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Design, DFT Calculations and Antimicrobial Activity of New Synthesized Piperazine Derivative

Sinan Mithat MUHAMMET[1*]

[1]*Department of Polymers Technology, Technology Sciences High School, Gazi University*

Keywords

Piperazine Derivatives
DFT Calculation
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MEP

Abstract

The title compound (2,2'-(piperazine-1,4-diyl)bis(N'-((E)-5-chloro-2-hydroxybenzylidene) acetohydrazide) ***(5-ClPAH)*** was synthesized by reacting 1,4-Piperazinediacetic acid, 1,4-dihydrazide and 5 -Chloro-2-hydroxybenzaldehyde. Mass spectrometry, ^{1}H, ^{13}C-NMR, IR results of the synthesized compound were examined. Many information about physical and chemical properties of ***5-ClPAH*** can be obtained by theoretical calculations. Density functional theory (DFT) is widely used theoretical method for predicting of chemical structures. The structure was optimized using DFT/6311G method with GAUSSIAN09. Frontier Molecular Orbitls (HOMO and LUMO) energies were calculated. Global reactivity descriptors and also electrophilic and nucleophilic regions were defined by molecular electrostatic potential surface. Antibacterial and fungal activity were evaluated.

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S. M. Muhammet, 0000-0001-6250-9476

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1. INTRODUCTION

The imine group (azomethine) of Schiff bases attracted much attention due to widely usage in biological studies and which was use in chemistry (Venkataramana et al., 2010; Yan et al., 2012; Gupta & Goklani, 2017; Ermiş & Durmuş, 2020). Piperazine ring is also an important class of N-heterocyclics having biological activities including antitumor (Xu et al., 2019), antiviral drugs (Hooshmand et al., 2021), antibacterial (Jalagari et al., 2019) and antifungal etc (Suryavanshi & Rathore, 2017) and pharmaceutical applications (Ullh et al., 2022). Piperazine derivatives have been widely used in the production of new drugs in recent years (Shaquiquzzaman et al., 2015).

In this paper, titled compound (5-ClPAH) was obtained and structural characterized by elemental analyses, IR, mass spectrometry, ^{1}H, ^{13}C-NMR NMR methods. Antibacterial activity against six bacteria [*B.cereus* 709 ROMA, *S. aureus* ATCC 29213, *K. pneumonia* ATCC 13883,*P. aeruginos*a ATCC 27853, *S. aureus* ATCC 25923, *E. coli* ATCC 25922] was conducted. Microdilution (as MICs) and disc diffusion method were used to determine the antibacterial activity. Morever, the 5-ClPAH was evaluated for the activities against three fungal pathogens (*C. tropicalis* M002, *C. parapsilosis* M006 and *C. albicans* ATCC 80018) by agar well diffusion technique. The structure-activity relationship (SAR) of the5-ClPAH was also analysized in the present work. Frontier molecular orbital energies (FMOs), Global reactivity descriptors and MEP were studied at B3LYP/6-311G level of theory.

*Corresponding Author, e-mail: mithat@gazi.edu.tr

2. MATERIAL AND METHOD

2.1. Physical Measurements

Used chemicals were purchased from Merck (with high purifitiy). Fourier Transform Infrared spectra of the was performed between 400 and 4000 $cm^{-1}$from KBr pellets on. NMR spectras were carried out by Bruker-Spectrospin Avance DPX-400 Ultra-Shıeld (using d6-DMSO solvent). Mass spectrometry was recorded on an Agilent 6470 QQQ LC-MS/MS @ 1290 INF HPLC. The m.p. values were determined using an Opti Melt apparatus. All reactions were watched using Merck silica gel (60 F 254) by thin-layer chromatography (TLC). The elemental analysis was implemented out on a LECO CHNS 9320 type analyzer.

2.2. Synthesis of 2, 2'-(piperazine-1,4-diyl)bis(N'-((E)-5-chloro-2-hydroxybenzylidene)aceto hydrazide *(5-ClPAH)*

The solution of 1,4-piperazinediacetic acid, 1,4-dihydrazide (0,9 g, 4.5 mmol) (Koparde et al., 2018) in 25 mL of water- ethanol (1-4 rate) was slowly added with hot solution (50 ºC) of 5-Chloro-2-hydroxy-benzaldehyde (1.4g, 8.9 mmol) in 30 mL of ethanol and refluxed for 24 h. Reactions were watched by TLC. The precipitated product was cooled to room temperature an ~ 23ºC to crystallized from the ethanol/water (4:1) mixture and dried in vacuo. The reaction equation was given Figure 1. Yield 87%. M.p. 198-199ºC. LC-MS (100 eV, APCl): 507.8 ($M+H^+$, 100%), 507.2 (M+, 82%), IR (KBr) ν/cm^{-1}: 2931 cm-1,C-H aliph; 3066, C-H arom.; 3187 , N-H. Elemental analysis for $C_{22}H_{24}Cl_2N_6O_4$ (MW:507.37 g/mol) (Calc.%) C: 52.08; H: 4.77; N; 16.56; Cl; 13.97, O; 12.61. (Found %) C, 51.01; H, 5.11; N, 17.04; O, 12.41; Cl, 14.44.

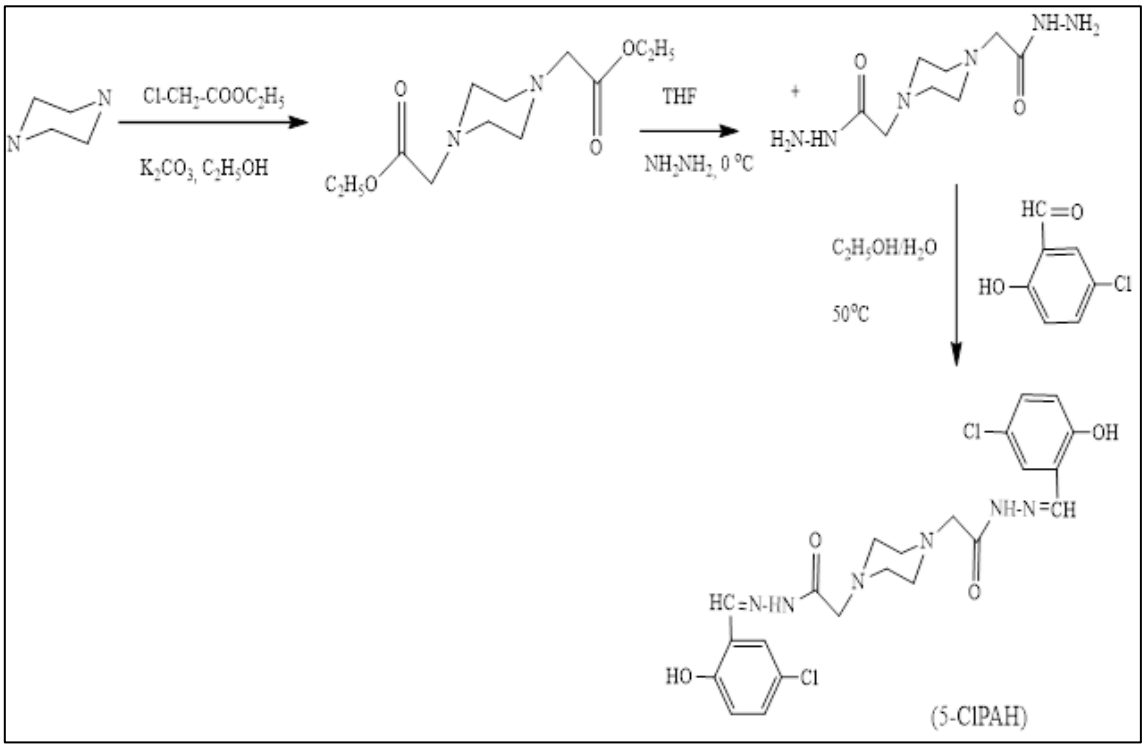

Figure 1. *Preparation of 5-ClPAH*

2.3. Procedure for Antibacterial Activity

Patogen bacterial cultures incubated for 24 hour at 37°C were acquired from Biology Department of Kırsehir Ahi Evran University.

The little discs 6 mm in diameter were treated with 50 µg Schiff bases and then were located on test plates. The paper discs containing 30 µg streptomycin and 50 µg Ampisilin were used as standard drugs. The **5-ClPAH** were tested against microorganisms three times and the activated zones were detected after a day (Luis Esaú et al., 2019).

A serial concentration was prepared by thin out the stock solution with 20 %DMSO solvent: 1250, 625, 312.5, 156.25, 78.125, 39.06, 19.53, 9.76, 4.88 µg mL^{-1}. After incubation at 37ºC for 24 h of the MIC of against microbial strain was defined by macroscopic observation (Koneman et al., 1997).

2.4. Antifungal activity (*in vitro*)

Candida albicans ATCC 80018, *Candida tropicalis* M002 and *Candida parapsilosis* M006 strains were obtained from Biology Department of Kırsehir Ahi Evran University. *In vitro*, antifungal activite was studied using agar well diffusion method (Suryavanshi & Rathore, 2017). The 50 µL of microbial suspension was disseminated over plate containing agar surface. The 5-ClPAH and standard drugs Ampisilin and Streptomycin were incubated at 303 K for 48 h.

2.5. Computation details

Molecular modeling of the compound was carried out by Gaussian 09 (Frisch et al., 2009) program using DFT/B3LYP (Prasad et al., 2022) density functional theory with 6-311G (Foresman & Frisch, 1996) base set and semi-emprical theory with AM1 (Austin Model 1) (Dewar et al., 1985) base set. HOMOs and LUMOs as frontier molecular orbitals computed by DFT method play a important role in the chemical reactivities, stabilities and electronic transition levels of molecules (Dennington et al., 2016).

3. RESULT AND DISCUSSION

3.1. Structure of the 5-ClPAH

IR spectrum of **5-ClPAH** is shown in Figure 2. As seen in Figure 2, a characteristic O-H stretching band is observed at 3344,40 cm^{-1}. Other stretching bands are observed at 3187 cm^{-1} (N-H), 3066 cm^{-1} (CH: aromatic), 1688cm^{-1} (C=O; amide), 1512 cm^{-1} (C=C: aromatic), 1294 cm^{-1} (C-N).

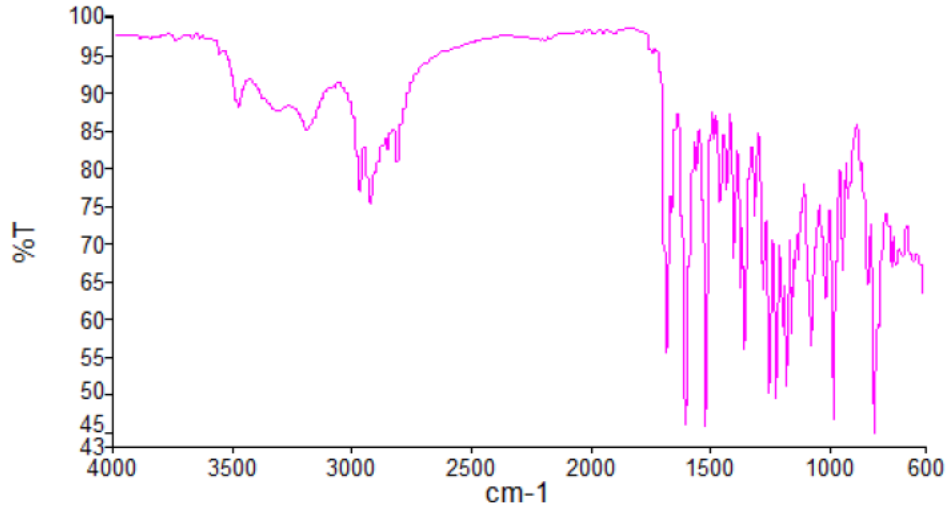

Figure 2**. IR spectrum of **5-ClPAH

The NMR spectrums (^{1}H, ^{13}C) of 5-ClPAH were obtained and interpreted in *d6* –DMSO. In ^{1}H-NMR, the protons of piperazine appeared at 2.38 (m, 4H) and 2.62 (m, 4H) ppm respectively. The protons belonging to N=CH (8.4 ppm), and C-NH (11.2–11.51 ppm) were detected a singlet. The aromatic-H peaks were observed between 6.94-7.60 ppm.

In ^{13}C NMR of Schiff base, piperazine ring peak and azomethine CH=N carbon peak were observed at 53.60 ,163.5 ppm, respectively. The aromatic-C peaks were also observed between 107.7-156.5 ppm.

The mass spectrum of 5-ClPAH is presented in Figure 3. Molecular ion $[M+H]^{+}$peak is observed as base peak at 507.8 (m/z). The main peaks (82%), $[M^{+}]$ at (m/z) 507.2 and (75%), $[M+3H]^{+}$.at (m/z) 509.8 are observed.

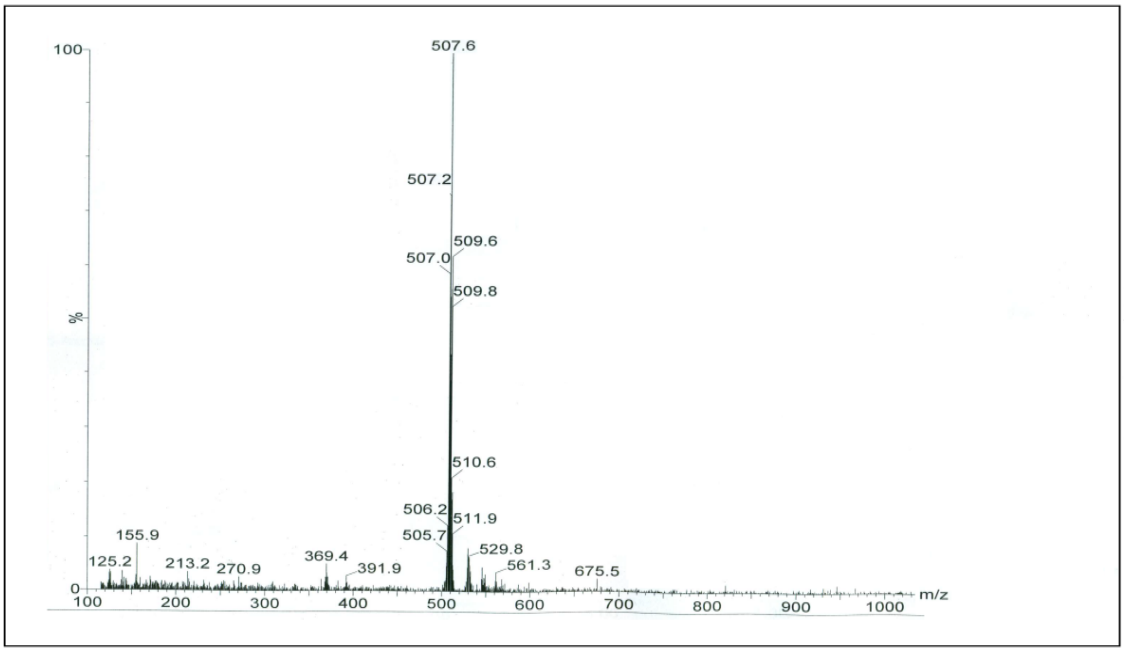

Figure 3**. Mass spectrum of **5-ClPAH

3.2. Antimicrobial activity results

The tested strains selected for this study were categorized as three Gram negative bacteria (*E. coli*, *K. pneumoni*, *P. aeruginosa)*, three Gram positive bacteria (*S. aureus**,*B. cereus*, *S. aureus)* and three fungi (*C. tropicalis*, *C. albicans* and *C. parapsilosis)*. The 5-ClPAH was dissolved in DMSO (20%) at proper concentration.The antibacterial results were given in Table 1, Table 2 by MICs and disc diffusion, respectively. The positive control used in this study as antibacterial and antifungal agents is Ampisilin, and Streptomycin and were compared **(**Figure 4**).**

As the disc diffusion technique results show that the compound exhibits a moderate inhibition effect against tested all bacteria. The compound was significant activity against *P. aeruginosa* ATCC whereas Ampisilin and Streptomycin, the drugs used as standart, have been determined a bit active (10-12 mm) against the six bacteria.

***Table 1.** Measured diameter (mm) of the compounds*

Compounds	***Gram-negative***			***Gram-positive***		
	K.pneumonie ATCC 13883	*E.coli* ATCC 25922	*P.aeruginoa* ATCC 27853	*B. cereus* Roma 709	*S. aureus* ATCC 29213	*S.aureus* ATCC 25923
5-ClPAH	15	15	16	10	10	8
Streptomycin	12	20	10	15	18	19
Ampisilin	12	16	12	15	18	18
<10: weak; >10: moderate; >16: significant Ampisilin (50 µg/disk) Streptomycin (30 µg/disk)						

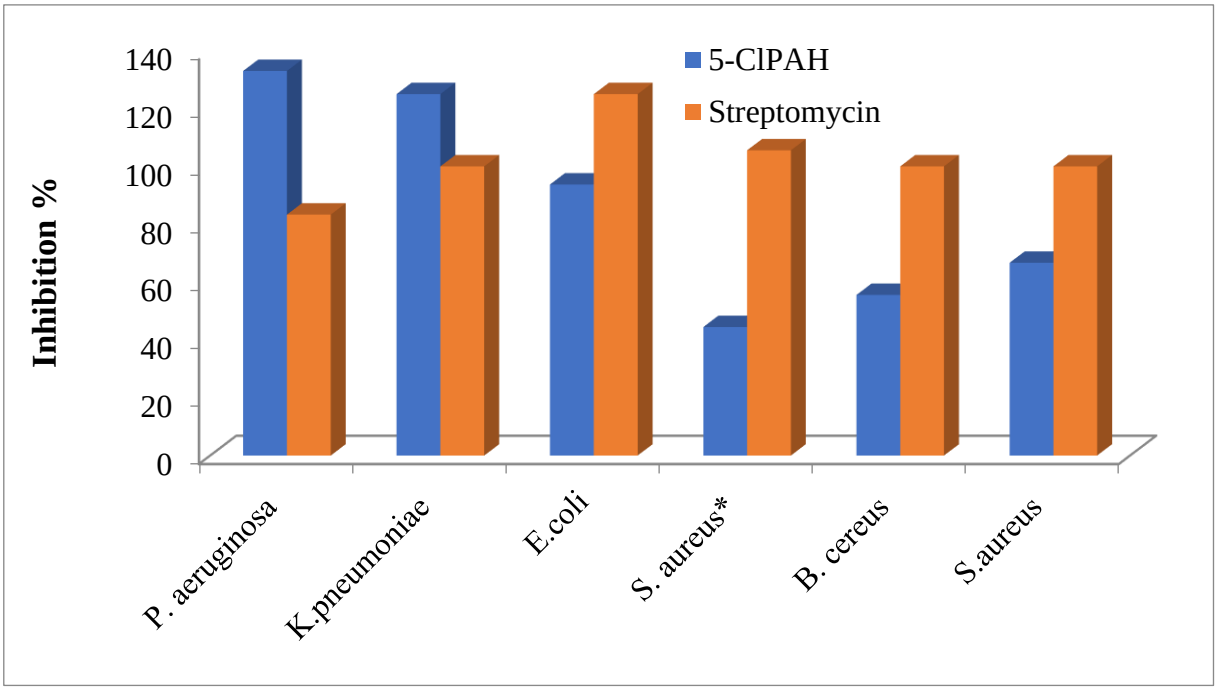

Figure 4. *Percentage of inhibition of 5-ClPAH and Streptomycin against Ampisilin*

The percentage of inhibition for 5-ClPAH molecule and standard drug Streptomycin showed in Figure 4. The 5-ClPAH molecule and Streptomycinshow good activity against Gram-negative *K. pneumonia* (125% 100%), respectively (Ampisilin was accepted 100% inhibition).

The 5-ClPAH molecule showed excellent inhibiting activity against bacterial strains with MIC value less than that of standard drugs Ampisilin and Streptomycin. The 5-ClPAH molecule was excellent inhibition (MIC=78.12 µg/mL) against *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213.

Table 2. *The MICs* ***(µg/mL)****,* ***(mM)*** *of antibacterial activity of 5-ClPAH and standard drugs*

Bacteria strain	**MICs *µg/mL (mM)***		
Gram- negative	**5-ClPAH**	**Ampisilin**	**Streptomycin**
P. aeruginosa ATCC 27853	156.25 **(0.308)**	93.75 **(0.268)**	93.75 **(0.161)**
E. coli ATCC 25922	156.25 **(0.308)**	93.75 **(0.268)**	46.87 **(0.0806)**
K.pneumoniae ATCC 13883	156.25 **(0.308)**	93.75 **(0.268)**	93.75 **(0.161)**
Gram-positive			
S. aureus ATCC 29213	78.125 **(0.154)**	46.87 **(0.134)**	46.87 **(0.0806)**
B. cereus Roma 709	156.25 **(0.308)**	93,75 **(0.268)**	93.75 **(0.161).**
S. aureus ATCC 25923	78.125 **(0.154)**	46,87 **(0.134)**	46.87 **(0.0806)**

The antifungal stud of the 5-ClPAH molecule was carried out *Candida tropicalis* M002, *Candida parapsilosis* M006 and *Candida albicans* ATCC 80018 fungal strains (Table 3) (Rahman et al., 2001). The antifungal activity results of Schiff base and standard drugs; Ampisilin and Streptomycin were exhibited in Table 3 (Figure 5). From the antifungal activity results, 5-ClPAH exhibited excellent inhibition against *Candida albicans*, *Candida parapsilosis* and *Candida tropicalis* with disck 10-22 mm than the standard drugs Ampisilin (7-8 mm), Streptomycin (10-12mm). As seen in Figure 5, The 5-ClPAH showed excellent activity against *C.albicans* (214.28 %) *C. parapsilosis* (314.28%) and *C. glabrata* (125%).

Table 3. *Inhibition zone diameter (mm) of 5-ClPAH and standard drugs*

Compounds	**Zone of Inhibition (mm)**		
	Candida tropicalis M002	*Candida parapsilosis M006*	*Candida albicans ATCC 80018*
5-ClPAH	10	22	15
Streptomycin	12	11	10
Ampisilin	8	7	7

The HOMOs and LUMOs which are responsible for chemical reactivities are known as the FMO. HOMO is the highest energy filled molecular orbital and LUMO is the lowest energy vacant molecular orbital. HOMO represents the desire to donate an electron while LUMO represents the desire to gain an electron (Fleming, 2010; Benabid et al., 2020). HOMO and LUMO have been carefully evaluated to understand the ligand reaction and to specify the reactive sites. These orbital energy levels are used to determine intermolecular charge transfers, and to calculate Global reactivity descriptors such as electron affinity, electronegativity, electrophilicity index, potential chemical reactivity and ionization (Ayers & Parr, 2008; Sultan et al., 2016; Oueslati et al., 2019). The energies of HOMO and LUMO orbitals of ***5-ClPAH*** molecule are -8,01eV and -5.52 eV, respectively. The HOMO and LUMO orbital energy gap (ΔE: E_{HOMO}-E_{LUMO}) calculated using B3LYP/6311G method is 2,49 eV(Table 4, Figure 6). The values of global softness (S), ionisation potential (IP=-HOMO), electronegativity (χ), electron affinity (EA=-LUMO), chemical hardness (η), and electrophilicity index (ω) for **5-ClPAH** molecule at B3LYP/6311 basis are given in Table 4.

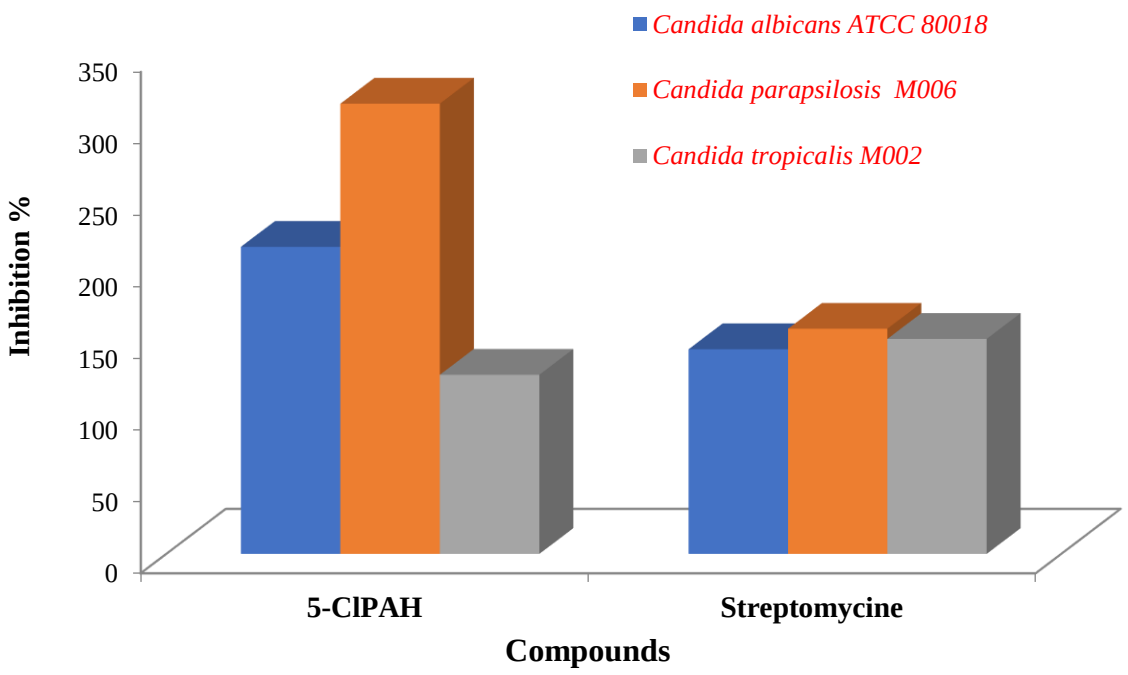

Figure 5. *Percentage of inhibition of 5-ClPAH and Streptomycin against Ampisilin*

Table 4. *The Traditional and Chemical Reactivity Descriptors of 5-ClPAH obtained using B3LYP with 6311G basis set*

Molecular Properties (eV)	**a.u.**	**ev**
E_{LUMO}	-0.20	-5.52
E_{HOMO}	-0.29	-8.01
$\Delta E_{HOMO-LUMO}$	-0.09	-2.49
IP	0.29	8.01
EA	0.20	5.52
η	0.05	1.25
χ	0.25	6.76
μ	-0.25	-6.76
S (ev^{-1})	10.92	297.06
ω	0.67	18.35

The MEP diagram for ***5-ClPAH*** molecule was shown in Figure 7. The red colors in the MEP indicates showing electrophilic reactivity Whereas the blue colors in the MEP show nucleophilic reactivity. From the MEP of the molecule, the positive and negative potential sites occur over nitrogen (N12, N16, N21,N34) and oxygen (O8, O11, O20) atoms, respectively.

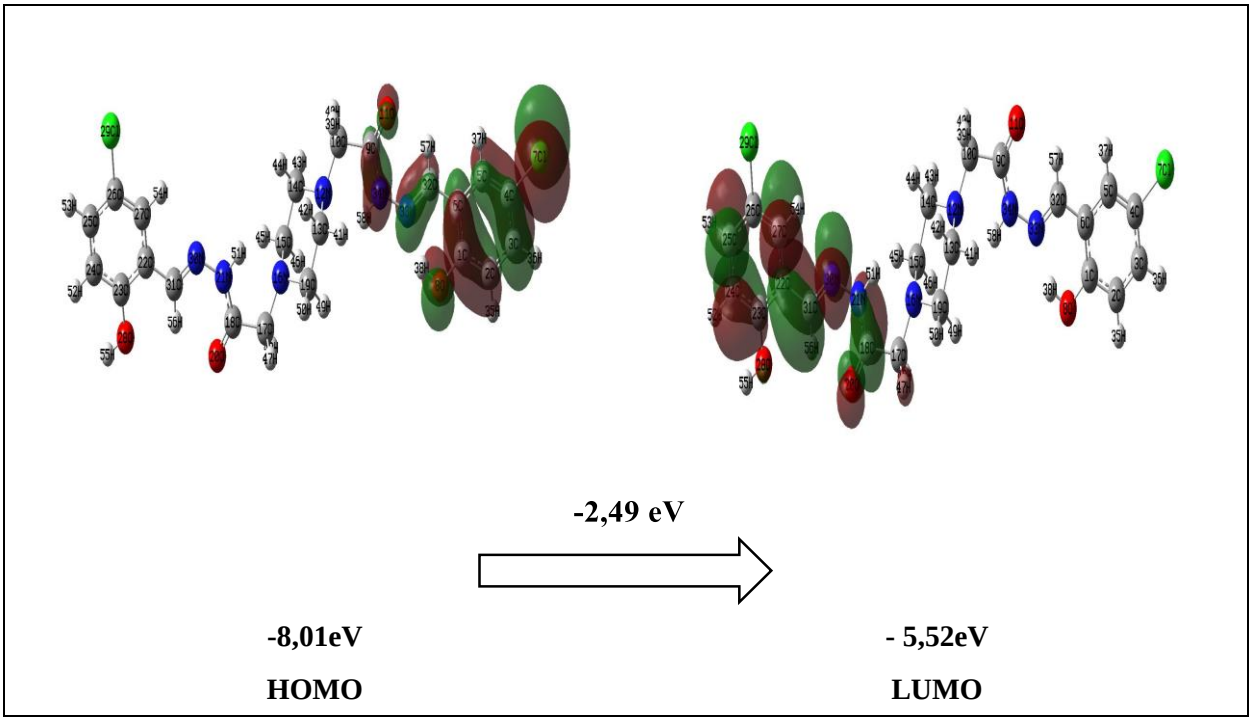

Figure 6. *Representation of Frontier orbitals of* ***5-ClPAH*** *molecule*

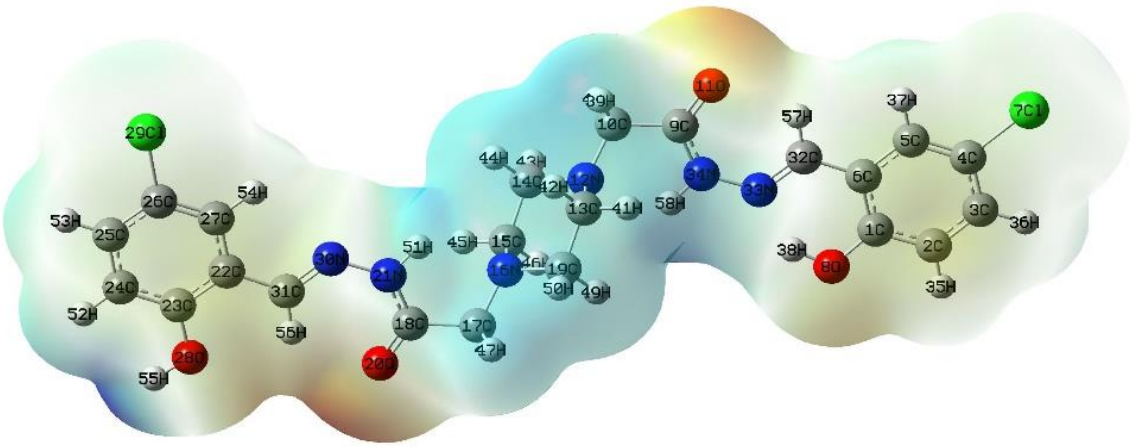

Figure 7. *Derived MEP surface analysis representation of 5-ClPAH molecule*

4. CONCLUSION

In this study, 5-ClPAH molecule with the chemical formula $C_{22}H_{24}Cl_2N_6O_4$. was synthesized and characterized. DFT based calculations were used to theoretically analyze the 3D optimized structure of 5-ClPAH molecule. The HOMO and LUMO energies were used to predict quantum chemical properties like hardness, electrophilicity and softness. Stable structure of 5-ClPAH molecule was obtained by PES analysis. The HOMO energy gap value for 5-ClPAH molecule was found to be -8.01 eV at B3LYP/6-311 G level of theory. In vitro biological tests show that 5-ClPAH molecule have moderate efficacy as antibacterial and antifungal reagents.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

REFERENCES

Ayers, P. W., & Parr, R. G. (2008). Local hardness equalization: Exploiting the ambiguity. *The Journal of Chemical Physics*, *128*(18), 184108. doi:10.1063/1.2918731

Benabid, W., Ouari, K., Bendia, S., Bourzami, R., & Ali, M. A. (2020). Crystal structure, spectroscopic studies, DFT calculations, cyclic voltammetry and biological activity of a copper (II) Schiff base complex. *Journal of Molecular Structure*, *1203*, 127313. doi:10.1016/j.molstruc.2019.127313

Dennington, R., Keith, T. A., & Millam, J. M. (2016). *GaussView 6.0. 16*. Semichem Inc.:Shawnee Mission, KS, USA.

Dewar, M. J. S., Zoebisch, E. G., Healy, E. F., & Stewart, J. J. P. (1985). Development and Use of Quantum Mechanical Molecular Models. 76. AM1: A New General Purpose Quantum Mechanical Model. *J. Am. Chem. Soc.*, *107*(13), 3902-3909. doi:10.1021/ja00299a024

Ermiş, E., & Durmuş, K. (2020). Novel thiophene-benzothiazole derivative azomethine and amine compounds: Microwave assisted synthesis, spectroscopic characterization, solvent effects on UV–Vis absorption and DFT studies. *Journal of Molecular Structure*, *1217*, 128354. doi:10.1016/j.molstruc.2020.128354

Fleming, I. (2010). *Molecular Orbitals and Organic Chemical Reactions, Reference Edition*. John Wiley & Sons. doi:10.1002/9780470689493

Foresman, J. B., & Frisch, A. (1996). *Exploring Chemistry with Electronic Structure Methods*. 2nd Edition. *Gaussian Inc*., Pittsburgh.

Frisch, M. J., Trucks, G. W., Schlegel, H. B., Scuseria, G. E., Robb, M. A., Cheeseman, J. R., Scalmani, G., Barone, V., Mennucci, B., & Petersson, G. A. (2009). *Gaussian 09, revision a. 02*, gaussian. Inc., Wallingford.

Gupta, A., & Goklani, P. (2017). Synthesis and Characterization of Various Alkyl, Aryl and Hetero Aryl Substituted Hydrazines and Study of their Biological Activity. *Oriental Journal of Chemistry*, *33*(2), 1041-1044. doi:10.13005/ojc/330262

Hooshmand, S. A., Ghobadi, M. Z., Hooshmand, S. E., Jamalkandi, S. A., Alavi, S. M., & Masoudi-Nejad, A. (2021). A multimodal deep learning-based drug repurposing approach for treatment of COVID-19. *Mol. Divers.*, *25*(3), 1717-1730. doi:10.1007/s11030-020-10144-9

Jalageri, M. D., Puttaiahgowda, Y. M., Parambil, A. M., & Kulal, A. (2019). Design of multifunctionalized piperazine polymer and its activity toward pathogenic microorganisms. *Journal of Applied Polymer Science*, *136*(19), 47521. doi:10.1002/app.47521

Koneman, E. W., Allen, S. D., Janda, W. M., Schreckenberger, P. C., & Winn Jr., W. C. (1997). *Color Atlas and Textbook of Diagnostic Microbiology*. 5th Edition. JB Lippincot Company.

Koparde, S., Hosamani, K. M., Kulkarni, V., Joshi, D. S. (2018). Synthesis of coumarin-piperazine derivatives as potent anti-microbial and anti-inflammatory agents, and molecular docking studies. *Chemical Data Collections*, *15-16*, 197-206. doi:10.1016/j.cdc.2018.06.001

Luis Esaú, L.-J., Christian Rodolfo, R.-G., Melissa, H.-D., Claudia Adriana, C.-C., Rodolfo, G.-C., & Rafael, F.-C. (2019). An alternative disk diffusion test in broth and macrodilution method for colistin susceptibility in Enterobacteriales. *Journal of Microbiological Methods*, *167*, 105765. doi:10.1016/j.mimet.2019.105765

Oueslati, Y., Kansız, S., Valkonen, A., Sahbani, T., Dege, N., & Smirani W. (2019). Synthesis, crystal structure, DFT calculations, Hirshfeld surface, vibrational and optical properties of a novel hybrid non-centrosymmetric material (C10H15N2)2H2P2O7. *Journal of Molecular Structure*, *1196*, 499-507. doi:10.1016/j.molstruc.2019.06.110

Prasad, H. S. N., Ananda, A. P., Lohith, T. N., Prabhuprasad, P., Jayanth, H. S., Krishnamurthy, N. B., Sridhar, M. A., Mallesha, L., & Mallu, P. (2022). Design, synthesis, molecular docking and DFT computational insight on the structure of Piperazine sulfynol derivatives as a new antibacterial contender against superbugs MRSA. *Journal of Molecular Structure*, *1247*, 131333. doi:10.1016/j.molstruc.2021.131333

Rahman, A.-u., Choudhary, M. I., & Thomsen, W. J. (2001). *Bioassay Techniques for Drug Development.* Harwood Academic Publishers, The Netherlands.

Shaquiquzzaman, M., Verma, G., Marella, A., Akhter, M., Akhtar, W., Khan, M. F., Tasneem, S., & Alam, M. M. (2015). Piperazine scaffold: A remarkable tool in generation of diverse pharmacological agents. *European Journal of Medicinal Chemistry*, *102*, 487-529. doi:10.1016/j.ejmech.2015.07.026

Sultan, M. A., Karama, U., Almansour, A. I., & Soliman, S. M. (2016). Theoretical study on regioselectivity of the Diels-Alder reaction between 1,8-dichloroanthracene and acrolein. *Molecules*, *21*(10), 1277. doi:10.3390/molecules21101277

Suryavanshi, H. R., & Rathore, M. M. (2017). Synthesis and biological activities of piperazine derivatives as antimicrobial and antifungal agents. *Org. Commun.*, *10*(3), 228-238. doi:10.25135/acg.oc.23.17.05.026

Ullh, Z., Al-Otaibi, J. S., Mary, Y. S., & Kwon, H. W. (2022). Computational study of furosemide-piperazine (FS – PZ) and 2,3,5,6-tetramethylpyrazine (FS-TP) co-crystals. *Journal of Molecular Liquids*, *360*, 119537. doi:10.1016/j.molliq.2022.119537

Venkataramana, H. S. C., Singh, A., Tiwari, A., & Tiwari, V. (2010). Synthesis of Phenyl Hydrazine Substituted Benzimidazole Derivatives and Their Biological Activity. *International Journal of Pharmaceutical Sciences and Research*, *1*(1), 34-38. doi:10.13040/IJPSR.0975-8232.1(1).34-38

Xu, Y., Liang, P., Rashid, H. ur, Wu, L., Xie, P., Wang, H., Zhang, S., Wang, L., & Jiang, J. (2019). Design, synthesis, and biological evaluation of matrine derivatives possessing piperazine moiety as antitumor agents. *Medicinal Chemistry Research*, *28*(10), 1618-1627. doi:10.1007/s00044-019-02398-2

Yan, T., Yu, S., Liu, P., Liu, Z., Wang, B., Xiong, L., & Li, Z-m. (2012). Design, Synthesis and Biological Activities of Novel Benzoyl Hydrazines Containing Pyrazole. *Chinese Journal of Chemistry*, *30*(4), 919-923. doi:10.1002/cjoc.201100347