Synthesis Of Pd (II) Complex by Using a Novel Reagent 3-((1H-Benzo[D]Imidazol-1-Yl) Methyl)-5-Amino1,3,4-Thiadiazole-2(3H)-Thione and Study of Biological Activities

Shraddha Parab [a], Dr.Willy Shah[1,2], Dr. Suhas Janwadkar[1], Dr. Dilip Yadav[1], Hardik Deshmukh[1]
[1]*Department of Chemistry, S. D. Arts, V. S. A. Commerce & M. H. M. Science college, Palghar-401404, Maharashtra, India*
[2]*Anna Saheb Vartak College of Arts, Kedarnath Malhotra College of commerce, E. S. Andrades, College of science, Vasai Road, Vasai-Mumbai-401202, Maharashtra, India*

Abstract- The research work was aimed to synthesis a reagent with the help of (5-amino-1, 3, 4-thiadiazole-2-thiol) and Benzimidazole. A reagent has been characterised by IR, H1 and C13 NMR and Mass Spectroscopy. Reagent screened for antibacterial activity against *Staphylococcus aureus* as gram positive bacteria and *Escherichia coli* as a gram negative bacteria. It has been observed that reagent give promising results. Reagent have good anti-inflammatory activity. The successful synthesis, structural characterization, and notable biological activities of the compound underscore its pharmaceutical potential. Further studies are warranted to optimize its bioactivity and explore additional therapeutic applications.

Keywords: (5-amino-1, 3, 4-thiadiazole-2-thiol), Benzimidazole, antibacterial activity

1. INTRODUCTION

The heterocyclic organic compounds received increasing attention from researchers in the field of organic and inorganic sciences due to their use in the various applications. [1-4]

A synthetic heterocyclic compounds exists as valuable intermediate in synthesis of numerous therapeutic applications.[5] Benzimidazole have emerged as important heterocyclic compound because of its broad spectrum of biological activities.[6-7] A variety of chemical modifications were carried out so far around the benzimidazole backbone (core) to improve its various biological activities [8-9].

Thiadiazole derivatives reported to have different interesting biological activities. Metal Complexes in heterocyclic moiety have been used as drug design and catalyst in various reactions such as polymerisation, oxidation etc. In this study, we successfully synthesized 3-((1H-benzo[d]imidazol-1-yl)methyl)-5-amino-1,3,4-thiadiazole-2(3H)-thione, a heterocyclic compound incorporating both benzimidazole and thiadiazole moieties. The synthesis was accomplished using 5-amino-1,3,4-thiadiazole-2-thiol as the core precursor, which was reacted with benzimidazole under optimized conditions to yield the target compound. The structural elucidation and purity assessment were conducted using Fourier Transform Infrared (FT-IR) spectroscopy, Nuclear Magnetic Resonance (^{1}H and ^{13}C NMR), and Mass Spectrometry (MS).

Given the well-documented pharmacological significance of benzimidazole and thiadiazole derivatives, the synthesized compound was systematically evaluated for its biological activities. The antibacterial potential was investigated against *Pseudomonas aeruginosa* (Gram-negative) using the agar well diffusion method, with Streptomycin as the reference standard. The antifungal activity was assessed against *Candida albicans* using the disc diffusion method, with a standard antifungal agent serving as a benchmark. Furthermore, the in-vitro anti-inflammatory activity was determined using the protein denaturation method, a widely accepted model for evaluating anti-inflammatory potential. The inhibition percentage of protein denaturation was quantified and compared with Diclofenac Sodium, a reference non-steroidal anti-inflammatory drug (NSAID).

The results demonstrated that 3-((1H-benzo[d]imidazol-1-yl)methyl)-5-amino-1,3,4-thiadiazole-2(3H)-thione exhibits significant antibacterial, antifungal, and anti-inflammatory

activities. These findings underscore its potential as a promising pharmacological candidate for further drug development and therapeutic applications.

2. EXPERIMENTAL

Materials:
All chemical were used which is of Sigma -Aldrich.
2.1 Synthesis of 3-((1H-benzo[d]imidazol-1-yl) methyl)-5-amino1,3,4-thiadiazole-2(3H)-thione:
Step 1:A mixture of benzimidazole (10 g, 84.7 mmol, 1.00 eq), paraformaldehyde (2.796 g, 93.2 mmol, 1.1 eq), and triethylamine (1 mL) was heated with stirring in an oil bath at 80 °C until the solid completely melted, forming a viscous residue. Further chilled to 0 °C, resulting in the formation of a white solid. The crude product, (1H-benzo[d]imidazol-1-yl)methanol (2), was obtained and used in the next step with further purification.

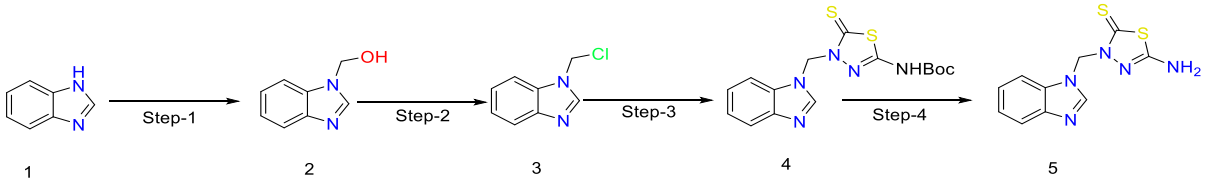
Spectral Data:
1H NMR (400 MHz, DMSO-d_6) data o: δ 8.26 (s, 1H), 7.67–7.59 (m, 2H), 7.29–7.19 (m, 2H), 5.67–5.61 (m, 1H).
Mass (ESI): m/z calculated for $C_8H_8N_2O$: 149.17 $[M+1]^+$, found: 149.1.
Step 2: (1H-Benzo[d]imidazol-1-yl) methanol (2) was cooled 0 °C under a nitrogen atmosphere. add SOCl2 dropwise carefully under stirring at 0 °C temperature. After complete addition of $SOCl_2$ heat the reaction mixture at 80^0C. Progress of the reaction was monitored on TLC (TLC phase: Ethyl acetate 100 %) cool reaction to gives 1-(Chloromethyl)-1H-Benzimidazole.
The resulting brown gum was dried for an additional 30 minutes, yielding crude 1-(chloromethyl)-1H-benzimidazole (3) (9.5 g, 84% yield), which was used immediately for the next step.
To a solution of 5-amino-1,3,4-thiadiazole-2(3H)-thione in a tBuOH:H_2O mixture (90 mL:90 mL), NaOH (1.63 g, 71.4 mmol, 1.0 eq) was added at 0 °C, followed by the dropwise addition of Boc_2O (27.1 g, 71.4 mmol, 1.0 eq) at the same temperature. The precipitate was filtered, washed with water, and dried thoroughly under vacuum or at 50–60 °C to remove residual moisture, yielding tert-butyl (5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)carbamate.

Spectral Data:
^{1}H NMR (400 MHz, DMSO-d_6):
δ 12.09 (bs, 1H), 8.13 (s, 1H), 7.83–7.81 (m, 2H), 7.72–7.65 (m, 2H), 6.62 (s, 2H), 1.47 (s, 9H).
^{13}C NMR (400 MHz, DMSO-d_6):
δ 163.12, 154.89, 144.71, 133.26, 123.19, 122.69, 120.11, 111.69, 82.81, 48.28, 28.24

Mass (ESI): m/z calculated for $C_{15}H_{17}N_5O_2S_2$: 364.08 $[M+1]^+$, found: 363.9.
Step 3:To a solution of tert-butyl (5-thioxo-4,5-dihydro-1,3,4-thiadiazol-2-yl)carbamate (5) (5.0 g, 21.4 mmol, 1.0 eq) in DMF (50 mL), TEA (10.8 g, 107.2 mmol, 5.0 eq) was added, followed by the addition of 1-(chloromethyl)-1H-benzo[d]imidazole (3) (3.91 g, 23.6 mmol, in 50 mL DMF, 1.10 eq) at room temperature. The reaction mixture was then heated to 80 °C and stirred for 16 hours.
After completion, the reaction mass was cooled, and cold water was added. The compound was extracted with DCM. The combined organic layers were washed with cold water. The separated organic layer was evaporated under vacuum to obtain the crude product. Purification was carried out using column chromatography.
Step 4: Protection of Boc removed by using 4N Dioxane in HCl.

Spectral Data:
1H NMR (400 MHz, DMSO-d_6): δ 8.08 (s, 1H), 7.65 (d, J = 8 Hz, 2H), 7.42 (s, 2H), 7.30–7.22 (m, 2H), 5.92 (s, 2H).
13C NMR (400 MHz, DMSO-d_6): δ 171.67, 144.60, 144.05, 133.27, 123.16, 122.65, 120.11, 111.68.
Mass (ESI): m/z calculated for $C_{10}H_9N_5S_2$: 264.34 $[M+1]^+$, found: 264.1.

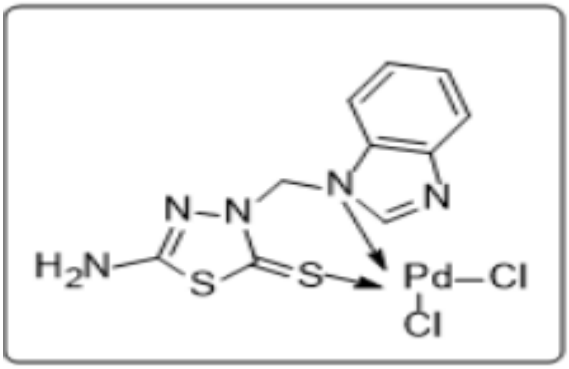
Reaction:



Preparation of Complex:
Ethanolic Solution of reagent and $PdCl_2$ (1.00 Eq) mix and reflux for 3-4 hrs at 80^0C. Cooled this reaction mixture to form precipitated.

Elemental analysis:

Instrument	ICP-OES	Unit	Result
Palladium(Pd)		%	8.84



3. BIOLOGICAL ACTIVITY EVALUATION (METHODOLOGY)

3.1 Antibacterial Activity of the Given Sample Against *Escherichia coli* and *Staphylococcus aureus* Using the Well Diffusion Method

3.1.1 Preparation of Bacterial Inoculum

The bacterial inoculum was prepared from pure cultures of *Escherichia coli* and *Staphylococcus aureus*. Fresh bacterial colonies were transferred into nutrient broth and incubated at 37°C for 18–24 hours to achieve an optimal bacterial growth phase. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard to ensure uniform bacterial density for testing.

3.1.2 Preparation of Agar Plates

A total of 15 mL of sterilized nutrient agar (HiMedia) was poured into clean, sterile Petri plates under aseptic conditions. The plates were allowed to cool and solidify at room temperature, ensuring a uniform agar surface.

3.1.3 Inoculation of Bacterial Strains

Once the agar had solidified, 100 µL of the prepared bacterial broth culture was pipetted onto the surface of the agar. The inoculum was spread evenly across the medium using a sterile glass spreader to ensure uniform bacterial lawn formation. The plates were left to dry under laminar airflow for a few minutes, allowing the bacterial suspension to be absorbed into the medium.

3.1.4 Application of Sample and Incubation

After the bacterial lawn had dried, sample slides containing the test compound were carefully placed on the agar surface at specific positions. The plates were then incubated at 37°C for 24 hours to allow bacterial growth and diffusion of the test compound into the surrounding agar.

3.1.5 Measurement of Antibacterial Activity

Following the incubation period, the antibacterial activity of the sample was assessed by measuring the diameter of the zone of inhibition (ZI) around each sample slide. The inhibition zones were measured in millimeters using a calibrated ruler or digital caliper.

3.1.6 Results and Conclusion

The given sample demonstrated antibacterial activity against both *S. aureus* and *Escherichia coli* (*E. Coli*) Among the tested concentrations, the 10 mg concentration exhibited significant inhibitory effects, showing a larger zone of inhibition compared to the standard control. This suggests that the sample possesses promising antibacterial properties against these bacterial strains.

Sr. No	Sample	Concentration (mg/ml)	Zone diameter (mm) E. coli	Zone diameter (mm) Stap. Aureus
1	Control	-	-	
2	Standard (streptomycin)	1 mg	30	28
3	Sample (7)	5 mg	08	04
4	Sample (7)	10 mg	13	06

Table-3: Antibacterial Activity of the Given Sample Against Escherichia coli and Staphylococcus aureus Using the Well Diffusion Method

3.2 Antifungal activity against Candida albicans via Agar well plate diffusion Method

3.2.1 Preparation of Stock Solution for Antifungal Activity

To assess the antifungal potential of the synthesized compounds, stock solutions were prepared at two different concentrations: 5 mg/ml and 10 mg/ml, These stock solutions were stored under refrigerated conditions until further use to ensure stability and efficacy. Antifungal activities of the sample were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of (Hufford et al., 1975). Sabouraud dextrose agar (Hi media) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is

prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121ºC for 15 minutes. Twenty five ml of molten (45^0C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish.For counting of spore (fungi) were suspended in normal saline to make volume up to1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened, 6mm wells were bored using a sterile cork borer. Then 0.1ml (100µl) from each stock solution of the sample having final concentration of 5 mg and 10mg was placed in each the well and the plates were incubated for 72 hour at 29oC. The antifungal activity was measured as the diameter (mm) of clear zone of growth inhibition.

3.2.2 Conclusion

The agar well diffusion assay provided a reliable assessment of the antifungal activity of the synthesized compounds. The diameter of the inhibition zones served as a key parameter in determining the effectiveness of the tested samples against fungal strains. Further comparative analysis with standard antifungal agents would help in evaluating their potential as therapeutic antifungal agents.

Sr. No.	Sample	Concentration. (mg/ml)	Zone diameter (mm) against Candida albicans
1	Control	-	18
2	Sample (7)	5 mg	05
3	Sample (7)	10 Mg	13

Table 4 : Antifungal activity against Candida albicans via Agar well plate diffusion Method

3.3 In Vitro Anti-Inflammatory Activity by Protein Denaturation Method:

3.3.1 Principle: Protein denaturation is a process in which proteins lose their stable native conformation due to external stress, such as heat or chemical agents. This phenomenon plays a crucial role in inflammation, as denatured proteins can trigger an immune response leading to inflammatory conditions. Evaluating the ability of compounds to inhibit protein denaturation provides insights into their anti-inflammatory potential.

Since denaturation of proteins is one of the key factors contributing to inflammatory diseases, substances that prevent or reduce this process can be considered potential anti-inflammatory agents.

3.3.2 Materials and Reagents: Fresh hen's egg albumin (source of protein), Phosphate Buffered Saline (PBS, pH 6.4), Synthesized Compounds (Sample A & Sample B) at a concentration of 1 mg/ml, Diclofenac Sodium (Standard reference drug) at a concentration of 1 mg/ml, Double-distilled water (Control), Incubator (set at 37°C ± 2°C), Water bath (set at 70°C), UV-Vis Spectrophotometer (measuring absorbance at 660 nm)

3.3.3 Experimental Procedure: Preparation of Reaction Mixture: A 1 mL reaction mixture was prepared in a test tube containing: 0.1 mL of fresh egg albumin (protein source), 0.5 mL of Phosphate Buffered Saline (PBS, pH 6.4), 0.4 mL of Sample A or Sample B (1 mg/mL concentration) A control sample was prepared using the same components, replacing the test sample with double-distilled water.

3.3.4: Conclusion

This protein denaturation assay effectively assesses the anti-inflammatory potential of synthesized molecules. The inhibitory effect of the compounds on protein denaturation suggests their possible application as anti-inflammatory agents. Further in vivo studies and molecular investigations are needed to confirm their pharmacological efficacy and mechanisms of action.

Sr. No.	Compounds	Conc.	O.D.	Mean	% inhibition
1	Blank	-	1.50 1.45 1.48	1.47	-
2	Standard (Diclofenac sodium)	1mg/ml	0.13 0.14 0.15	0.14	90.47
3	Sample (7)	1mg/ml	0.26 0.28 0.29	0.27	81.63

Table 5: In Vitro Anti-Inflammatory Activity by Protein Denaturation Method

ACKNOWLEDGEMENT

The authors express their gratitude to the Principal, Head of the Department, and staff of the Department of Chemistry, S. D. Arts, V. S. A. Commerce & M. H. M. Science College, Palghar-401404, Maharashtra, India, for their support in this research work.

REFERENCE

[1] B.D. Salih, M.A. Alheety, A.R. Mahmood, A. Karadag, D.J. Hashim, Hydrogen storage capacities of some new Hg (II) complexes containing 2-acetylethiophene, Inorg. Chem. Commun. 103 (2019) 100–106.

[2] A.A. Hameed, M.A. Alheety, A.R. Mahmood, S.A. Al-Jibori, A. Karadag, H2 storage abilities of some novel Pd (II) complexes containing 2H [1, 4] benzothiazin-3 (4H)- one, Inorg. Chem. Commun. 106 (2019) 11–17.

[3] S.A. Al-Jibori, M.A. Ulghafoor, A. Karadağ, A. Aydın, H. Akbaş, S.G. Ruiz, Synthesis, characterization and anti-tumor activity of Pd (II) complexes with 4, 5-benzo-3H-1, 2-dithiole-3-thione, Transition Met. Chem. 44 (2019) 575–583.

[4] C. dos Santos Chagas, F.L. Fonseca, I.A. Bagatin, Quinoline-derivative coordination compounds as potential applications to antibacterial and antineoplasic drugs, Mater. Sci. Eng., C 98 (2019) 1043–1052.

[5]Bansal R. Heterocyclic chemistry. 4th ed. Tunbridge Wells: Anshan Ltd; 2007. p. 1-2.

[6] Krishnanjaneyulu IS, Saravanan G, Vamsi J, Supriya P, Bhavana JU, Sunil Kumar MV. Synthesis, characterization and antimicrobial activity of some novel benzimidazole derivatives. J Adv Pharm Technol Res 2014;5:21-7.

[7] Rangaswamy R, Chakrabhavi DM, Toreshettahally RS, Swamy J, Kanchugarakoppal SR. Synthesis, characterization, and in vivo biological evaluation of novel benzimidazoles as potential anticancer agents. Asian J Pharm Clin Res 2014;7:309-13

[8]swara Rao G, Srinivasa Babu P, Sai Koushik O, Sharmila R, Vijayabharathi M, Maruthikumar S, et al. A review on chemistry of benzimidazole nucleus and its biological significance. Int J Pharm Chem Biol Sci 2016;6:227-32.

[9] Sivakumar R, Pradeepchandran R, Jayaveera KN, Kumarnallasivan P, Vijaianand PR, Venkatnarayanan R. Benzimidazole: an attractive pharmacophore in medicinal chemistry. Int J Pharm Res 2011;3:19-31.

[10] Pullagura M Krishna Parasad, Avdhut Kanvinde S. , Raja S. " Potent Biological Agent Benzimidazole-A review", International Journal of Pharmacy and Pharmaceutical

[11] Oluwakemi Ebenezer , Funsho Oyetunde-Joshua , Oluwadamilare

D. Omotoso , Micheal Shapi, Benzimidazole and its derivatives: Recent Advances (2020–2022) Results in Chemistry, Volume 5, January **2023**, 100925, https://doi.org/10.1016/j.rechem.2023.100925

[12] Baral and A. Chakravorty synthesized Trinuclear metallic complexes ofCu (II) and studied their spectral and magnetic properties in the year 1979-80 S. 39 (1980) l-8.