Asian Pub. Corp.

www.asianpubs.org

ARTICLE

Microwave Assisted Synthesis and Computational Approach of 5-Nitrothiophene-2-carboxaldehyde Derived Schiff Bases as Antibacterial Agents

Susmitha Kasula✉ and Sanem Gyaneshwari

Asian Journal of Organic & Medicinal Chemistry

Volume: 7 Year: 2022
Issue: 3 Month: July–September
pp: 273–279
DOI: https://doi.org/10.14233/ajomc.2022.AJOMC-P399

Received: 28 July 2022
Accepted: 21 September 2022
Published: 30 September 2022

Author affiliations:

Department of Chemistry, St. Francis College for Women, Begumpet, Hyderabad-500016, India

✉To whom correspondence to be addressed:

E-mail: susmithakasula@gmail.com

Available online at: http://ajomc.asianpubs.org

ABSTRACT

Schiff bases were synthesized by condensation of the aldehyde group of 5-nitro thiophen-2-carboxaldehyde with different fluoro substituted aromatic primary amines. All the synthesized compounds (**3a-c**) were characterized by various spectral techniques and the completion of reaction were confirmed by TLC. *In vitro* antimicrobial activity of the synthesized compounds was evaluated using minimum inhibitory concentration against Gram-positive and Gram-negative microbial strains. The results of antimicrobial study revealed that compounds **3a** and **3c** were active and exhibited better inhibitory activities as compared with standard drug levofloxacin. The molecular docking studies have higher binding affinity with the receptors enzymes enoyl-ACP reductase. Density functional theory (DFT) calculations at the B3LYP method and 6-31G(d,p) basis set have been carried out to investigate the equilibrium geometry of the ligands. Moreover, total energy, energy of HOMO and LUMO and MEP and other quantum parameters were also calculated. The DFT calculations suggested the lowest energy gap of the studied compounds, which were chemically reactive and may serve as potential drug candidates.

KEYWORDS

Schiff bases, Microwave synthesis, Fluoro substituted amines, 2-Amino thiazole, 5-Nitrothiophene-2-carboxaldehye, DFT analysis, Molecular docking.

INTRODUCTION

Conventional techniques of organic synthesis typically call for prolonged heating times, complex apparatus setups that are time-consuming and expensive. This drives up the cost of the procedure and causes environmental contamination [1]. Due to its quickness, cleanliness and simplicity of operation, microwave aided processes in solvent or solvent free conditions have grown in popularity [2]. Microwaves are being used in organic/pharmaceutical synthesis by pharmaceutical corporations for drug discovery [3-5].

Ionic liquids have been used as a solvent, co-solvent and/or catalyst in the microwave-assisted organic synthesis [6,7]. 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 [8-10]. Despite these benefits, microwave chemistry has scarcely been applied to the production of new materials. Therefore, a new technique for the quick, controlled synthesis of Schiff base derivatives generated from 5-nitro thiophene has been devised in the current study using microwave heating.

Schiff bases exhibit typical biological behaviours such as antibacterial, antifungal, anticancer and herbicidal capabilities [11-18]. Numerous therapeutically significant drugs contain thiophene nuclei [1-3]. Particularly, compounds with 5-nitro-thiophene moiety have been shown to have therapeutic potential due to their antibacterial [4,5,8], antifungal [22-24], antimicrobial [23,24], anticancer [25,26], antiprotozoal [27,28] and antileishmanial [29] activities. Thus, keeping in mind all the aforementioned applications of Schiff bases, in the current study, 5-nitrothiophen derived Schiff bases toward the potential therapeutic target enoyl-ACP reductase enzyme (PDB: 1C14) have also been conducted in addition to microwave assisted synthesis, characterization, antibacterial activities and the molecular docking studies.

EXPERIMENTAL

All reagents and starting chemicals were commercially available and used without additional purification. Synthesis of Schiff bases was performed using a microwave synthesizer. On the pre-coated plates (silica gel 60 F_{254}), analytical thin-layer chromatography (TLC) was performed and spots were noted using ultraviolet light. On a Tensor27 FT-IR spectrometer (Bruker Optics, Germany), FT-IR spectra were analyzed using KBr disc. Trimethyl silane as standard solvent was used for recording ^{1}H NMR spectra on an Avance-III 400 MHz NMR spectrometer (Bruker Biospin, Germany). The LCMS was carried out using a direct insertion probe with an Alignent 6540ba Q to f infinity 1290 M/S Alignent Technology.

General procedure for synthesis of 5-nitrothiophene-2-carboxaldehyde derivatives: A reaction of 5-nitro thiophene-2-carboxaldehyde (**1**, 1.57, 0.01 mol) with 2-aminothiazole (**2a**, 100.1 g, 0.01 mol)/4-fluro aniline (**2b**, 4.8972 g, 0.01 mol)/trifluro-methyl aniline (**2c**), for 15 min at 180 watts in ethanol (15 mL). Following cooling, precipitated crude product was filtered, ethanol was added and it was then recrystallized (**Scheme-I**). The mixture was then refluxed for 10 to 20 min with a water condenser. The TLC was used to check the reaction's development and after 30 min, a single spot was observed, signifying the complete conversion of reactants to products. The resulting product was filtered and recrystallized with absolute ethanol.

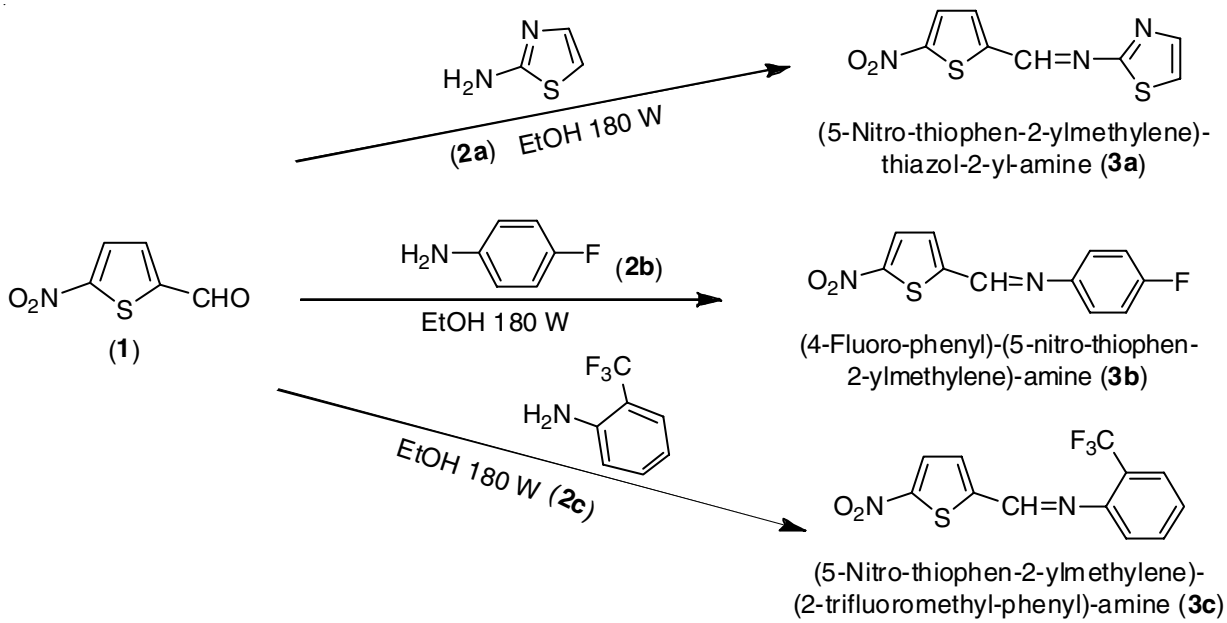
(5-Nitro-thiophen-2-yl methylene)-thiazol-2-yl-amine (3a): Black solid, m.f.: $C_8H_5N_3O_2S_2$, IR (KBr, ν_{max}, cm^{-1}): 3100 (arom. C=H), 1502 (CH=N), 1583 (C=C). ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 9.19 (s, 1H, CH=N), 7.94 (d, 1H, thiazole), 7.5 (d, 1H, thiazole), 7.38 (m, 1H, thiophene), 7.26 (s, 1H, thiophene), UV (360 nm); Mass: *m/z* 239.98.

(4-Fluoro-phenyl)-(5-nitro-thiophen-2-ylmethylene)-amine (3b): Orange solid, m.f.: $C_{11}H_7FN_2O_2S$, IR (KBr, ν_{max}, cm^{-1}): 3116 (arom. C=H), 1591 (CH=N), 1531 (C=C), ^{1}H NMR (300 MHz, $CDCl_3$, δ ppm): 8.53, (s, 1H, CH=N), 7.92 (d, 1H, thiophene), 7.38 (d, 1H, thiophen), 7.27-7.29 (m, 2H, Ar-H), 7.08-7.14 (t, 2H, Ar-H); UV (360 nm); Mass: *m/z* 251.028.

5-Nitro-thiophen-2-ylmethylene)-(2-trifluoromethyl-phenyl)amine (3c): Yellow crystal, m.f.: $C_{12}H_7F_3N_2O_2S$, IR (KBr, ν_{max}, cm^{-1}): 3126 (arom. C=H) , 1537 (CH=N), 1505 (C=C). ^{1}H NMR (300 MHz $CDCl_3$, δ ppm), 8.46 (1s, 1H, CH=N) 7.92 (1d, 1H, thiophene), 7.70 (d, 1H, thiophene), 7.43 (t, 2H, Ar-H) 7.36 (d, 1H, Ar-, H), 7.11 (t, 2H, Ar-H), 7.09 (d, 1H, Ar-H), UV (390 nm); Mass: *m/z*: 301.025.

Biological activity

Determination of minimum inhibitory concentration: The *in vitro* antimicrobial activity of the synthesized compounds was assessed against *Staphylococcus aureus* 29213, *Escherichia coli* 25922, *Pseudomonas aeruginosa* 27853, *Acinetobacter baumannii* 1605 and *Klebsiella pneumoniae*



Scheme-I: Synthesis of 5-nitrothiophene-2-carboxaldehyde derived Schiff bases (**3a-c**)

1705 using the MIC technique. By using the serial dilution approach, the MIC values of the synthesized compounds (**3a-c**) were identified in accordance with the established CLSI recommendations [30]. In brief, Mueller-Hinton cation-supplemented broth was used to cultivate the bacterial cultures. After measuring the optical density (OD_{600}) of the cultures, the concentration was diluted to ~ 10^6 cfu/mL. This inoculum was put into a number of test wells in a microtitre plate containing test compound concentrations ranging from 64 to 1 µg/mL. Levofloxacin was used as a reference standard and the cells with media (without chemical or cells) serve as controls. Plates were incubated at 37 ºC for 16-18 h, after which the MIC values were determined. The MIC values were calculated separately in triplicate.

Quantum computational studies: Utilizing Spartan 20 software, the DFT analysis of the synthesized Schiff bases (**3a-c**) was carried out. Using a B3LYP/6-31G(d,p) level basis set without any symmetrical constraints, the structural coordinates were optimized. The improved geometry yielded the compounds' energies and molecular electrostatic potential map. The energy gap, related reactive parameters and the highest and lowest occupied molecular orbitals (HOMO and LUMO) were all estimated using the Koopman's approach (electronegativity, chemical potential, hardness, softness, electrophilicity).

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. Using the graphical user interface application FLAREV5 from CRESSET, U.K., the docking stimulation was set up, carried out and assessed.

Preparation of protein: The protein was prepared using FLAREV5 and the PDB structure (1c14) and 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: Structures of 5-nitrophene-2-carboxaldehyde derivatives were drawn using Chem Draw and saved in mol2 format and then convert into three-dimensional structure and minimize the ligand geometry.

RESULTS AND DISCUSSION

All the synthesized Schiff base derivatives of 5-nitro thiophene-2-carboxaldehyde (**3a-c**) using different fluoro substituted aromatic primary amines were successfully synthesized and characterized. All of the spectral data of the synthesized Schiff bases (**3a-c**) were found to be compatible with the anticipated results. The characteristic absorption bands were visible in the IR spectra at 3100, 3116, 3126, 1502, 1591, 1537, 1583 and 1531 cm^{-1} regions, indicating the existence of aromatic (C-H), azomethine (C-H), (C=N) and (C=C), respectively. A band at 1650-1500 cm^{-1}, a significant absorption band for (C=N) also appeared. The stretching frequency of the aromatic ring was represented by the absorption bands at 3139-3012 and 1568–1547 cm^{-1}.

Additionally, in the ^1H NMR spectra of Schiff bases, we found a singlet of integration intensity 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 of thiophene, thiazole and aromatic ring yield numerous signals.

Biological studies: The most substantial activity was shown by compounds **3a** and **3b**. Additionally, compound **3a**, which had a MIC range of 32, 8 and 16 µg/mL, displayed action against *E. coli*, *S. aureus* and *A. bahummani* that was comparable to that of conventional levofloxacin among all synthesized 5-nitro thiophene-2-carboxaldehyde derivatives. Additionally, compound **3b** also showed good efficacy against *K. puneumonia*, *A. baumannii*, *S. aureus* and *E. coli*, with a MIC value of 64. None of the bacterial strains are affected by compound **3c** (Table-1). Thus, as compared to standared levofloxacin, thiazole (**3a**) and fluro (**3b**) derivatives of 5-nitro thiophene-2-carboxaldehyde showed the best antibacterial efficacy against strains of organism.

Molecular calculations: The molecular chemical stability is indicated by the energy difference between HOMO and LUMO. Tables 2 and 3 provide a summary of the molecular characteristics and HOMO and LUMO energies determined using the B3LYP/6-31G basis set computation. The findings revealed that every molecule had the smallest energy gap (E),

TABLE-1
ANTIBACTERIAL BIOASSAY

Compound No.	*E. coli* ATCC 25922	*S. aureus* ATCC 29213	*K. pneumonia* BAA 1705	*A. bahmani* BAA 1605	*P. aeruginosa* ATCC 27853
3a	32	8	> 64	16	> 64
3b	16	8	64	8	> 64
3c	> 64	> 64	> 64	> 64	> 64
Levofloxacin	0.0156	0.125	64	4	1

TABLE-2
CHEMICAL REACTIVE DESCRIPTORS FOR SYNTHESIZED COMPOUND (**3a-c**)

Compd. No.	E_{HOMO} (eV)	E_{LUMO} (eV)	ΔE = (I-A) band gap	Ionization potential	Electron affinity	Electronegativity	Global hardness	Softness	Electrophilicity
				$I = [-E_{HOMO}]$	$A = [-E_{LUMO}]$	$\chi = (I+A)/2$	$\eta = (I-A)/2$	$\delta = 1/n$	$\omega = \chi^2/2\eta$
3a	6.42	3.27	3.15	6.42	3.27	4.845	1.575	0.63492063	7.452071429
3b	6.34	3.14	3.20	6.34	3.14	4.740	1.600	0.62500000	7.021125000
3c	6.68	3.17	3.51	6.68	3.17	4.925	1.755	0.56980057	6.910434473

TABLE-3
MOLECULAR DOCKING RESULTS OF SYNTHESIZED COMPOUNDS AGAINST ENOYL ACP REDUCTASE ENZYME (PDBID:1c14)

Ligand	LF rank score	LF dG	LFV score	LF LE
3a	-7.280	-7.470	-7.609	-0.499
3b	-7.776	-6.802	-7.896	-0.400
3c	-6.620	-6.219	-7.467	-0.310

pointing to great chemical reactivity and significant intramolecular charge transfer from an electron donor (HOMO) to electron acceptor (LUMO) group. These correspond to the energies of ligand **3a** (-6.42 eV and 3.27 eV), ligand **3b** (-6.34 eV and -3.13 eV) and ligand **3c** (-6.68 eV and -3.17 eV). According to the computed HOMO-LUMO energy gap value for ligand **3a**, compound **3a** is more reactive at 3.15 eV. As compared to ligands **3a**, **3b** and **3c** have the energy gap values of 3.2 and 3.51 eV, respectively (Table-4). To comprehend the bonding structure of existing compounds, the surfaces for the frontier orbitals are shown in Fig. 1.

Molecular electrostatic potential analysis: The MEP surface is displayed using the B3LYP/6-31G basis set to anticipate the reactive sites for the synthesized Schiff base mole-

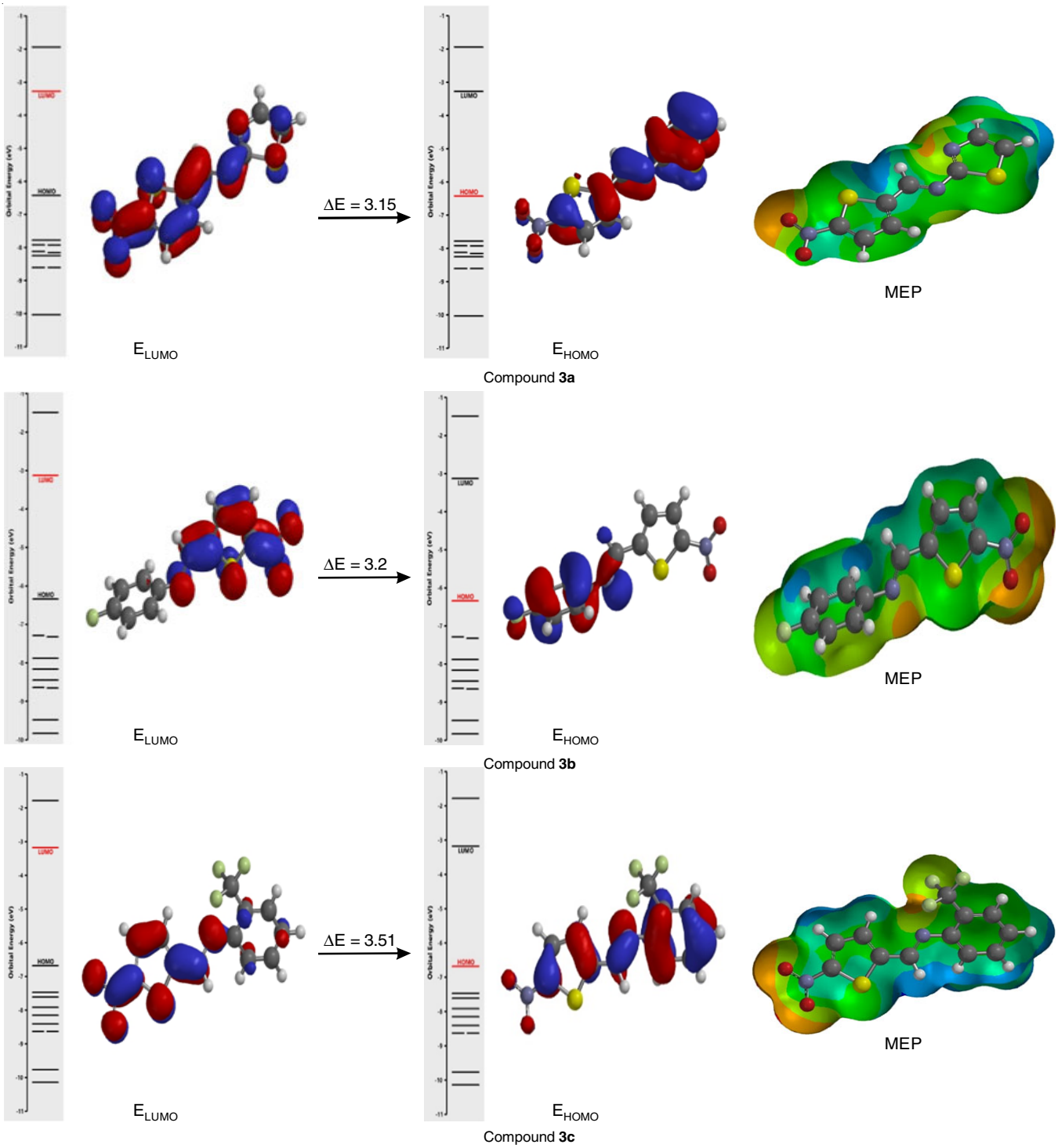
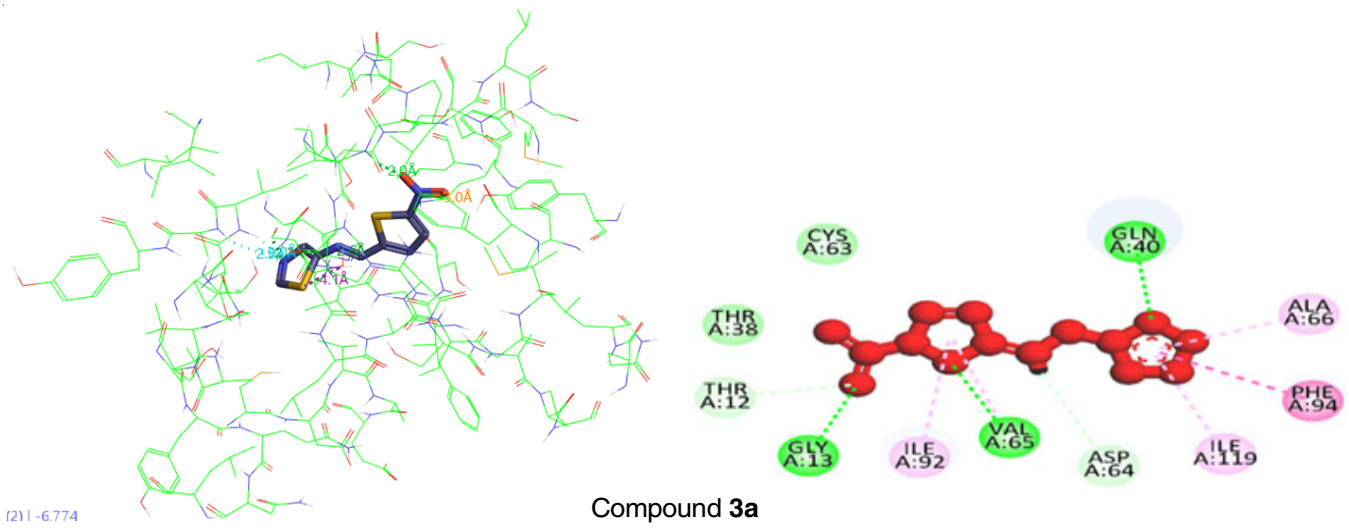


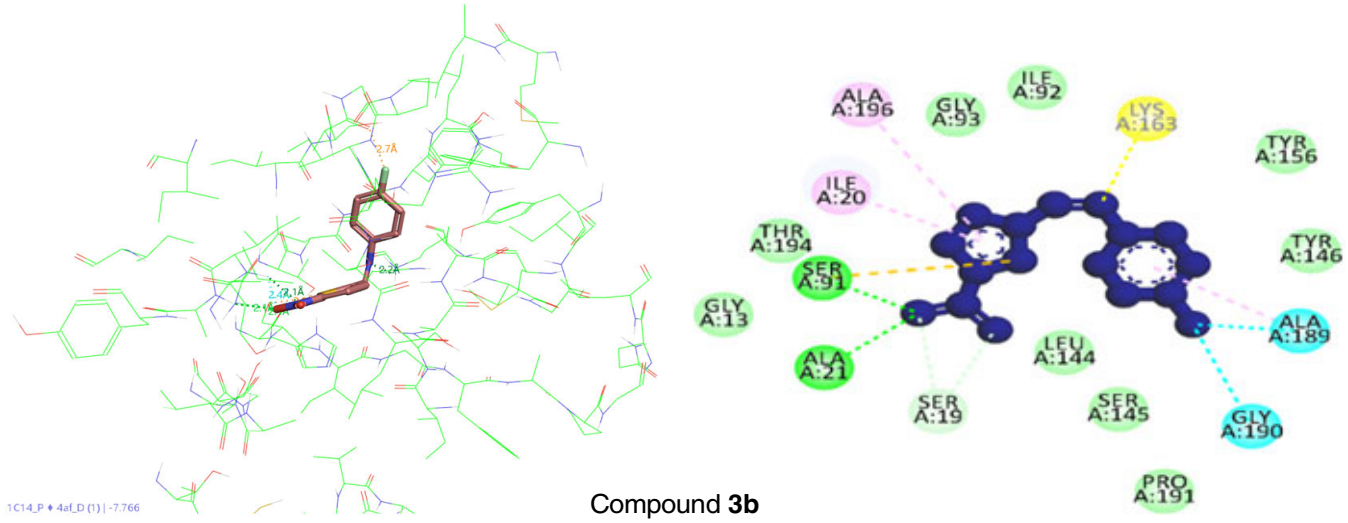
Fig. 1. HOMO, LUMO orbitals and MEP for compounds **3a-c**. The images were achieved by SPARTAN'20 software

TABLE-4
QUANTUM-CHEMICAL PARAMETERS OF SYNTHESIZED COMPOUNDS (**3a-c**) COMPUTED WITH DFT AT B3LYP/6-31G LEVEL

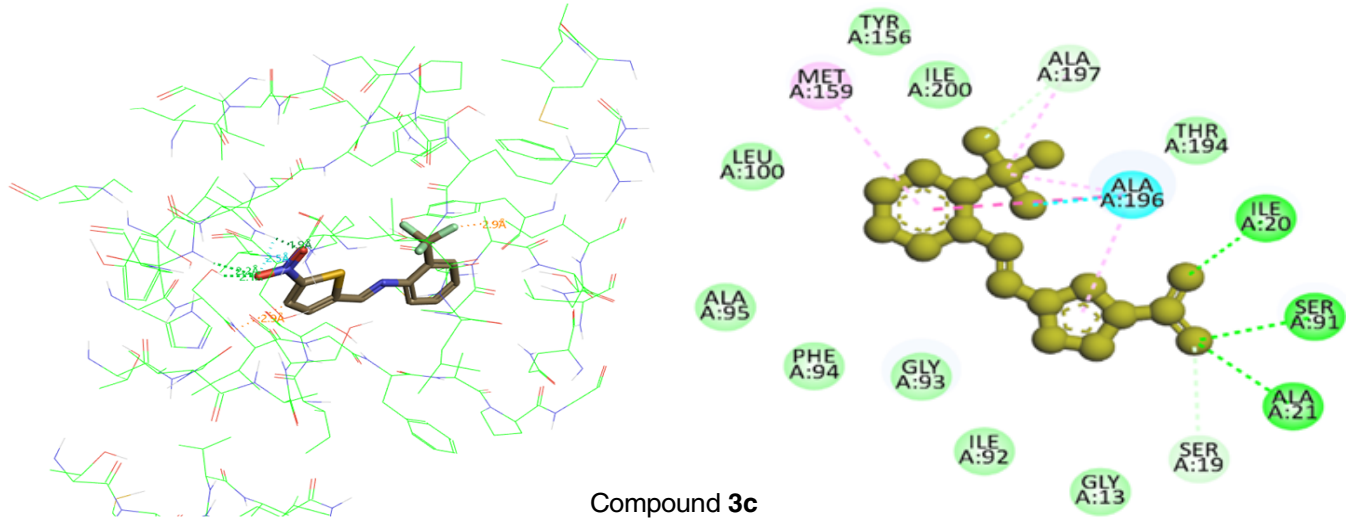
Compd. No.	Energy (amu)	E_{HOMO} (eV)	E_{LUMO} (eV)	Dipole (debye)	log P	Polarizability	HBA count	HBD count
3a	-1418.81	-6.42	-3.27	5.40	3.26	56.81	0	7
3b	-1181.23	-6.34	-3.13	4.69	4.37	58.73	0	5
3c	-1419.04	-6.68	-3.17	5.29	5.13	60.86	5	0



Compound **3a**



Compound **3b**



Compound **3c**

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

cules' electrophilic and nucleophilic attack. The molecules have a number of potential sites for electrophilic and nucleophilic attack (the electrophilic sites are most electronegative and are depicted as red hue) (the nucleophilic sites are most positive and are represented as blue colour). The red colour indicated an electrophilic area, where the oxygen and nitrogen atoms behave. The nucleophilic area was coloured blue as well. As indicated by the green colour, the areas above the circles are neutral. Intermolecular interactions are shown by these regions.

Molecular docking studies: In order 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. The protein-binding ligands were discovered to have LF dG scores between -7.5 and -6.0 kcal/mol. 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 10 amino acid residues in the active site were Ser91, Ala21, Lys163, Gly93, Ile92, Asp64, Gln40, Thr194, Val65 and Met159. The molecules of the synthesized 2D and 3D ligands obtained from 5-nitro thiophene-2-carbaoxaldehyde are shown in Fig. 2, whereas the binding energies of three Schiff base compounds are displayed in Table-4. All of the compounds had a reasonable amount of binding energy between -7.5 and -6.0 kJ mol^{-1} towards the target protein.

Conclusion

Using microwave irradiation, a unique approach for the synthesis of three Schiff bases **3a-c** was developed, which had significant improved yield percentage and reaction time. All the synthesized Schiff bases were characterized using UV, ^{1}H NMR, IR and mass spectra. The *in vitro* antibacterial activity of the synthesized compounds against Gram-positive and Gram-negative microbial strains were also evaluated, where compounds **3a** and **3c** were active and shown stronger inhibitory activities than the standard antibiotic levofloxacin. The synthesized compounds were also docked *in silico* against the protein enoly ACP reductase enzyme (PDB: 1c14). Furthermore, according to DFT studies, the energy gap between the three ligands was identical, which suggests that the investigated compounds had the lowest gap energies and were chemically reactive.

ACKNOWLEDGEMENTS

The authors thank the Management of St. Francis College for Women, Osmania University, Hyderabad, India for providing the research facilities. Thanks are also due to the Central Facilities for Research and Development (CFRD), Osmania University and NIPER, Hyderabad, India for providing the necessary spectral analyses facilities.

REFERENCES

1. J.F. Collados, E. Toledano, D. Guijarro and M. Yus, Microwave-Assisted Solvent-Free Synthesis of Enantiomerically Pure *N*-(*tert*-Butylsulfinyl)-imines, *J. Org. Chem.*, **77**, 5744 (2012); https://doi.org/10.1021/jo300919x
2. B, Borah, K.D. Dwivedi, B. Kumar and L.R. Chowhan, Recent Advances in the Microwave- and Ultrasound-Assisted Green Synthesis of Coumarin-Heterocycles, *Arab. J. Chem.*, **15**, 103654 (2022); https://doi.org/10.1016/j.arabjc.2021.103654
3. F. Mavandadia and A. Pilotti, The Impact of Microwave-Assisted Organic Synthesis in Drug Discovery, *Drug Discov. Today*, **11**, 165 (2006); https://doi.org/10.1016/S1359-6446(05)03695-0
4. M. Larhed, C. Moberg and A. Hallberg, Microwave-Accelerated Homogeneous Catalysis in Organic Chemistry, *Acc. Chem. Res.*, **35**, 717 (2002); https://doi.org/10.1021/ar010074v
5. B. Floris, F. Sabuzi, P. Galloni and V. Conte, *Catalysts*, **7**, 261 (2017); https://doi.org/10.3390/catal7090261
6. M.B. Gawande, S.N. Shelke, R. Zboril and R.S. Varma, Microwave-Assisted Chemistry: Synthetic Applications for Rapid Assembly of Nanomaterials and Organics, *Acc. Chem. Res.*, **47**, 1338 (2014); https://doi.org/10.1021/ar400309b
7. P. Lidstrom, J. Tierney, B. Wathey and J. Westman, Microwave Assisted Organic Synthesis: A Review, *Tetrahedron*, **57**, 9225 (2001); https://doi.org/10.1016/S0040-4020(01)00906-1
8. R. Martinez-Palou, Ionic Liquid and Microwave-Assisted Organic Synthesis: A Green and Synergic Couple, *J. Mex. Chem. Soc.*, **51**, 252 (2007).
9. S. Balasubramanian, G. Aridoss, P. Parthiban, C. Ramalingan and S. Kabilan, Synthesis and Biological Evaluation of Novel Benzimidazol/Benzoxazolylethoxypiperidone Oximes, *Biol. Pharm. Bull.*, **29**, 125 (2006); https://doi.org/10.1248/bpb.29.125
10. S. Savithiri, M.A. Doss and R.V. Thanikachalam, Synthesis, Spectral, Stereochemical and Antimicrobial Evaluation of Some 3t-pentyl-2r,6c-diarylpiperidin-4-one Thiosemicarbazone Derivatives, *Can. Chem. Trans.*, **2**, 403 (2014).
11. A.A.A. Abu-Hussen, Synthesis and Spectroscopic Studies on Ternary bis-Schiff-base Complexes having Oxygen and/or Nitrogen Donors, *J. Coord. Chem.*, **59**, 157 (2006); https://doi.org/10.1080/00958970500266230
12. M.S. Karthikeyan, D.J. Prasad, B. Poojary, K. Subrahmanya Bhat, B.S. Holla and N.S. Kumari, Synthesis and Biological Activity of Schiff and Mannich Bases bearing 2,4-Dichloro-5-fluorophenyl Moiety, *Bioorg. Med. Chem.*, **14**, 7482 (2006); https://doi.org/10.1016/j.bmc.2006.07.015
13. K. Singh, M.S. Barwa and P. Tyagi, Synthesis, Characterization and Biological Studies of Co(II), Ni(II), Cu(II) and Zn(II) Complexes with Bidentate Schiff Bases Derived by Heterocyclic Ketone, *Eur. J. Med. Chem.*, **41**, 147 (2006); https://doi.org/10.1016/j.ejmech.2005.06.006
14. N. Bhattacharyya, D. Dutta and J. Biswas, A Review on Synthesis and Biological Activity of Schiff Bases, *Indian J. Chem.*, **40B**, 225 (2005).
15. S.K. Sridhar, M. Saravanan and A. Ramesh, Synthesis and Antibacterial Screening of Hydrazones, Schiff and Mannich Bases of Isatin Derivatives, *Eur. J. Med. Chem.*, **36**, 615 (2001); https://doi.org/10.1016/S0223-5234(01)01255-7
16. S.N. Pandeya, D. Sriram, G. Nath and E. DeClercq, Synthesis, Antibacterial, Antifungal and Anti-HIV Activities of Schiff and Mannich Bases Derived from Isatin Derivatives and *N*-[4-(4'-chloro-phenyl)-thiazol-2-yl] thiosemicarbazide, *Eur. J. Pharm. Sci.*, **9**, 25 (1999); https://doi.org/10.1016/S0928-0987(99)00038-X
17. R. Mladenova, M. Ignatova, N. Manolova, T. Petrova and I. Rashkov, Preparation, Characterization and Biological Activity of Schiff Base Compounds Derived from 8-Hydroxyquinoline-2-carboxaldehyde and Jeffamines ED®, *Eur. Polym. J.*, **38**, 989 (2002); https://doi.org/10.1016/S0014-3057(01)00260-9
18. O.M. Walsh, M.J. Meegan, R.M. Prendergast and T. Al Nakib, Synthesis of 3-Acetoxyazetidin-2-ones and 3-Hydroxyazetidin-2-ones with Antifungal and Antibacterial Activity, *J. Med. Chem.*, **31**, 989 (1996); https://doi.org/10.1016/S0223-5234(97)86178-8
19. K. Arora and K.P. Sharma, Studies on High-Coordination Complexes of Dioxouranium(VI) sith a Schiff Base, *Synth. React. Inorg. Met.-Org. Chem.*, **32**, 913 (2003); https://doi.org/10.1081/SIM-120005611
20. P.A. Vigato and S. Tamburini, The Challenge of Cyclic and Acyclic Schiff Bases and Related Derivatives, *Coord. Chem. Rev.*, **248**, 1717 (2004); https://doi.org/10.1016/j.cct.2003.09.003

21. T. Katsuki, Catalytic Asymmetric Oxidations using Optically Active (salen)manganese(III) Complexes as Catalysts, *Coord. Chem. Rev.*, **140**, 189 (1995); https://doi.org/10.1016/0010-8545(94)01124-T
22. L.N. de Araújo Neto, M. do Carmo Alves de Lima, J.F. de Oliveira, E.R. de Souza, M.D.S. Buonafina, M.N. Vitor Anjos, F.A. Brayner, L.C. Alves, R.P. Neves and F.J.B. Mendonça-Junior, Synthesis, Cytotoxicity and Antifungal Activity of 5-Nitro-thiophene-thiosemicarbazones Derivatives, *Chem. Biol. Interact.*, **272**, 172 (2017); https://doi.org/10.1016/j.cbi.2017.05.005
23. R.C. Hartkoorn, O.B. Ryabova, L.R. Chiarelli, G. Riccardi, V. Makarov and S.T. Cole, Mechanism of Action of 5-Nitrothiophenes against *Mycobacterium tuberculosis*, *Antimicrob. Agents Chemother.*, **58**, 2944 (2014); https://doi.org/10.1128/AAC.02693-13
24. A. Foroumadi, Z. Kiani and F. Soltani, Antituberculosis agents VIII: Synthesis and *in vitro* Antimycobacterial Activity of Alkyl α-[5-(5-Nitro-2-thienyl)-1,3,4-thiadiazole-2-ylthio]acetates, *Il Farmaco*, **58**, 1073 (2003); https://doi.org/10.1016/S0014-827X(03)00158-7
25. J.R. Rodrigues, J. Charris, J. Camacho, A. Barazarte, N. Gamboa and F. Antunes, Cytotoxic Effects of *N*'-Formyl-2-(5-nitrothiophen-2-yl) benzothiazole-6-carbohydrazide in Human Breast Tumor Cells by Induction of Oxidative Stress, *Anticancer Res.*, **32**, 2721 (2012).
26. K.M. Roque Marques, M.R. do Desterro, S.M. de Arruda, L.N. de Araújo Neto, M. do Carmo Alves de Lima, S.M.V. de Almeida, E.C.D. da Silva, T.M. de Aquino, E.F. da Silva-Júnior, J.X. de Araújo-Júnior, M. de M. Silva, M.D. de A. Dantas, J.C.C. Santos, I.M. Figueiredo, M.-A. Bazin, P. Marchand, T.G. da Silva and F.J.B. Mendonça Junior, 5-Nitro-Thiophene-Thiosemicarbazone Derivatives Present Antitumor Activity Mediated by Apoptosis and DNA Intercalation, *Curr. Top. Med. Chem.*, **19**, 1075 (2019); https://doi.org/10.2174/1568026619666190621120304
27. N. Bharti, K. Husain, M.T. Gonzalez Garza, D.E. Cruz-Vega, J. Castro-Garza, B.D. Mata-Cardenas, F. Naqvi and A. Azam, Synthesis and *in vitro* Antiprotozoal Activity of 5-Nitrothiophene-2-carboxaldehyde Thiosemicarbazone Derivatives, *Bioorg. Med. Chem. Lett.*, **12**, 3475 (2002); https://doi.org/10.1016/S0960-894X(02)00703-5
28. F. Delmas, M. Gasquet, P. Timon-David, N. Madadi, P. Vanelle, A. Vaille and J. Maldonado, Synthesis and *in vitro* Anti-protozoan Activity of new 5-Nitrothiophene Oxime Ether Derivatives, *Eur. J. Med. Chem.*, **28**, 23 (1993); https://doi.org/10.1016/0223-5234(93)90075-P
29. A. Tahghighi, S. Emami, S. Razmi, F. Rezazade Marznaki, S. Kabudanian Ardestani, S. Dastmalchi, F. Kobarfard, A. Shafiee and A. Foroumadi, New 5-(Nitroheteroaryl)-1,3,4-thiadiazols containing Acyclic Amines at C-2: Synthesis and SAR Study for their Antileishmanial Activity, *J. Enzyme Inhib. Med. Chem.*, **28**, 843 (2013); https://doi.org/10.3109/14756366.2012.689297
30. P. Sanna, A. Carta, M. Loriga, S. Zanetti and L. Sechi, Synthesis of Substituted 2-Ethoxycarbonyl- and 2-Carboxyquinoxalin-3-ones for Evaluation of Antimicrobial and Anticancer Activity, *Il Farmaco*, **53**, 455 (1998); https://doi.org/10.1016/S0014-827X(98)00044-5