Microwave Assisted Synthesis, DFT and Molecular docking Computations, DNA Binding of Schiff Bases from Nitrothiophene analogs and Antibacterial screening.

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Abstract

The current work focuses on the synthesis and characterization of three Schiff bases synthesized under microwave conditions from 5-nitrothiophene-2-carboxaldehyde and substituted aromatic amines. These compounds were successfully synthesized with high yields, and they were examined using FTIR, NMR (^{1}H), and mass spectroscopic techniques. Density functional theory (DFT) was used to theoretically examine the quantum chemical characteristics of synthesized compounds employing the B3LYP/6-31G(d,p) method. These substances were also tested for antibacterial activity. Few of the microorganisms under investigation were found to be resistant to these compounds. Chemical reactivity characteristics and molecular docking investigations have been linked to the antibacterial properties. Additionally, using the electronic absorption titration method, the compounds' DNA-binding capabilities have been studied experimentally. The outcomes are very encouraging, so we can say that these compounds are biologically less active and can be used with structural modifications towards drug development.

Key words: Microwave assisted synthesis, Antibacterial activity, Molecular docking, DFT analysis, DNA Binding studies.

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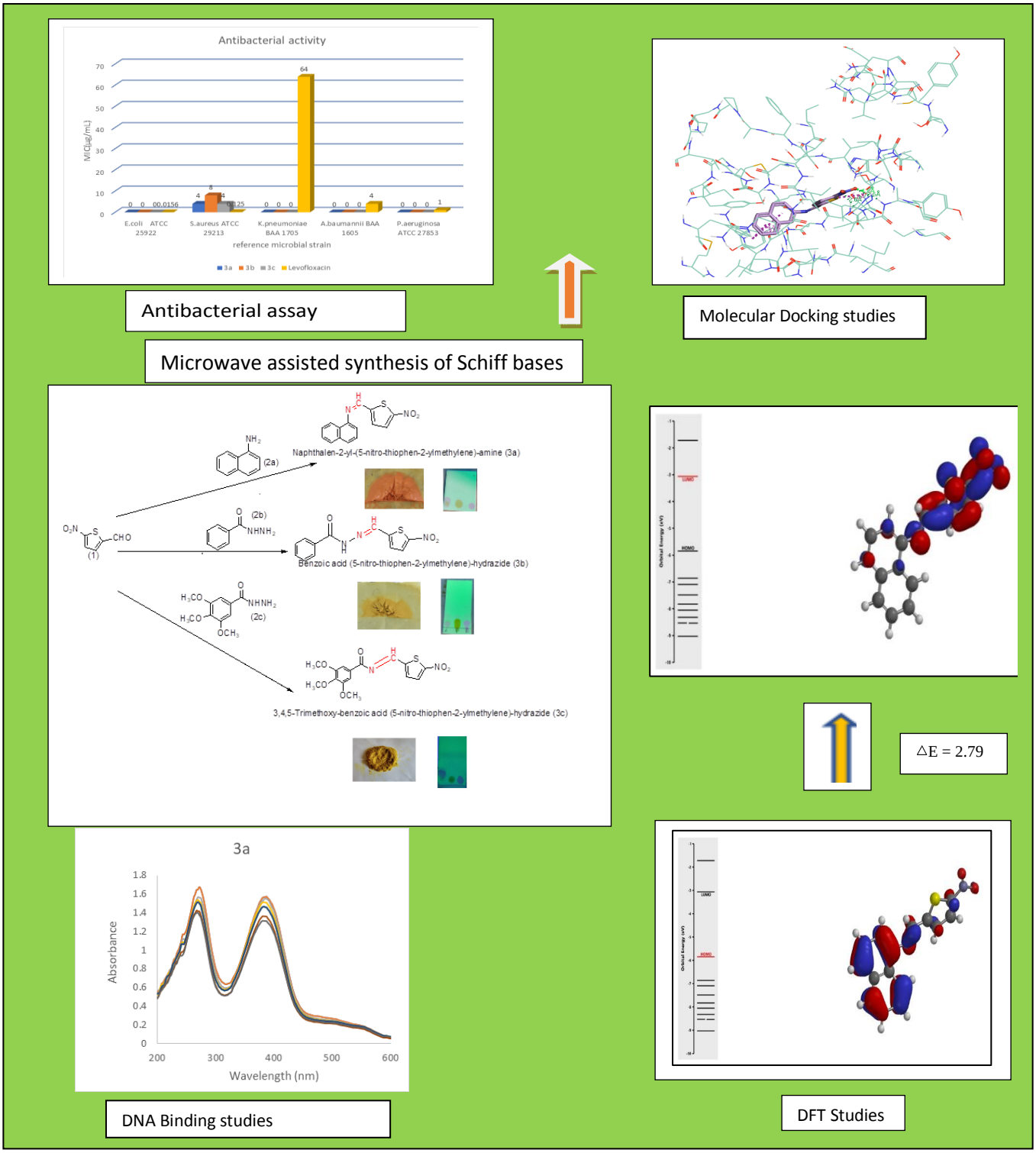
Graphical abstract



1. Introduction

The microwave-assisted organic reactions have greater yields and have been shown to be environmentally beneficial. Consequently, one of the recognised fields in green chemistry. Microwave assisted synthesis is a useful and practical tool for both industry and academic research owing to its quick reaction times, broad range of reactions, little exposure to dangerous chemicals, and high efficiency. Due to its quickness, cleanliness, and simplicity of operation, microwave aided processes in solvent or solvent free conditions have grown in popularity. Because of its benefits, microwave assisted organic reactions are of tremendous interest and was used to modify fundamental basic reactions like Borsche–Drechsel cyclization, Benzillic acid rearrangement, Biginelle reaction , Claisen-Smith condensation of Pyrazoline Cyclization , Nimentowski reaction etc using microwave method. [1-5] Microwaves are being used in organic/pharmaceutical synthesis by pharmaceutical corporations for drug discovery and screening. Pharmaceutical drugs with a wide range of biological activities, such as analgesic, antihypertensive, central nervous system depressant, antiviral, bactericidal, and fungicidal activities, have been explored among the diverse range of drug molecules for the development of microwave assisted synthetic processes. [6-10].
Schiff bases especially those linked with heterocyclic moiety exhibited various pharmacological and biological activities such as antibacterial, cytotoxic effects, antifungal, antimalarial, anticonvulsant, antioxidant, and anti-inflammatory [11]. Drugs contain thiophene nuclei has numerous therapeutic significances. [12] Particularly, compounds with a 5-nitro-thiophene moiety have been shown to have therapeutic potential due to their antibacterial [13–15], antifungal [16], antimicrobial [17–18], anticancer [19-21] and antiprotozoal activities[22].Keeping in view the importance of 5- nitro thiophene derivatives our work emphasizes on microwave assisted green chemical synthesis[23, 24] of three new Schiff bases of 5- nitro thiophene 2- carboxaldehyde with aromatic aldehydes (3a-3c).Conventional methods reported [25] suffered from longer reaction time but microwave method completes in short time. Synthesized compounds were characterized using IR, NMR, and Mass spectroscopy. Molecular docking studies have been carried out to understand the behaviour of 5-nitrothiophen derived Schiff bases toward the possible therapeutic target enoyl-ACP reductase enzyme (PDB: 1C14). Density functional theory (DFT) is a quantum mechanical semi – imperial approach for theoretical investigations on electronic structure of a molecule. It gives a comprehensive explanation of the global and local reactivity descriptors and MEP of 5-nitro thiophene derived Schiff bases compounds. The in vitro antimicrobial activity was performed using MIC method each compound was determined against *Staphylococcus aureus* 29213, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, *Acinetobacter baumannii* 1605, *Klebsiella pneumoniae* 1705. Levofloxacin was used as standard. The antibacterial activities have been correlated with chemical reactivity parameters and molecular docking results.

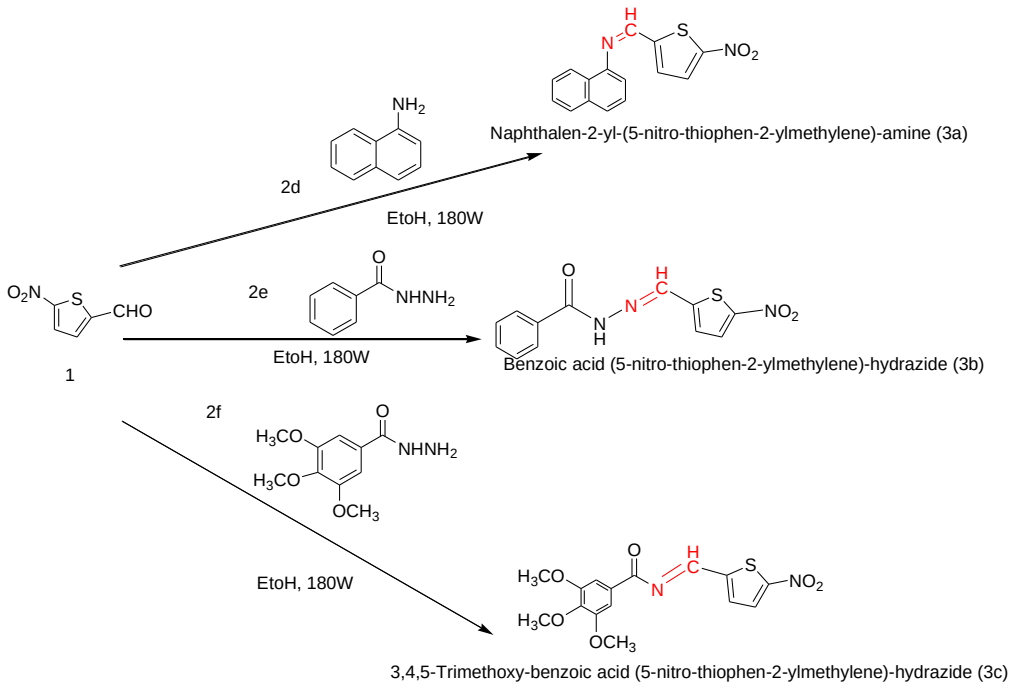
2. Materials and Methods

All reagents and starting ingredients were commercially available and used without additional purification. Synthesis was performed using a Start Synth microwave synthesiser. On precoated plates (silica gel 60 F254), analytical thin-layer chromatography (TLC) was performed, and spots were seen using ultraviolet (UV) light. On a Tensor27 FT-IR spectrometer made by Bruker Optics in Germany, FT-IR spectra were captured using KBr disc. TMS was used as the standard for recording ^{1}H NMR spectra on an Avance-III 400MHz NMR Spectrometer (Bruker Biospin, Germany), and ESI Mass was carried out

using Micromass QTof MicroTM, SL no.-YB389, Maker- WATERS II) Model- Maxis Impact, Maker- Bruker (NIPER, Hyderabad).

I) General procedure for synthesis of 5-Nitrothiophene-2-Carboxaldehyde Derivatives

The aromatic aldehyde 5-nitro thiophene-2-carboxaldehyde (1,1.57g, 0.01mol) are mixed various substituted amines 1- Naphthylamine (2a, 1.431g, 0.01mol), benzhydrazide (2b, 1.363g, 0.01mol), trimethoxy benzhydrazide (2c, 2.26g, 0.01mol) in 15ml ethanol for 15 minutes in StartSYNTH microwave synthesiser at 180 watts. The TLC was used to track the reaction's development. A single spot was seen, signifying the complete conversion of reactants to products seen in scheme 1. To assess the product's purity further thoroughly, ^{1}H NMR, IR, and mass spectra were employed.



Scheme 1: Synthesis of 5-Nitrothiophene-2-Carboxaldehyde Derived Schiff bases (3a-3c)

3. Biological Activity

Determination of minimum inhibitory concentration: Each compound's in vitro antimicrobial activity was assessed against Staphylococcus aureus 29213, Escherichia coli 25922, Pseudomonas aeruginosa 27853, Acinetobacter baumannii 1605, and Klebsiella pneumoniae 1705 using the MIC technique. Levofloxacin was the standard medication. By using the serial dilution approach, the MIC values of the compounds (3a-3f) were identified [26]. It is described as the lowest dose of the test substance needed to stop microorganisms from growing visibly. The newly synthesised compounds were tested for antibiotic susceptibility by establishing the Minimum Inhibitory Concentration (MIC) in accordance with established CLSI recommendations. The lowest concentration of a substance (MIC) required to suppress clearly apparent bacterial growth. Mueller-Hinton cation-supplemented broth was used to cultivate bacterial cultures (CAMHB). After measuring the optical density (OD600) of the cultures, the concentration was diluted to ~ 106cfu/mL. This inoculum was put into a number of test wells in a microtitre plate that had test compound concentrations ranging from 64 to 1 μg/mL. Levofloxacin is used as a reference standard and cells with media (without chemical or cells) serve as controls. Plates were incubated at 37°C for 16–18 hours, after which the MIC values were determined by determining whether or not visible growth was present. The MIC values were calculated separately three times with duplicate samples for each chemical.

4. Quantum Computational Studies:

The DFT (density functional theory) analysis of synthesized compounds was performed using Spartan 20 software. The structural coordinates of the lead compounds were optimized using B3LYP/6–31 G (d,p) level basis set without any symmetrical constraints. The molecular electrostatic potential map and energies of the compounds were obtained from the optimized geometry. Koopman's ap- proximation was used to estimate the HOMO-LUMO energy gap and related reactive parameters (electronegativity, chemical potential, hardness, softness, and electrophilicity). [27-30]

5. Docking Studies

A clear understanding of any compound's properties, such as binding energy, electron distribution, hydrogen bonds and donor acceptors, polarizability, hydrophobicity, and protein ligand interaction with selectivity/affinity for the target, drug likeness, and lead determination, is provided by molecular docking studies. [31-32]

Software: Using the Graphical User Interface application FLAREV5 from CRESSET. UK, the docking stimulation was set up, carried out, and assessed.

Preparation of protein: The protein is prepared using FLAREV5 and the PDB structure (1c14) and 1BNA is retrieved from www.rcsb.org. Energy minimization is then performed using an open MM force field. The protein underwent preprocessing to get rid of any bound water molecules, cofactors, ligands, and to fill loop holes with the Freed Loop Builder by Python Extension in Flare.

Preparation of ligand: The structures of 5-nitrophene-2-carboxaldehyde derivatives were drawn using Chem Draw and saved in mol2 format. Make it a three-dimensional structure and minimise the ligand geometry. The steps in this process are as follows:

- Using Chem Draw, 2D structures of ligands were created.
- 3D models were created from 2D structures.
- Conformers were produced and improved.
- For docking, the lowest energy conformer was chosen.
- FLARE GUI handled docking

6. DNA Binding Studies

Electronic absorption spectroscopic studies: The 5-nitrophene-2-carboxaldehyde derivativeswere kept at a constant concentration of 10 M throughout the experiment (5 mM Tris-HCl/50 mM NaCl buffer at pH 7.4) for the UV-Vis titration studies.The CT-DNA in tris HCl-NaCl buffer solution gave a ratio of 1.8-1.9 of UV absorbance at 260 and 280 nm, showing that the DNA was adequately free of protein .The source DNA is present at a concentration of 1 mg/mL. After 1:30 dilution of the source DNA with a 5 mM Tris-HCl/50 mM NaCl buffer at pH = 7.2, we used a molar absorptivity (6600 $M^{-1}cm^{-1}$) at A_{260} nm to calculate the concentration of the CT-DNA stock solution. Therefore, the estimated concentration of the source DNA (1 mg/mL) is 5700µ M. In trials, the overall volume of the reaction mixture was constant while the concentration of CT-DNA was changed between 0 and 10µ M. (3 mL).Following each addition of CT-DNA to the derivatives of 5-nitrophene-2-carboxaldehyde, the resultant solution was allowed to equilibrate at 25°C for 5 min before the absorption spectra was recorded.By plotting [DNA]/(εb − εf) and [DNA], the binding constants (Kb) were determined from the spectroscopic titration data [33-34].

Results and Discussion:

i) Spectral Data of Synthesized Schiff bases

With the aid of their 1H NMR, UV, IR, and mass spectrometric analyses, the structures of the all-synthesized Schiff base derivatives of 5-nitro thiophene-2-carboxaldehyde were described. According to the spectrum data, all of the compounds (3a-c) were effectively synthesized.

All of the spectrum data of the synthesized Schiff bases (3a-3f) were found to be compatible with the anticipated results, as per the literature review.Characteristic absorption bands were shown in the IR spectra at approximately 3100, 3116, 3126, 1502, 1591, 1537, 1583, and 1531 cm^{-1}, indicating the existence of the following compounds: aromatic (C-H), azomethine (C=N), and (C=C), respectively. The overall IR data for the Schiff base derivatives were presented in Table 1 and matched the expected range.

Table 1: IR spectral data (cm $^{-1}$)

Compound	(N-H	Aromatic C-	CH=N	NO2	(C-N	(C-S

	stretch)	H		stretching	stretch)	Stretching)
3a	-	3051	1602-1535	1437-1331	1265	768
3b	3255	3072	1646-1548	1432-1332	1261	731
3c	3212	3001	1650-1525	1325-1357	1232	731

Additionally, in the ^{1}H NMR spectra of Schiff bases, we found a singlet equivalent to one hydrogen at 8.2–9.3 ppm, confirming the existence of azomethine proton(-CH=N). At 7.2–7.9 ppm, the peaks are due to thiophene, thiazole, and aromatic ring protons. The compound's purity can be further confirmed using mass spectra.

3a) Naphthalene -2-yl- (5-nitrothiophene-2-ylmethylene)amine

FT-IR (KBr):$\bar{\nu}$ Wavenumber cm^{-1} 3051(C-H aromatic stretching), 1602-1535(CH=N stretching, 1437-1331(Nitro stretch), 1265(C-N stretch), 768 (C-S Stretching) cm^{-1}

^{1}HNMR (400MHz,CDCl3):δChemical shift 8.59(s,1H),8.35(d,1H),7.89(d,1H),7.13-7.77(multiplet, 8H).

ESI mass =283.0[M+1]

3b) **Benzoic acid (5-nitrothiophene -2-ylmethylene)-hydrazide**

FT-IR(KBr):$\bar{\nu}$ Wavenumber cm^{-1}3255(N-H stretch), 3072(C-H aromatic stretching), 1646-1548(N=CH stretching), 1600-1492(C=C aromatic stretching), 1432-1332(Nitro stretch), 1261(C-N stretch), 731(C-S Stretching) cm^{-1}

^{1}HNMR: 400MHz, CDCl3):δChemical shift 12.2(s,1H deuterium exchangeable) ,8.7(s,1H),8.2 (d,1H),7.8(d,1H), 7.3-7.75(multiplet,5H).

ESI mass =277 [M+1]

3c) 3, 4, 5 –Trimethoxy benzoic acid (5-nitrothiophene -2-yl methylene)-hydrazide

FT-IR (KBr): $\bar{\nu}$ Wavenumber cm^{-1}3212(N-H stretch), 3001(C-H aromatic stretching), 1650-1525(N=CH stretching), 1579-1499(C=C aromatic stretching), 1325-1357(Nitro stretch), 1232(C-N stretch), 1124(C-O-C stretching), 731(C-S Stretching) cm^{-1}

^{1}HNMR: 400MHz, CDCl3):δChemical shift 9.2(s,1H),8.7(s,1H),8.2 (d,1H),7.8(d,1H), 7.3-7.75(S,2H).3.9(S,9H)

ESI mass: 366 [M+1]

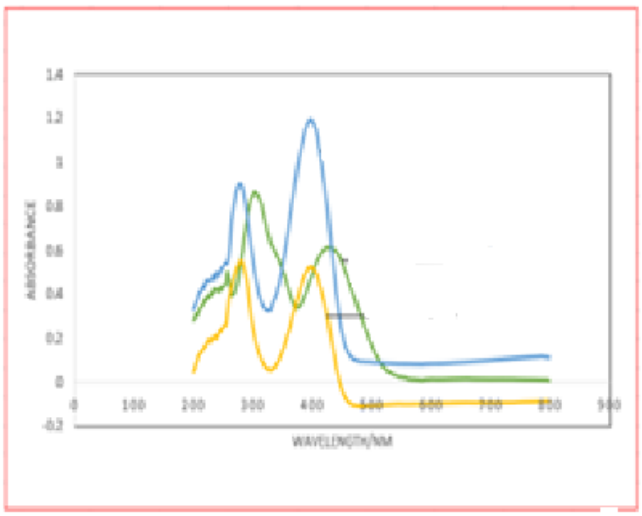
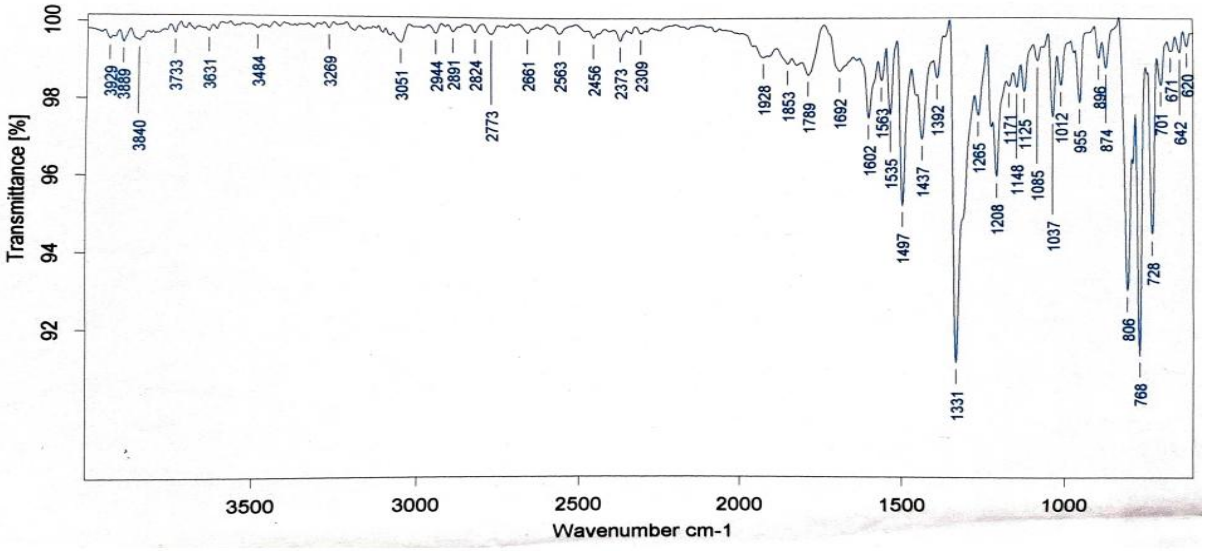
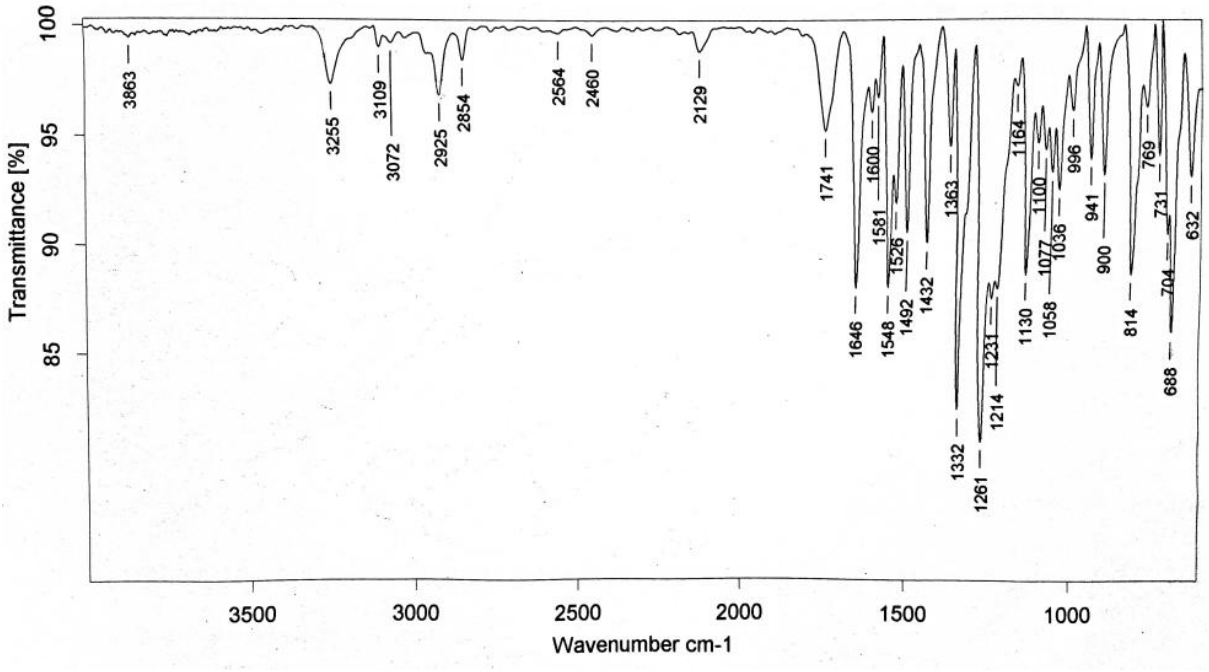


Figure 1 UV Absorption spectrum of synthesized 5- nitro thiophene -2-carboxaldehyde derived Schiff bases (3a-3c)

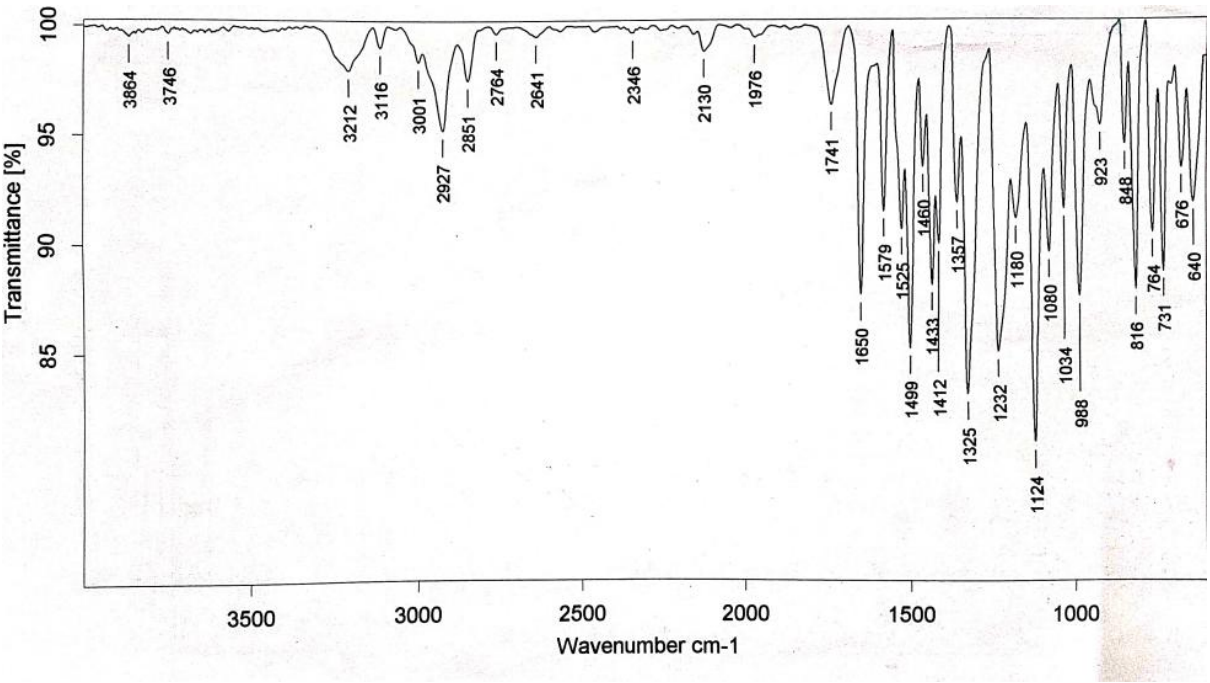
IR Spectrum of synthesized derivatives (3a-3c)



3a: Naphthalene -2-yl- (5-nitrothiophene-2-ylmethylene)amine



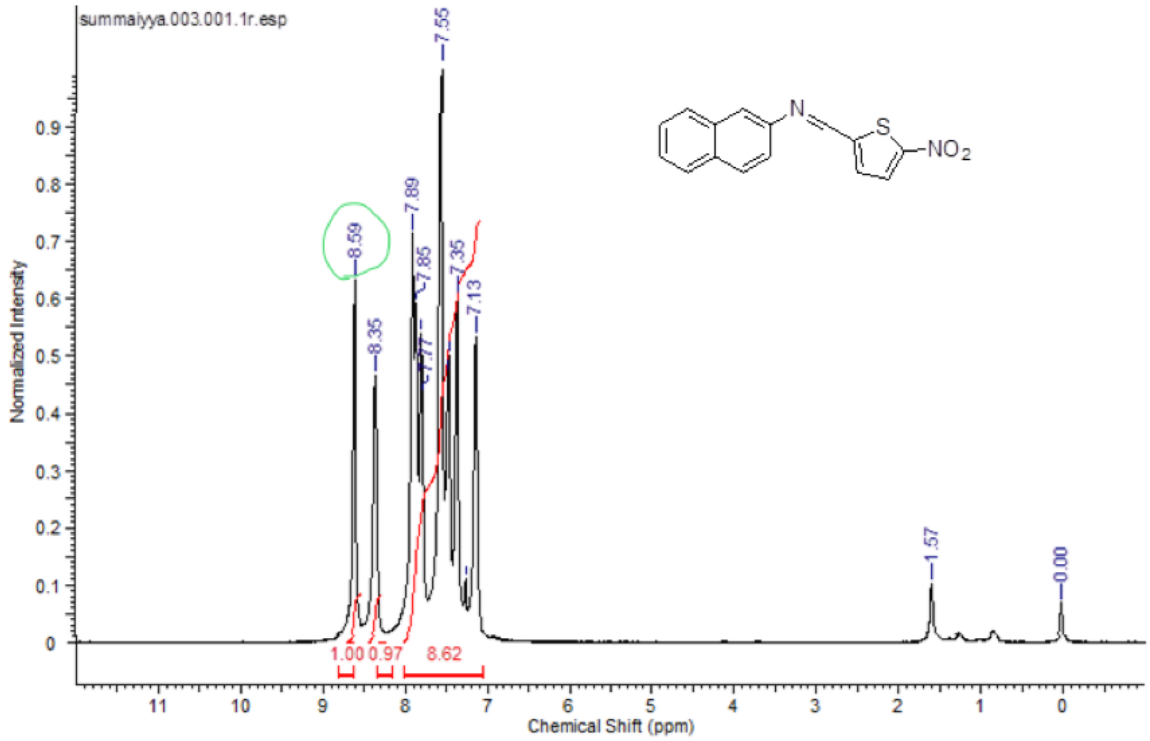
3b: Benzoic acid (5-nitrothiophene -2-ylmethylene)-hydrazide



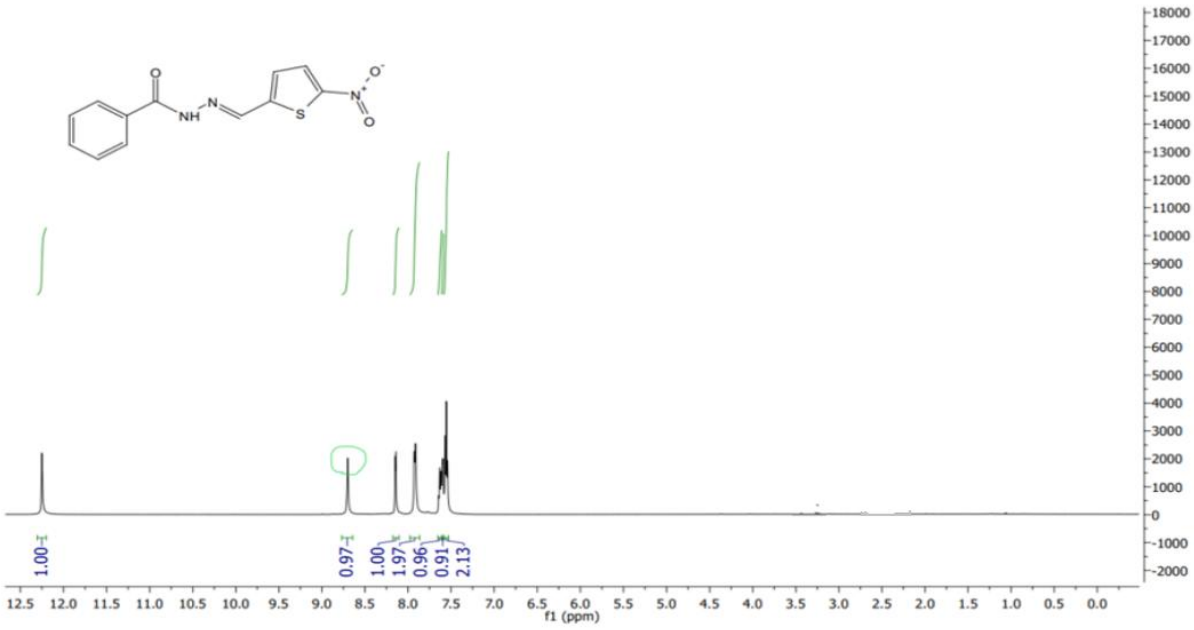
3c: 3, 4, 5 –Trimethoxy benzoic acid (5-nitrothiophene -2-yl methylene)-hydrazide

Figure -2 IR Spectrum of synthesized compounds (3a-3c)

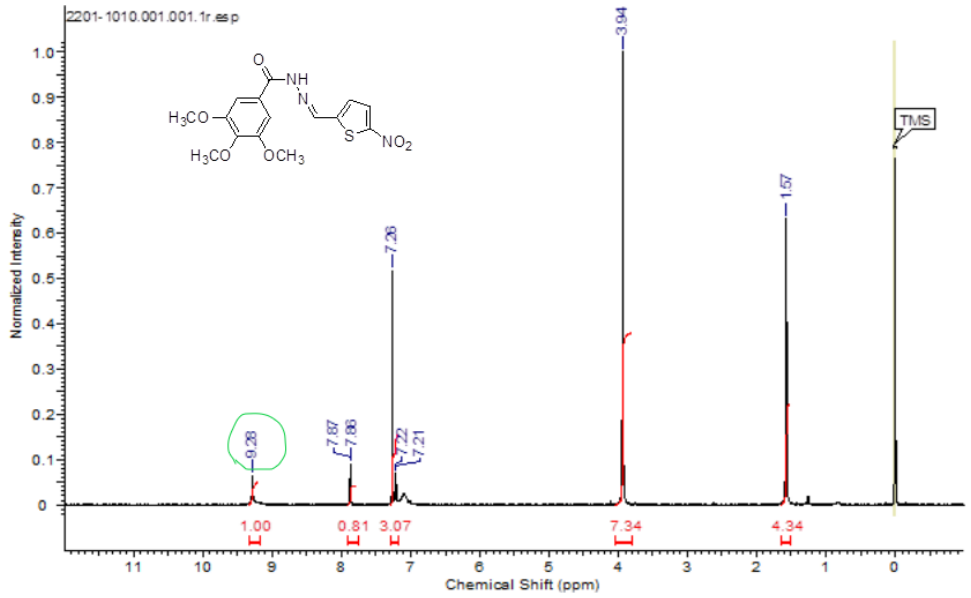
NMR Spectrum of synthesized derivatives (3a-3c):



3a: Naphthalene -2-yl- (5-nitrothiophene-2-ylmethylene)amine



3b: Benzoic acid (5-nitrothiophene -2-ylmethylene)-hydrazide

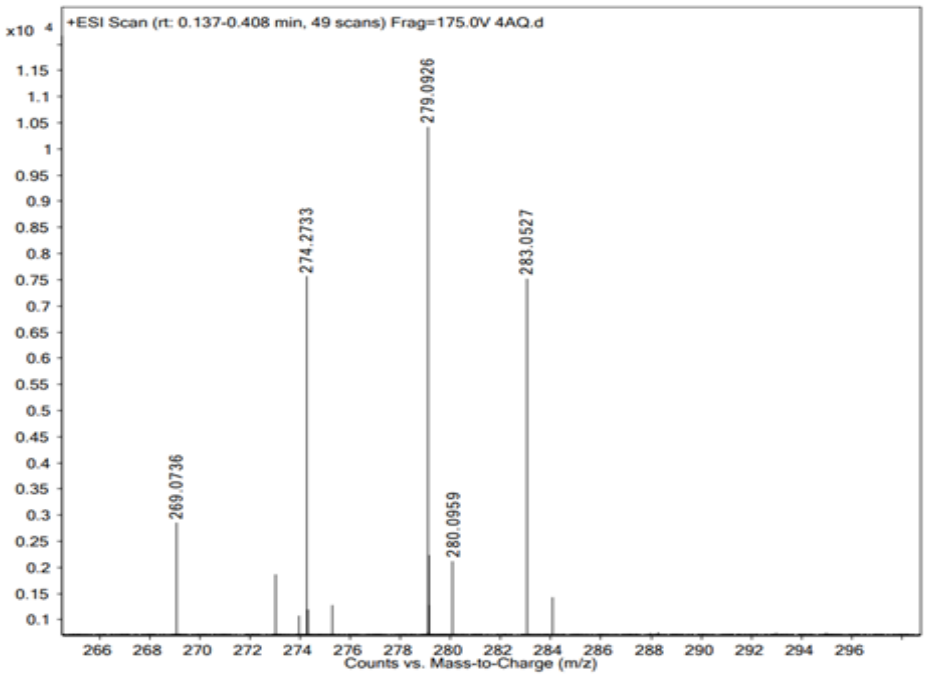


3c: 3, 4, 5 –Trimethoxy benzoic acid (5-nitrothiophene -2-yl methylene)-hydrazide

Figure -3 NMR Spectrum of synthesized compounds (3a-3c)

Mass Spectra of Ligands (3a-3c)

3a



3b

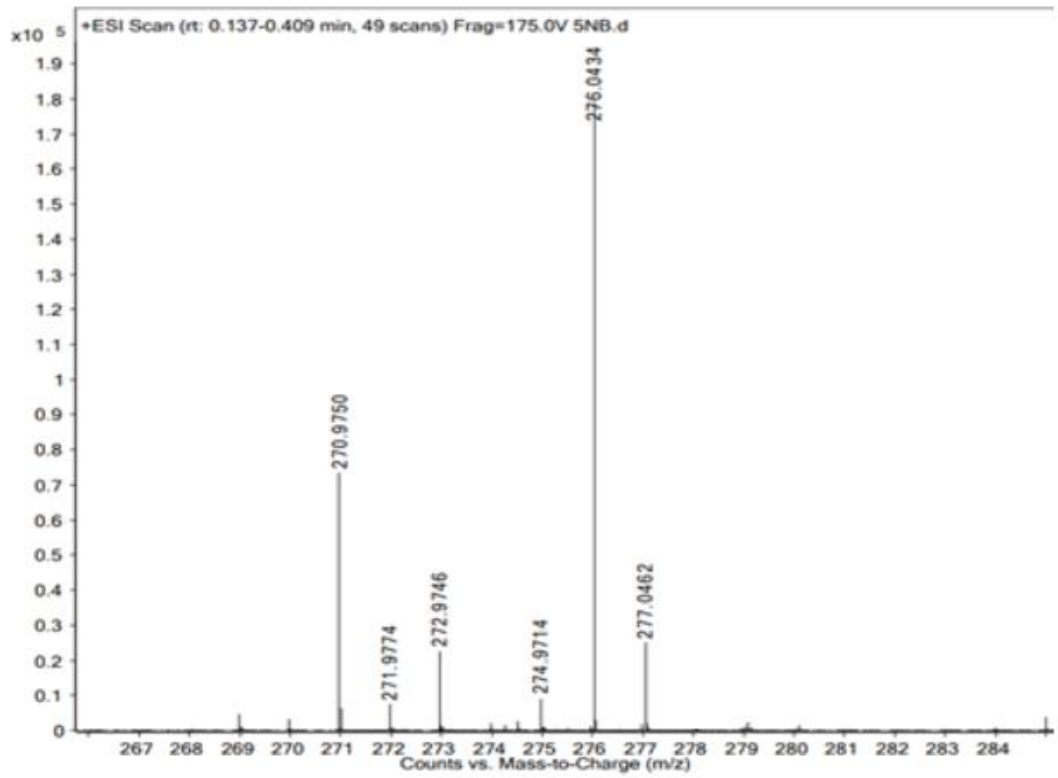


Fig-4 ESI Mass spectra of 3c

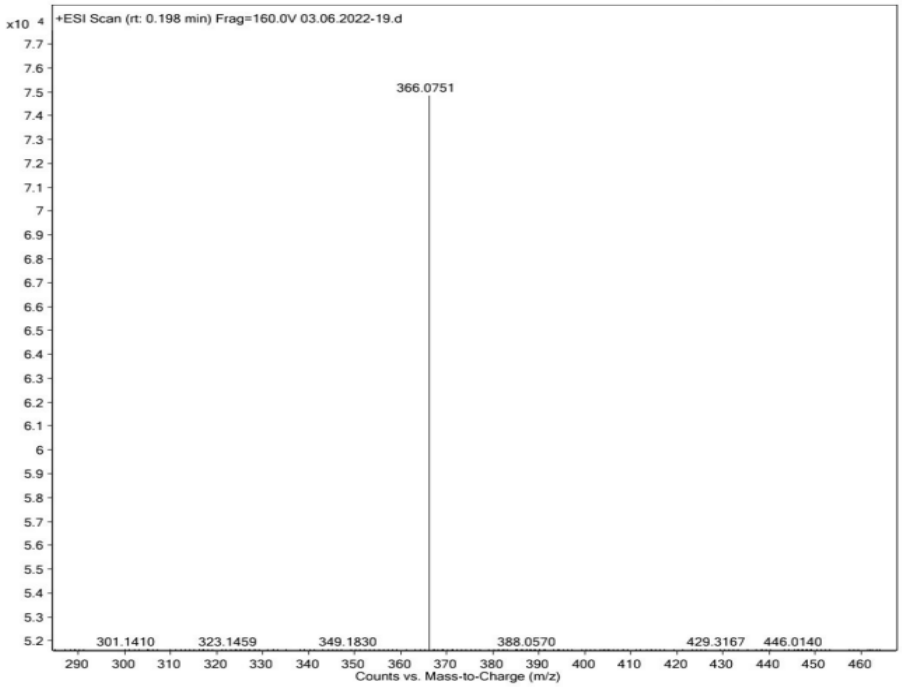


Figure 4 ESI Mass spectra of synthesized compounds (3a-3c)

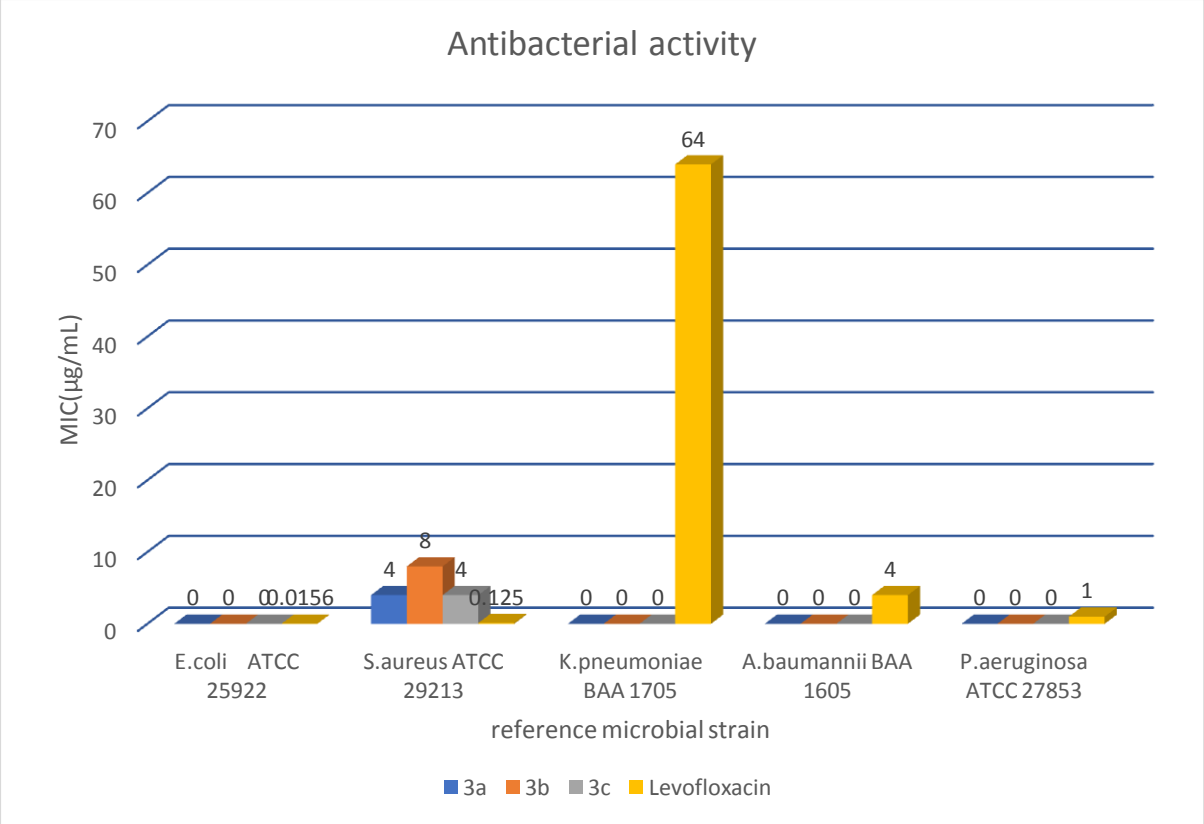
2. Biological studies:

The antibacterial study's findings made it abundantly evident that the majority of the tested substances were active and significantly inhibited both gramme positive and gramme negative bacterial strains. (Table 2). All three synthesized compounds (3a-3c) which had a MIC range of 4, 8 and 4 µg/ mL, displayed action against only S. aureus, that was comparable to that of conventional Levofloxacin among all produced 5-nitro thiophene-2-carboxaldehyde derivatives. In conclusion, as compared to normal levofloxacin, all three derivatives of 5-nitro thiophene-2-carboxaldehyde showed that the antibacterial efficacy against only strain of S. aureus 29213-8 organism.

Table -2 Anti-bacterial bioassay

S.No	Mol Formula	Mol Wt	Sample Wt (mg)	Solubility	*E.coli* ATCC 25922	*S.aureus* ATCC 29213	*K.pneumoniae* BAA 1705	*A.baumannii* BAA 1605	*P.aeruginosa* ATCC 27853
3a	$C_{15}H_{10}N_2O_2S$	282.32	1.21	DMSO	>64	4	>64	>64	>64
3b	$C_{12}H_9N_3O_3S$	275.28	1.87	DMSO	>64	8	>64	>64	>64
3c	$C_{15}H_{15}N_3O_6S$	365.36	1.34	DMSO	>64	4	>64	>64	>64
Levofloxacin					0.0156	0.125	64	4	1

Figure 5 Graphical representation of antibacterial activity



3. FMO Analysis:

The HOMO and LUMO orbitals are essential in quantum chemistry calculations. The HOMO energy determines the electron donating ability while the LUMO determines the electron accepting ability and the HOMO–LUMO energy gap is a significant value for stability index. A large energy gap implies a good thermodynamic stability of the compound, in the sense of its lower reactivity in chemical reactions. However, the magnitude of the HOMO-LUMO gap has significant chemical implications, even if qualitatively evaluated. The energy gap between HOMO and LUMO indicates the molecular chemical stability. The molecular parameters and values of HOMO, LUMO energies according to B3LYP/6-31G basis set calculation are summarized in Table 3, 4, 5 These are the corresponding energy values for Ligand 3a (-5.85eV and -3.06eV), Ligand 3b (-6.51eV and -3eV) and ligand 3c (-6.26eV and -3eV). The calculated HOMO-LUMO energy gap value is found to be 2.79 eV for 3a, 3.51eV for 3b and 3.26eV for 3c which suggests that all studied compounds exhibited the least energy gap (ΔE), suggesting high chemical reactivity and considerable intramolecular charge transfer from an electron donor (HOMO) to electron acceptor (LUMO) groups. The surfaces for the frontier orbitals were drawn to understand the bonding scheme of present compounds. The features of these molecular orbitals can be seen in (Figure 6).

Table 3: Quantum-chemical parameters of synthesised compounds (3a-3c) computed with DFT at B3LYP/ 6-31G basis set.

COMPOUNDS	**E HOMO (eV)**	**E LUMO (eV)**	**Dipole (debye)**	**Log P**	**Polarizability**	**HBD Count**	**HBA Count**
3a	-5.85	-3.06	5.12	5.21	62.6	0	5
3b	-6.51	-3	3.75	3.68	60.94	1	7
3c	-6.26	-3	370.51	67.66	1	10	0

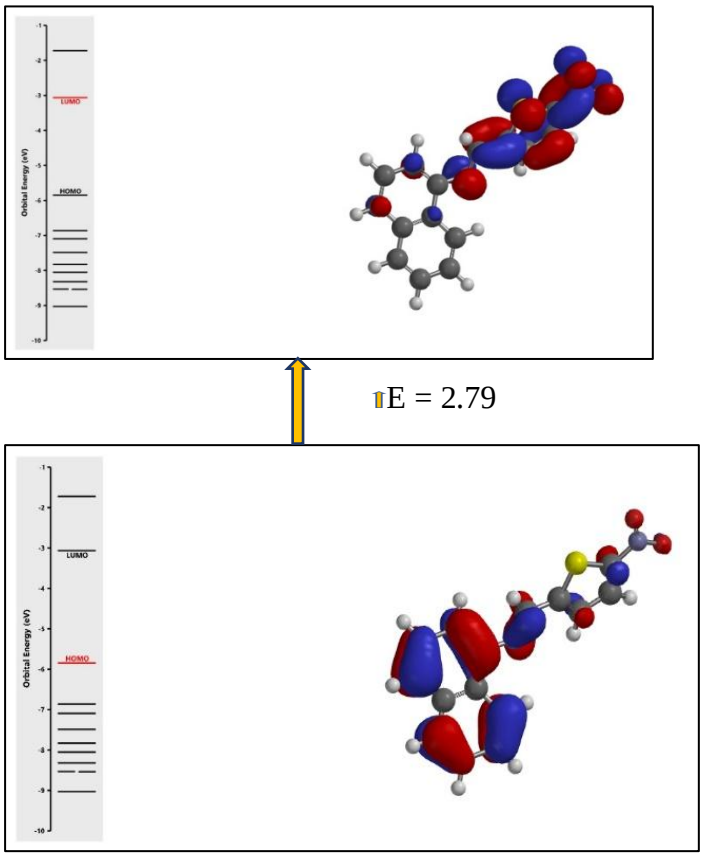
Table 4: HOMO and LUMO orbitals energy values for 5-nitrophene-2carboxaldehyde derived ligands

S.no	Label	E HOMO (eV)	E LUMO (eV)	ΔE =(I-A) BAND GAP
1	3b	-6.51	-3	3.51
2	3c	-6.26	-3	3.26
3	3a	-5.85	-3.06	2.79

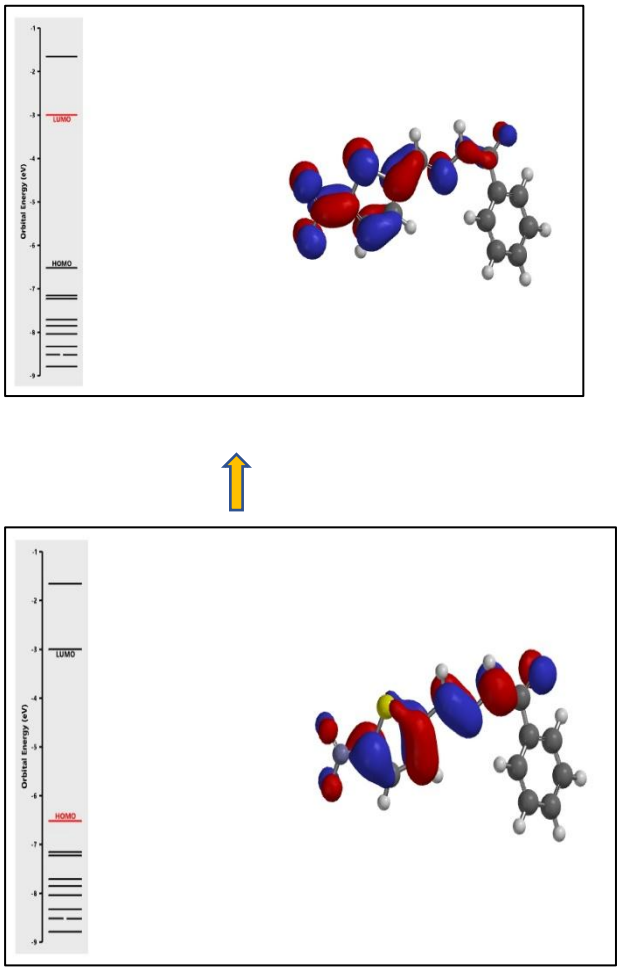
Table 5: Chemical reactivity descriptors

Ionisation potential	Electron affinity	Electronegativity	Global hardness	Softness	Electrophilicity
I= [-E HOMO]	A= [-E LUMO]	$\chi=(I+A)/2$	$\eta=(I-A)/2$	$\delta=1/\eta$	$\omega=\chi^2/2\eta$
6.51	3	4.755	1.755	0.569800	6.4416025
6.26	3	4.63	1.63	0.613496	6.575736
5.85	3.06	4.455	1.395	0.716845	7.113629

3a


LUMO
HOMO
Orbital Energy (eV)
E = 2.79
LUMO
HOMO
Orbital Energy (eV)

3b



E = 3.51

3c

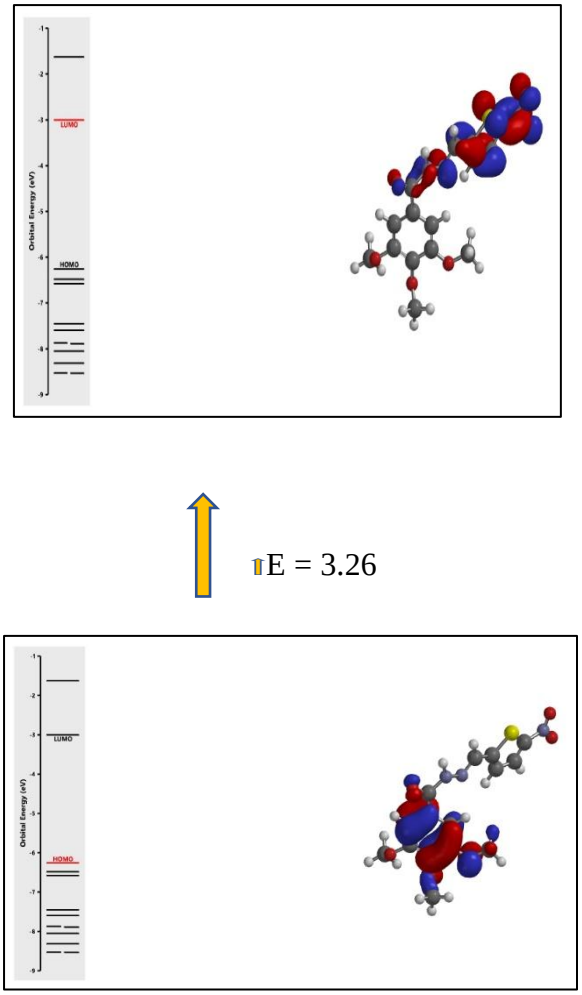


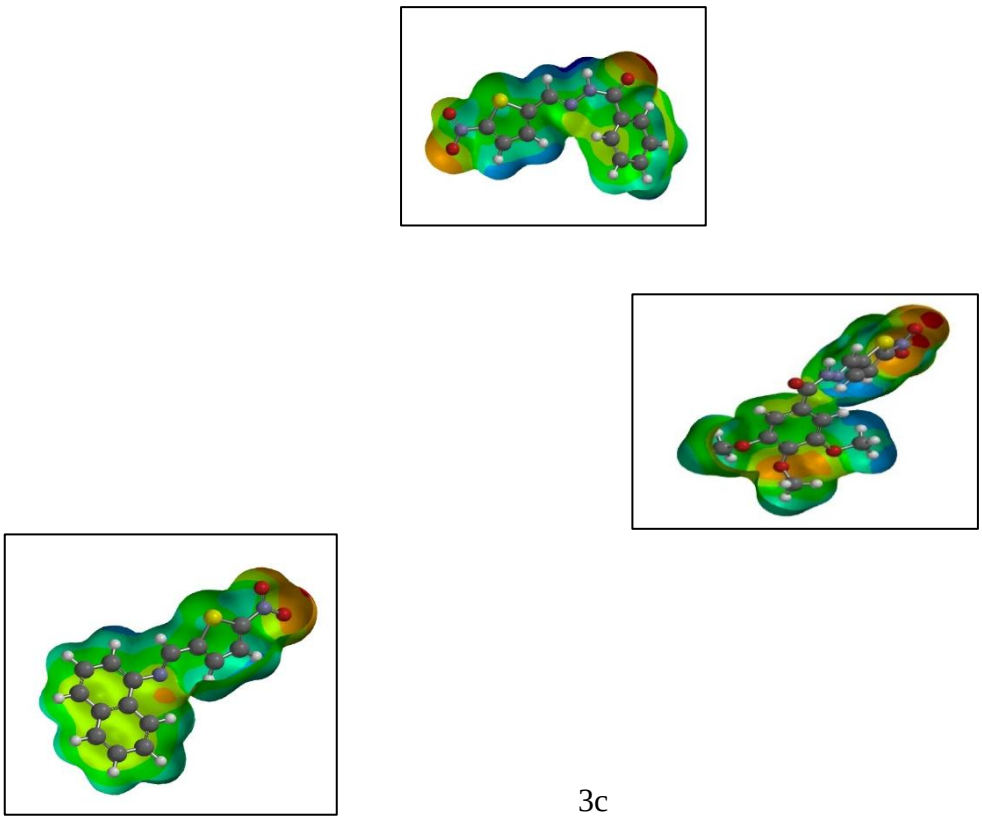
Figure 6: The HOMO and LUMO orbitals and energy gap ΔE of compounds (3a-3c) obtained by the DFT / B3LYP method

Molecular Electrostatic Potential Analysis:

Molecular electrostatic potential (MEP) provides information about the net electrostatic effect produced at that point by total charge distribution (electron + proton) of around the molecule Furthermore, MEP surface helps to identify the reactivity of wide variety of chemical systems in both electrophilic and nucleophilic reactions, hydrogen bonding interactions and the study of biological recognition processes. It also helps visual understanding of relative polarity of the molecule. An electron density iso-surface mapped with electrostatic potential surface predicts the size, shape, charge density and reactive sites of the molecules. The different values of the electrostatic potential at the surface are represented by different colours; red indicates regions of most electro negative electrostatic potential, blue indicates regions of most positive electrostatic potential and green indicates regions of zero potential. The electrostatic potential increases in the order: red < orange < yellow < green < blue.

To predict the reactive sites for electrophilic and nucleophilic attack of our Schiff's base molecules, MEP surface is plotted using DFT / B3LYP basic set. Figure 7 shows the Molecular Electrostatic Potential maps of our ligands. As seen, the ligand molecules have several possible sites for electrophilic (the electrophilic sites are most electro negative and are represented as red color) and nucleophilic attack (the nucleophilic sites are most positive and are represented as blue color). We concluded that, the oxygen and nitrogen atom behave as an electrophilic region and it denoted in red color. Likewise, the nucleophilic region was in blue color. The regions over the rings are neutral as represented in green color. These regions give information about intermolecular interactions.

3a 3b



3c

Figure 7: Molecular Electrostatic Potential maps for Ligands 3a, 3b, 3c. The blue colour represents the positive region and the red colour represents the negative region. They were obtained employing Spartan'20 Software

4. Molecular docking studies:

By predicting the non-covalent interaction between the drug molecule and the receptor, molecular docking is a crucial method for the rational design of novel chemotherapeutic medicines. The structure-based computer-assisted drug discovery is a powerful tool for developing concepts based on precise projected ligand or protein interactions and ligand binding poses, which may be used to guide the synthesis of improved small molecules. It is crucial that computational ligand docking methods accurately identify protein ligand

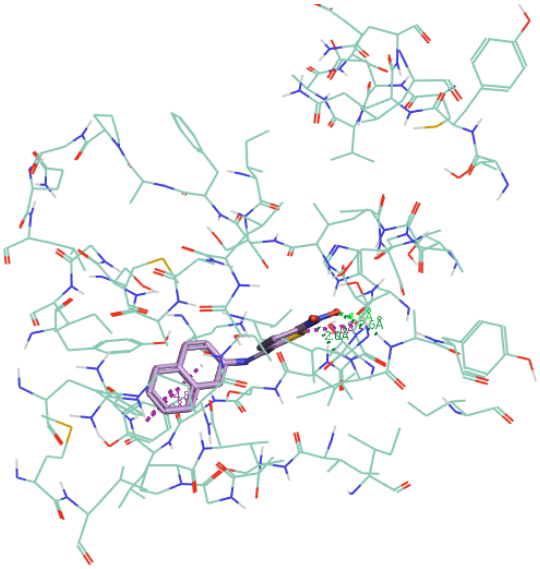
interactions.In order to determine the energy, hydrogen bonds, and hydrophobic interaction between the chemicals and the receptor, the conformation of the docked small molecule (ligand) was examined. The lowest energy was used to suggest the compound's ideal position inside the receptor, and better affinity between the drug molecule and the macromolecule was used to determine the compound's ideal position. 5 nitrothiophene-2-carboxaldehyde is integrated into many therapeutically significant compounds in medicinal chemistry. Particularly, it has been shown that compounds containing the 5-nitro-thiophene moiety have significant chemotherapeutic effects, such as antibacterial, antifungal, and anticancer characteristics.

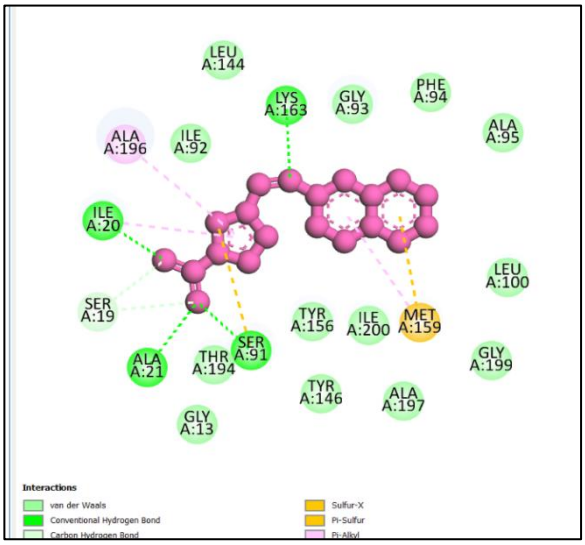
To better understand ligand-protein interaction, molecular docking experiments on synthetically produced 5-nitro thiophene-2-carboxaldehyde derived compounds with proteins (PDB-1C14) were conducted using the FLARE V5 software, an interactive molecular docking programme. Synthesized substances and proteins interact. Protein-binding ligands were discovered to have LF dG scores between -7.5 and -6.0 kcal/mol. The compound 3a, one of the three newly produced ligands, exhibits strong binding energies to the target protein. The one or more amino acids in the receptor active pockets have created linkages, according to the docking of receptor 1c14 with newly produced potential ligands. The 2D and 3D interactions and ligands superimposability are shown in Figures 8, 9. The binding energies and interaction of the three compounds are listed in Table 6 and 7. According to the experiments, all of the compounds had a reasonable amount of binding energy between -7.7 and -6.89 kJ mol^{-1}towards the target protein.

Table 6: Molecular docking results of synthesized compounds against enoyl ACP reductase enzyme (PDBID:1c14)

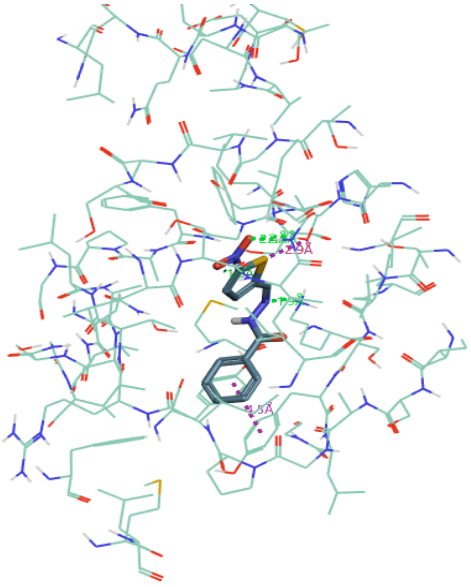
S.NO	Ligand	LF rank score	LF dG	LFV Score	LF LE
1.	3a	-9.553	-7.58	-8.442	-0.379
2.	3b	-7.89	-6.896	-7.836	-0.363
3.	3c	-8.736	-7.65	-8.719	-0.306

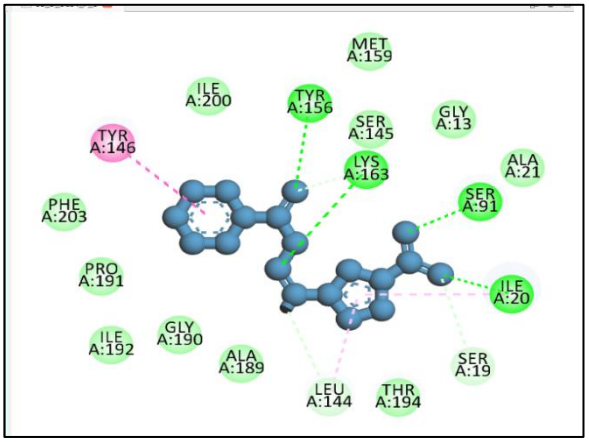
3a




LEU A:144
LYS A:163
GLY A:93
PHE A:94
ALA A:196
ILE A:92
ALA A:95
ILE A:20
LEU A:100
SER A:19
TYR A:156
ILE A:200
MET A:159
ALA A:21
THR A:194
SER A:91
GLY A:199
TYR A:146
ALA A:197
GLY A:13
Interactions
van der Waals
Conventional Hydrogen Bond
Carbon Hydrogen Bond
Sulfur-X
Pi-Sulfur
Pi-Alkyl

3b




MET A:159
ILE A:200
TYR A:156
SER A:145
GLY A:13
TYR A:146
LYS A:163
ALA A:21
PHE A:203
SER A:91
PRO A:191
ILE A:20
GLY A:190
ILE A:192
ALA A:189
SER A:19
LEU A:144
THR A:194

3c

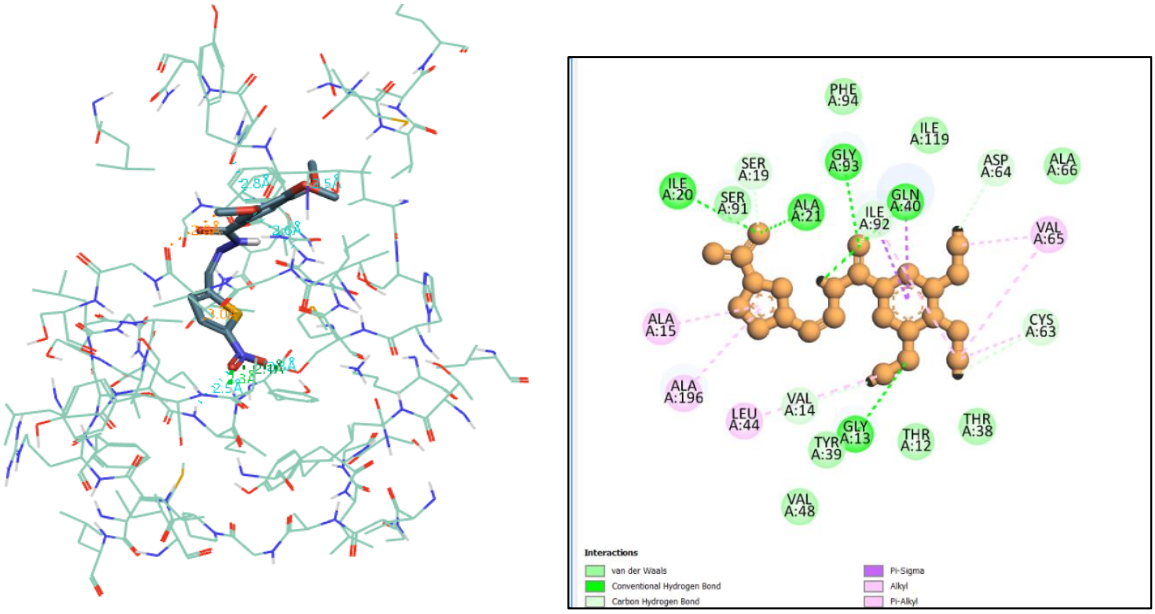


Figure 8 Computational studies;3D and 2D interaction between active site residues of 1c14 and 5-Nitro thiophene -2- carboxaldehyde Schiff base derivatives (3a-3c).

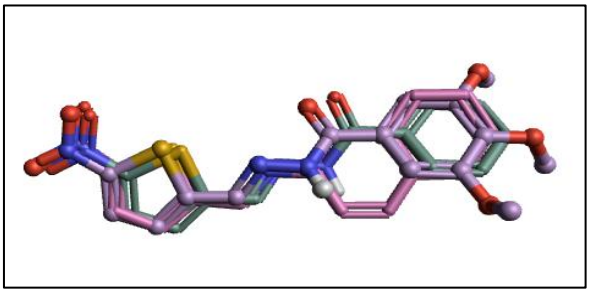


Figure 9 Schiff base Ligands (3a-3c) superimposed

Table-7 Molecular docking results of Synthesized ligands and its interactions

S.No	Ligands	Binding affinity (Kcal/mol)	Hydrogen Bonding	Residual Interactions	Vander walls Interactions
3a	$C_{15}H_{10}N_2O_2S$	**-7.673**	**Ala21, Ser91, Ile20, Lys163.**	**Pi-alkyl – ala196** **Sulphur-X- Met159**	**Ile92, leu144, gly93, phe94, ala95, leu100, gly199, ala197,**
3b	$C_{12}H_9N_3O_3S$	-6.734	Tyr156, Lys163, Ser91, Ile20	Pi-pi stacked- tyr146.	Phe203, pro191, ile192, gly190, ala189, thr-194, ala21, gly13, ser145, met159, ile200.
3c	$C_{15}H_{15}N_3O_6S$	-8.573	Ala21, Ile20, Gly93, Gln40, Gly13	Pi-alkyl- ala196, ala15. Alkyl-alkyl- val65, cys63, leu44	Ala66, ile119, phe94, ser91, ser19, val48, thr12, thr38, tyr39, ile92.

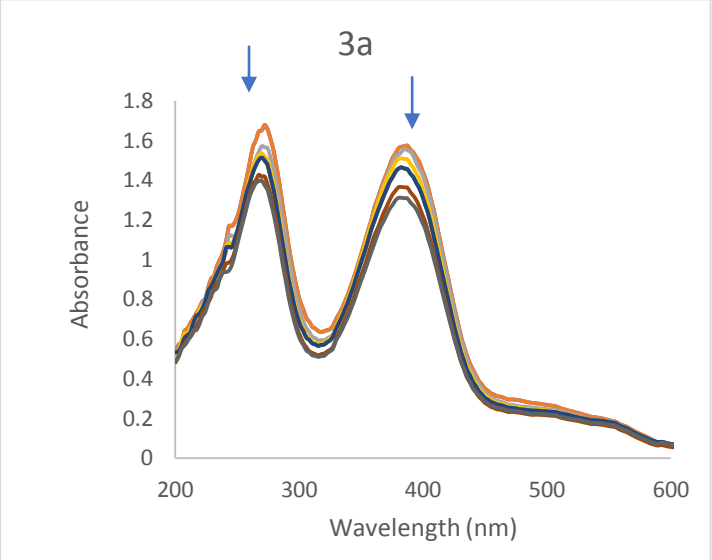
5. DNA binding studies:

Electronic absorption spectroscopic studies: One of the most trustworthy methods for determining the binding affinities and modes of metal complexes with CT-DNA is the electronic absorption investigation. This study varies the CT-DNA absorption titration concentration while maintaining a constant Schiff base concentration.A Schiff base absorption spectra typically exhibits a red shift and hypochromism, which indicates that the mode of binding between the compounds and DNA is intercalation. By observing the change in 5-Nitro thiophene-2-carboxaldehyde Schiff base derivative absorbance when the concentration of CT-DNA was raised, the binding tendency was ascertained. Figure 10a displays the 3a absorption spectra. Due to a strong stacking contact between the aromatic chromophore of the 5-Nitro thiophene-2-carboxaldehyde Schiff base derivatives and the neighbouring base pairs of DNA, absorbance reduced (hypochromism) and wavelength moved towards long wavelength (red shift) when the concentration of CT-DNA in the compounds increased(Fig.10a).The degree of the intercalative binding frequently reflects the extent of the hypochromism. The electronic absorption spectra of 3a (300nm, 420nm), 3b (270nm , 386nm) , 3c (274nm,399nm) show intense absorption bands. The intrinsic binding constant Kb of the complexes with CT-DNA was established in accordance with the equation 1 in order to compare the quantitative DNA binding propensity of the compounds.

$$[DNA]/ (\varepsilon a - \varepsilon f) = [DNA]/(\varepsilon b - \varepsilon f) + 1/Kb(\varepsilon b - \varepsilon f) \quad (1)$$

Here, Kb is the binding constant, [DNA] is the concentration of DNA in the base pairs, εa is apparent coefficient equal to Aobsd/[complex], εf and εb correspond to the extinction coefficients of the free and fully bound forms of the compound, respectively. Using this formula, the binding constants Kb were calculated and found to be, $1.57 \pm 0.16 \times 10^5$ M^{-1} for

compound 3a, 1.09 ±0.16×10^4 $M^{-1}$for compound 3b,1.0±0.16×$10^4M^{-1}$for compound 3c.This absorption spectral result reveals that the Schiff base (3a) have greater binding affinity than other two compounds.



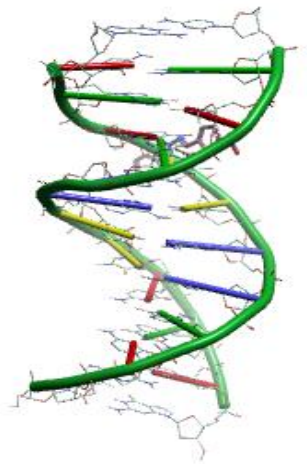


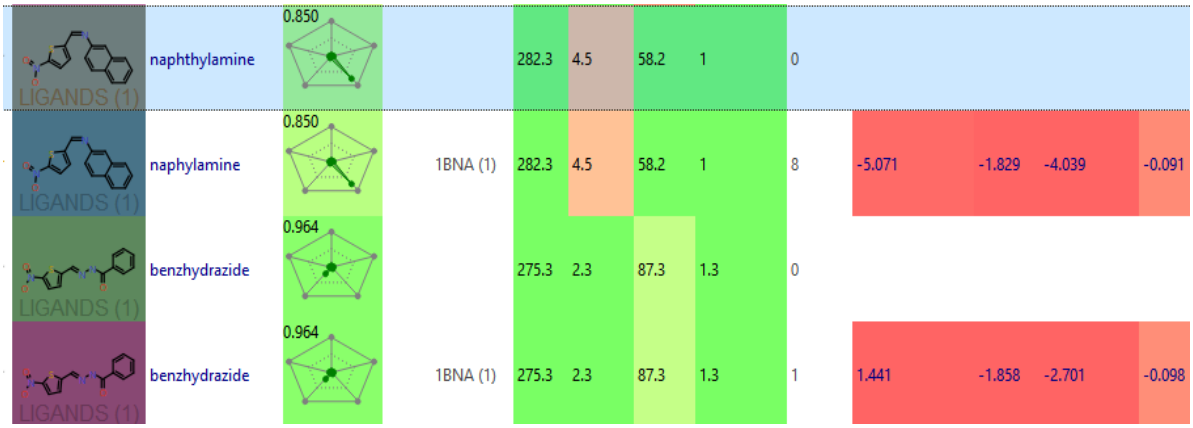
Figure10a Absorption spectra of 5-nitrophene-2-carboxaldehyde Schiff base derivatives 3a-3c (In Tris-HCl/NaCl buffer) on addition of increasing concentration of CT-DNA (0-260µl). Arrow shows changes in absorbance upon increasing amounts of CT DNA.

Figure 10b Molecular docking interactions of synthesized compounds (3a) with CT- DNA (PDB ID 1BNA)

Molecular Docking Studies

Molecular docking is the most suitable way for theoretical understanding of molecular mechanism and for the elucidation of binding mode/modes of a compound with DNA through non-covalent interactions. This theoretical approach is considered most appropriate for structure based- drug design. Molecular docking of compounds (3a,3b) was performed to predict its binding with DNA. The docked conformation of the compounds with LFdG Score ranging from -2.50 to - 5.5 kcal/mol shown in Table 5 and best pose view images are shown in Fig. 10b. Results obtained from the molecular docking study demonstrated that among the synthesized derivatives **3a** showed a strong binding affinity towards DNA (1BNA). The non-covalent interactions include H-bond, pi-pi stacking, pi-cation and pi-donor. The compound 3a with highest LFdG Score was found to form H-bonds with DT A8, DC A9, Aromatic Pi-Pi, Pi-cation, Pi-anion interactions with DG A10 , DC A11, DT B19, DT B20, were other important bindings observed. The results obtained suggest that among the synthesized derivatives 3a might be used as good intercalating agent.

Table 8 Molecular Docking data of Synthesized Compounds (3a, 3b) against CT- DNA (PDB ID 1BNA)



Conclusions:

In light of the large decrease in the use of organic solvents, which has led to less toxic residues, this microwave-assisted synthesis method appears to be more environmentally friendly. With the help of microwave irradiation, our current research has developed a unique approach for the synthesis of 5-Nitro thiophene -2- carboxaldehyde derived Schiff bases with high percentage yield and less reaction time. The study shows that among three compounds 3a showed high chemical reactivity and moderate antibacterial property both from invitro and docking studies. DNA binding studies were carried out both experimentally and theoretically and showed that out of three compounds synthesised two compounds (3a, 3b) showed good binding interactions. The results are very encouraging, so we can say that these products exhibited weak antibacterial activities, so more structural modifications are required to enhance the efficacy of the synthesized compounds.

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Conflicting interests: The authors declares that the publishing of this paper does not include any conflicts of interest.

DATA AND SOFTWARE AVAILABILITY

DFT calculations are done using trial version of SPARTAN 20 parallel suite software from Wavefunction, Inc.18401 Von Karman Ave., Suite 435, Irvine. Molecular Docking studies are done using Flare V5 CRESSET, New Cambridge House, Bassingbourn Rd, Litlington, Royston SG8 0SS, United Kingdom. The Department of Chemistry, St.Francis College for Women, Begumpet , Hyderabad , India, purchased term license of Cresset Flare V5 software from suppliers ***Neotel Systems & Services***. SCO 409-410, Sector 35-C. Chandigarh, India.

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