 0.5	12 ± 0.5	14 ± 0.4	11 ± 0.3
2	1B	17 ± 0.3	18 ± 0.4	21 ± 0.3	22 ± 0.3
3	1C	14 ± 0.4	15 ± 0.3	18 ± 0.4	15 ± 0.3
4	1D	16 ± 0.3	18 ± 0.4	20 ± 0.4	21 ± 0.3
5	1E	21 ± 0.3	22 ± 0.3	18 ± 0.4	16 ± 0.3
6	1F	12 ± 0.4	10 ± 0.4	12 ± 0.2	14 ± 0.3
7	1G	16 ± 0.4	15 ± 0.3	17 ± 0.3	17 ± 0.2
8	1H	24 ± 0.2	25 ± 0.3	22 ± 0.3	23 ± 0.2
9	1I	20 ± 0.3	21 ± 0.3	15 ± 0.3	16 ± 0.2
	Control DMSO (Used Sterile Disc) (Cl)	00.00	00.00	00.00	00.00
Reference	Tetracycline	25	27	24	26

Table – 1

Compound 1H shows the strongest antibacterial activity among all synthesized derivatives, with zones of 23 ± 0.2 and 25 ± 0.3 mm against *E. coli* and *S. typhi* and 22 ± 0.3 and 24 ± 0.2 against *S. aureus*, and *P.acnes*. Compound 1E also show notable activity against gram negative bacteria, consistent with the presence of an additional nitro acceptor region. In contrast, phenolic compound 1B and 1D showed comparatively stronger antibacterial activity against Gram-positive bacteria, its indicates that hydrogen-bond donor capacity and molecular structure may influence their antimicrobial activity. However, inhibition zone values alone cannot be used to determine MIC or exact mechanism of action.

C. Structure-pharmacophore relationship

The pharmacophore models help to explain the observed difference in biological activity among all synthesized compounds. Compound 2B showed three hydrophobic regions, two hydrogen-bond (HBD) sites associated with indole NH and phenolic OH, and one hydrogen-bond acceptor (HBA) site related to the imine/thiadiazole system. Compound 2D showed a similar arrangement with an additional HBA feature due to the methoxy group. Compound 2E also shows increased acceptor character due to presence of nitro group. In contrast, compound 2H show the highest biological activity containing extra Cl heteroatom. This higher activity may be due to presence of favourable hydrophobic environment shown by two aromatic rings, chloro substituent, methyl-imine group and thiadiazole sulfur, along with one HBA and one indole-NH HBD. Or we can say that 2-chloroaryl substituent provides a favourable balance between hydrophobic surface, molecular shape and the retained donor/acceptor arrangement of the indole–thiadiazole–imine framework. Its pharmacophore model showed a hydrophobic region was about 2.4 Å from HBD region while the HBA region was about 4.4 Å distance from a hydrophobic region. These distances are model-based observations and require further validation using larger set of aligned compounds.

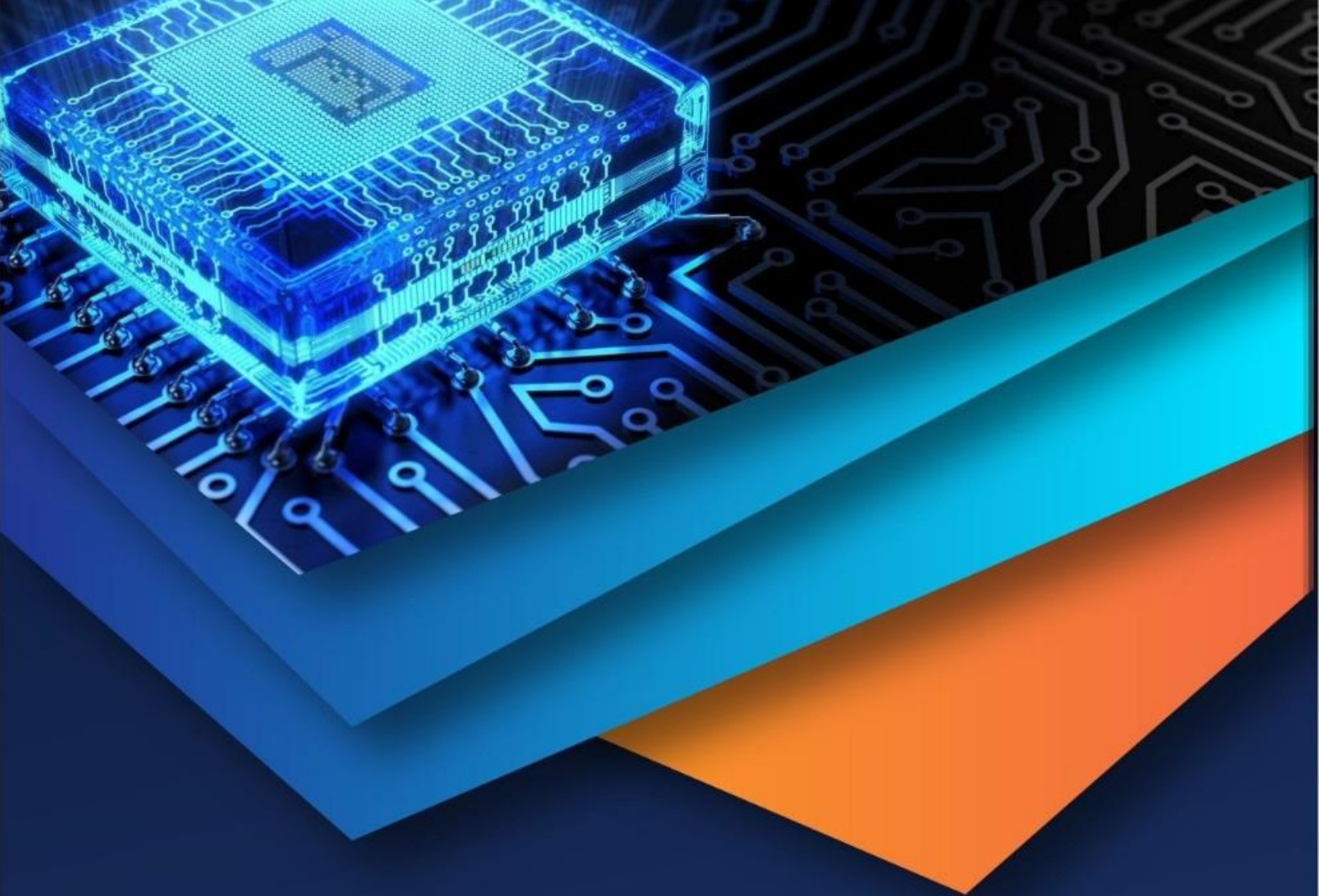
V. CONCLUSION

A series of indole -linked 1,3,4-thiadiazole Schiff base of aromatic aldehyde/ketone was synthesized and characterized by FT-IR, ¹HNMR and mass spectrometry. The presence of imine, indole NH, and substituent -specific functional group were identified successfully. Disc-diffusion screening showed that derivative 2H was the most active against both gram positive and negative bacteria. Compound 2E showed good antibacterial activity against gram negative bacteria, whereas 2B and 2D showed promising activity against Gram-positive bacteria. Pharmacophore analysis indicated a common hydrophobic aromatic–thiadiazole framework with substitution-dependent donor and acceptor features.

Overall, Schiff base derivative 2H showed the highest biological activity and can be consider a promising lead compound for further study. Further research, including MIC determination, evaluation of additional compounds, and molecular modelling, is useful to better understand the structure-activity relationship.

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