Novel Fabrication of Paracetamol Derivative and Evaluation of its Antimicrobial Activity Against Antibiotic-Resistant Bacteria

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Abstract

This study explores the novel fabrication of a paracetamol derivative compound [P] and evaluates its antimicrobial activity against antibiotic-resistant bacteria. Several established synthetic routes for paracetamol production are reviewed, including the direct p-aminophenol acetylation, phenol-based synthesis, para-nitrochlorobenzene reduction, and Beckmann rearrangement methods, with emphasis on their advantages, limitations, and environmental impacts. A derivative, 1-phenyl-azo-2-paracetamol, was synthesized via diazotization and coupling reactions using para-aminobenzoic acid and paracetamol. The compound was structurally characterized by chemical, physical, and spectroscopic methods (FTIR, UV-Vis, NMR). Antimicrobial assays were performed against E. coli, Staphylococcus aureus (clinical isolates), and Pseudomonas aeruginosa using agar well diffusion. Results showed that the paracetamol derivative exhibited stronger antibacterial activity compared to paracetamol and para-aminobenzoic acid, with enhanced inhibition zones particularly at higher concentrations. The findings suggest that structural modification of paracetamol can improve its pharmacological profile, providing dual benefits of analgesic/antipyretic activity and antimicrobial potential, especially valuable in combating multidrug-resistant bacterial infections.

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1. Introduction

The introduction of antimicrobial agents into clinical practice has transformed modern medicine by providing effective therapeutic strategies for the treatment of infectious diseases. Since their discovery and widespread commercialization, these agents have significantly improved patient outcomes and reduced mortality associated with microbial infections (Wright 2023) [11]. Despite the broad range of antimicrobial drugs currently available, the rapid emergence and dissemination of antimicrobial resistance (AMR) have become a major global health concern. Resistance has been reported against nearly every class of antimicrobial agents, and alarmingly, resistant strains may develop shortly after the clinical introduction of newly approved drugs (C Reygaert 2018) [1]. Consequently, the implementation of the "One Health" concept, which advocates the responsible and rational use of antimicrobial agents across human, animal, and environmental health sectors, has highlighted the urgent need for innovative pathogen-specific therapeutic approaches capable of overcoming the complex mechanisms of microbial resistance (Eirini Christaki, Markella Marcou 2020) [4].

The excessive and often inappropriate use of antimicrobial drugs remains one of the principal factors driving the development of antimicrobial resistance. Because many antimicrobial agents exhibit relatively low toxicity and are perceived as highly effective with minimal adverse effects, they are frequently prescribed or consumed unnecessarily. Such misuse has accelerated

the emergence of multidrug-resistant (MDR), extensively drug-resistant (XDR), and pandrug-resistant (PDR) microorganisms. Unfortunately, the development of new antimicrobial compounds has not progressed at a rate sufficient to counterbalance the increasing prevalence of resistant pathogens (Spellberg *et al* 2011) [10]. Addressing this challenge requires the establishment of comprehensive antimicrobial resistance surveillance programs, implementation of effective antimicrobial stewardship strategies, and stricter regulation of antibiotic usage in both medical and agricultural settings. Among these interventions, the discovery and development of novel antimicrobial agents remain the most critical priority. However, only a limited number of new antibiotics have received approval from the U.S. Food and Drug Administration (FDA) in recent years, underscoring the growing disparity between the emergence of resistant pathogens and the availability of effective therapeutic options.

To overcome the lengthy and costly process associated with conventional drug discovery, drug repurposing, also referred to as drug repositioning, has emerged as a promising alternative strategy. This approach investigates the potential antimicrobial properties of existing non-antibiotic medications, including non-steroidal anti-inflammatory drugs (NSAIDs), thereby reducing both development time and financial investment compared with the discovery of entirely new drugs (Islam *et al*. 2021) [6]. Consequently, increasing research efforts have focused on evaluating the antibacterial potential of NSAIDs and structurally related compounds, with the objective of identifying additional therapeutic applications beyond their traditional pharmacological uses (Omar. M. Alia, 2016).

Paracetamol (acetaminophen), which is included in the World Health Organization (WHO) Model List of Essential Medicines, possesses a unique pharmacological profile among analgesic and antipyretic agents. It is widely recognized for its potent fever-reducing and pain-relieving properties while exhibiting only minimal anti-inflammatory activity (Salam R AL-Ayash and Taghreed H AL-Noor 2022) [9]. Owing to its excellent safety profile, paracetamol is considered a first-line treatment for pain and fever across a broad range of patient populations, including children, pregnant women, older adults, and neonates (Dhahir, *et al*., 2013) [2]. Beyond its well-established clinical applications, accumulating evidence suggests that paracetamol may also possess antimicrobial activity. Several studies have demonstrated that it can potentiate the efficacy of conventional antibiotics through synergistic antibacterial interactions, inhibit biofilm formation, suppress microbial virulence factors, and modify bacterial susceptibility to antimicrobial agents (Dmitry Gil,et al., 2020) [3].

Given the widespread clinical use of NSAIDs for analgesic and antipyretic purposes, frequently alongside antimicrobial therapy, these agents may represent valuable adjunctive treatments capable of enhancing the effectiveness of antimicrobial regimens without significantly increasing the risk of adverse effects. Therefore, combining NSAIDs such as paracetamol with conventional antibiotics may provide an effective strategy for improving antibacterial prophylaxis and therapeutic outcomes while maintaining an acceptable safety profile (Mark Mcintyre *et al*., 2026) [7].

The present study was designed to investigate the antimicrobial potential of a newly synthesized paracetamol derivative against a panel of standard Gram-positive and Gram-negative bacterial strains and to compare its antimicrobial efficacy with that of conventional antibiotics under clean experimental conditions. Furthermore, this research sought to provide additional insight into the antimicrobial properties of paracetamol-derived compounds and their potential as alternative or complementary therapeutic agents. The findings contribute to a deeper understanding of the antibacterial capabilities of paracetamol derivatives, expanding the therapeutic applications of paracetamol, a drug that has remained one of the most widely prescribed and trusted analgesic and antipyretic medications worldwide for many decades.

2. Methodology

All the substances and solvents were pure, supplied by commercial sources (Scarula lab, Spanish co.), and used without further purifica tion. Para aminobenzoic acid (PABA, M.wt= 137.14 g/mol), Paracetamol (M.wt= 151.163 g/mol), Conc. Hydrochloric acid (HCl, 37%, M.wt= 36.46 g/mol), Sodium nitrate (NaNO2, M.wt= 69 g/mol, Sodium hydroxide (NaOH, M.wt=40), Distilled water (DW).

2.1. Preparation of paracetamol (P) compound derivative [P= 4-[(4-acetamido-2-hydroxyphenyl) diazenyl] benzoic acid] (Hamza Salman *et al*., 2019) [5].

1. 0.007 mol (0.95g) of para-aminobenzoic acid compound was dissolved in (5 ml) of concentrated hydrochloric acid and (5 ml) of water, in a small beaker.
2. (0.5 g) of sodium nitrite in (4 ml) of water was added to the mixture in step 1. The mixture was kept in an ice bath (0-5°C).
3. 0.65g of paracetamol in (10 ml) of (10%) sodium hydroxide (aq) was kept in an ice bath as well.
4. The cooled mixture in step 2 was slowly added to the paracetamol solution.
5. A new color developed, and crystals of 1-phenyl-azo-2-paracetamol separated
6. After completion of the addition, the mixture was kept in an ice bath for 30 minutes with occasional stirring.
7. The precipitate was filtered through a Buchner funnel, washed with water, and dried.
8. The precipitated dye was weighed to calculate the percentage yield.

2.2. Characterization techniques

The novel prepared compound (P) has been diagnosis using: An exact melting point determination was carried out via the Stuart SMP10 melting point apparatus. The purity of the compounds was achieved using the general recrystallisation method. The functional groups of compound P were determined using Fourier transform infrared (FTIR) spectra analysis [Thermo Mattson 300 infrared spectrophotometer]. The optical properties of compound P were recorded by an ultraviolet–visible spectroscopy (UV-VIS-i1900 UV, Ninevah university-Iraq). In addition, 1H,13C NMR spectra were measured via a Bruker DMX 500 NMR spectrophotometer (400MHz) with solvent (DMSO d6) in δ ppm using TMS as a standard.

2.3. Anti-bacterial assay

1. The process involved prepared eight Nutrition Agar plates, in two of which we planted E. coli, two staphylococci (patient Azhar), two staphylococci (patient ghofran), and two pseudomonades aeruginosa.

2. We made cavities inside the dishes (each dish contains four cavities) and placed the standard antibiotic, which is in the form of a disc, in the middle of each one.
1. 4 dishes of (E. coli, staphylococci patient Azhar, staphylococci patient ghofran, pseudomonas aeruginosa) contain:

cavity 1:100 microliters of (drug derivative 0.04g dissolved in 3ml ethanol)
cavity 2:100 microlitersof (para aminobenzoic acid 0.04 g dissolved in 3ml ethanol)
cavity 3:100microluter of (paracetamol 0.04g dissolved in 3ml ethanol)
cavity 4: 100 microliters of (3ml ethanol in 7 ml of water).

Another 4 dishes of (E. coli, staphylococci patient Azhar, staphylococci patient ghofran, pseudomonas aeruginosa) contain:
cavity 1:100 microliters of (drug derivative 0.06g dissolved in 3ml ethanol)
cavity 2:100 microliter of (para aminobenzoic acid 0.06 g dissolved in 3ml ethanol)
cavity 3:100 microliter of (paracetamol 0.06g dissolved in 3ml ethanol)
cavity 4: 100 microliters of (7ml ethanol in 3ml of water).
1. Place the dishes in the incubator for 38 hours.
2. The result: The observed values and zones of inhibition were recorded and presented in table 1.

Table 1: Antibacterial assay of compound P against different type of isolated bacteria

Type of bacteria	**Sensitivity Zone of derivative drug**	**Sensitivity Zone of para aminobenzoic acid**	**Sensitivity Zone of paracetamol**	**Sensitivity Zone of ethanol**	**Zone of standard (nitrofurantoin)**
E. coli	Sensitivity 20 mm at conc.0.04 g in 3ml eth.	Sensitivity 15 mm at conc.0.04 g in 3 ml eth.	Sensitivity 9 mm at conc.0.04 g in 3 ml eth.	Resistant at 3 ml eth. In 7ml DW.	Sensitivity 14 mm at conc. 100 mcg.
Pseudomonas aeruginosa	Sensitivity 10 mm at conc.0.04 g in 3 ml eth.	Sensitivity 9 mm at conc.0.04 g in 3 ml eth.	Sensitivity 5mm at conc.0.04 g in 3 ml eth.	Resistant at 3 ml eth. In 7 ml DW.	Resistant at conc. 100 mcg.
Staphylocci (patient ghofran).	Sensitivity 9 mm at conc.0.04 g in 3 ml eth.	Resistant at conc.0.04 g in 3 ml eth.	Resistant at conc.0.04 g in 3 ml eth.	Resistant at 3 ml eth. In 7 ml DW.	Resistant at conc. 100 mcg.
Staphylocci (patient Azhar).	Sensitivity 5mm at conc.0.04g in 3 ml eth.	Sensitivity 8 mm at conc.0.04 g in 3 ml eth.	Resistant at conc.0.04g in 3 ml eth.	Resistant at 3 ml eth. In7ml DW.	Sensitivity 15 mm at conc. 100 mcg.
Type of bacteria	**Sensitivity Zone of derivative drug**	**Sensitivity Zone of para aminobenzoic acid**	**Sensitivity Zone of paracetamol**	**Sensitivity Zone of ethanol**	**Zone of standard (nitrofurantoin)**
E. coli	Resistant at conc.0.06 g in 10 ml eth.	Sensitivity 16mm at conc.0.06 g in 10ml eth.	Resistant at conc.0.06g in 10 ml eth.	Resistant At 7 ml eth. in 3 ml DW.	Sensitivity 8 mm at conc. 100 mcg
Pseudomonas aeruginosa.	Sensitivity 14 mm at conc.0.06 g in 10 ml eth.	Resistant at conc.0.06 g in 10 ml eth.	Resistant at conc.0.06g in 10ml eth.	Resistant at 7ml eth. in 3 ml DW.	Resistant at conc. 100 mcg.
Staphylococci (patient Azhar).	Sensitivity 10 mm at conc.0.06 g in 10ml eth.	Sensitivity 18 mm at conc.0.06 g in 10 ml eth.	Sensitivity 4 mm at conc.0.06g in 10 ml eth.	Resistant at 7 ml eth. in 3 ml DW.	Sensitivity 15 mm at conc. 100 mcg.
Staphylococci (patient ghofran).	Resistant at conc.0.06 g in 10 ml eth.	Sensitivity 8mm at conc.0.06 g in 10 ml eth.	Resistant at conc.0.06 g in 10 ml eth.	Resistant at 7 ml eth. in 3 ml DW.	Sensitivity 20 mm at conc. 100 mcg.

3. Results

3.1. Physical Descriptive of obtained paracetamol derivatives [P]

The new derivative for paracetamol (P) compound (P= 4-[(4-acetamido-2-hydroxyphenyl) diazenyl] benzoic acid] which prepared by reaction of paracetamol with PABA compound was established as shown in figure 1. The physical properties and formula structure of the new paracetamol derivative [p] were identified using obtained melting point and new color formation as explain in table 2.

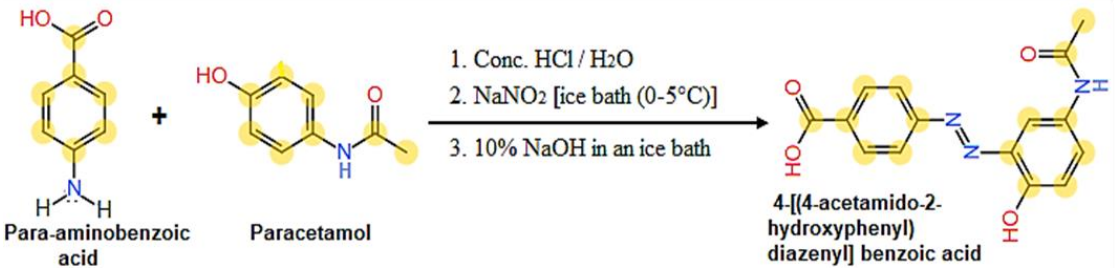

Fig 1: General chemical equation of preparation of Paracetamol derivative [P] compound.

Table 2: Physical analysis of para-amino benzoic acid, Paracetamol, and 4-[(4-acetamido-2-hydroxyphenyl) diazenyl] benzoic acid (paracetamol derivative).

Compound type	Molecular formula	Melting point (m.p) (^{0}C)	Observed color	Live image	Yield %
Para-amino benzoic acid	$C_7H_7NO_2$	187–189 °C	White powder		-
Paracetamol	$C_8H_9NO_2$	169-170 °C	Whaite powder		-
Obtained paracetamol [P] derivative	$C_{15}H_{13}N_3O_4$	289-290 °C	Reddish powder		89

3.2. Anti-bacterial assay

Figure 3 showed the results of antimicrobial activity against antibiotic-resistant bacteria at concentration 0.04 g/ml for utilized [para-aminobenzoic acid, pure paracetamol, paracetamol derivative, and nitrofurantoin] as follow:

3.2.1. E. coli

- Paraaminobenzoic acid sensitivity (15 mmol) at a concentration of 0.04 g in 3 ml Ethanol.
- Paracetamol_ sensitivity (9 mmol) at a concentration of 0.04 g in 3 ml Ethanol.
- Paracetamol derivative_ sensitivity (20 mmol) at a concentration of 0.04 g in 3 ml Ethanol.
- Ethanol resistant (3 ml Ethanol in 7 ml DW).
- Standard (nitrofurantoin)_sensitivity (14 mmol) at a concentration of 100 mcg.

3.2.2. Staphylococci (patient ghofran).

- Paraaminobenzoic acid resistant at a concentration of 0.04g in 3ml Ethanol.
- Paracetamol resistant at a concentration of 0.04g in 3ml Ethanol.
- Paracetamol derivative sensitivity (9 mM) at a concentration of 0.04g in 3ml Ethanol.
- Ethanol resistant (3ml Ethanol in 7ml DW).
- Standard (nitrofurantoin) resistant at a concentration of 100 mcg.

3.2.3. Pseudomonas aeruginosa.

- Para-aminobenzoic acid sensitivity (9 mM) at a concentration of 0.04 g in 3 ml Ethanol.
- Paracetamol sensitivity (5 mM) at a concentration of 0.04 g in 3 ml Ethanol.
- Paracetamol derivative sensitivity (10 mM) at a concentration of 0.04 g in 3 ml Ethanol.
- Ethanol resistant (3 ml Ethanol in 7 ml DW).
- Standard (nitrofurantoin) resistant at a concentration of 100 mcg.

3.2.4. Staphylococci (patient Azhar)

- Para-aminobenzoic acid_ sensitivity (8 mM) at a concentration of 0.04 g in 3 ml Ethanol.
- Paracetamol_resistant at a concentration of 0.04 g in 3 ml Ethanol.
- paracetamol derivative_ sensitivity (5 mM) at a concentration of 0.04 g in 3 ml Ethanol.
- Ethanol_resistant (3 ml Ethanol in 7 ml DW).
- Standard (nitrofurantoin)_ sensitivity (15 mM) at a concentration of 100 mcg.

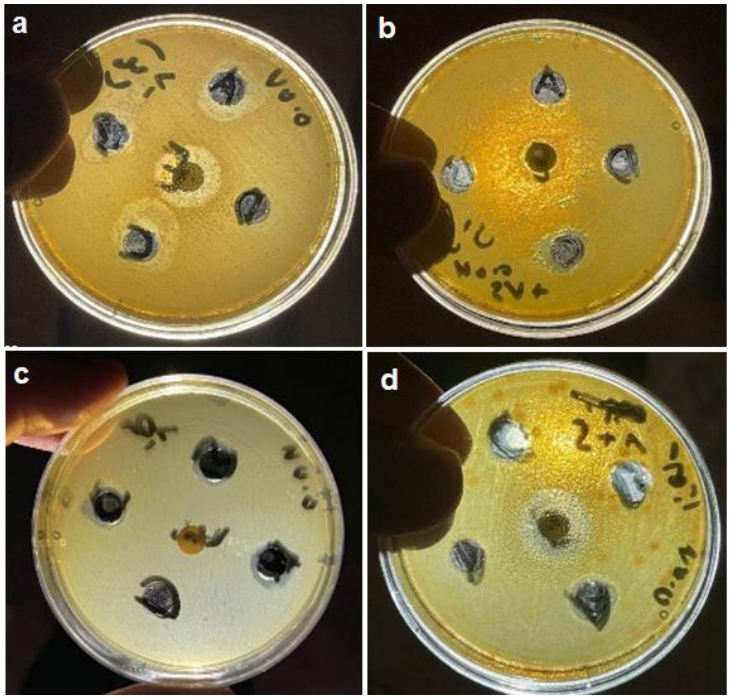

Fig 2: At concentration 0.04, antimicrobial activity against: (a) E. coli, (b) Staphylococci, (c) Pseudomonas aeruginosa, (iv) Staphylococci

While in Figure 4, the obtained results at concentration 0.06 g/ml of showed follow antimicrobial activity against antibiotic-resistant bacteria:

3.2.5. E. coli

- Paraaminobenzoic acid sensitivity (16 mM) at a concentration of 0.06 g in 10 ml Ethanol.
- Paracetamol resistant at a concentration of 0.06 g in 10 ml Ethanol.
- Paracetamol deriva ve resistant at a concentration of 0.06 g in 10 ml Ethanol.
- Ethanol resistant (7 ml Ethanol in 3 ml DW).
- Standard (nitrofurantoin) sensitivity (8 mM) at a concentration of 100 mcg

3.2.6. Pseudomonas

- Para-aminobenzoic acid - resistant at a concentration of 0.06g in 10ml Ethanol.
- Paracetamol - resistant at a concentra on of 0.06g in 10ml Ethanol.
- Paracetamol derivative - sensitivity (14mm) at a concentration of 0.06g in 10ml Ethanol.
- Ethanol - resistant (7ml Ethanol in 3ml DW).
- Standard (nitrofurantoin) - resistant at a concentration of 100 mcg.

3.2.7. Staphylococci (patent Azhar)

- para-aminobenzoic acid_ sensitivity (18 mM) at a concentration of 0.06 g in 10 ml Ethanol.
- Paracetamol_ sensitivity (4 mM) at a concentration of 0.06 g in 10 ml Ethanol.
- Paracetamol derivative_ sensitivity (10 mM) at a concentration of 0.06 g in 10 ml Ethanol.
- Ethanol-resistant (7 ml Ethanol in 3 ml DW).
- Standard (nitrofurantoin) sensitivity (15 mM) at a concentration of 100 mcg.

3.2.8. Staphylococci (patient ghofran)

- Para-aminobenzoic acid_ sensitivity (8 mM) at a concentration of 0.06 g in 10 mL of ethanol.
- Paracetamol_resistant at a concentration of 0.06 g in 10 mL of ethanol.
- Paracetamol derivative resistant at a concentration of 0.06 g in 10 mL of ethanol.
- Ethanol_resistant (7 mL of ethanol in 3 mL of DW).
- Standard (nitrofurantoin)_sensitivity (20 mM) at a concentration on of 100 mcg

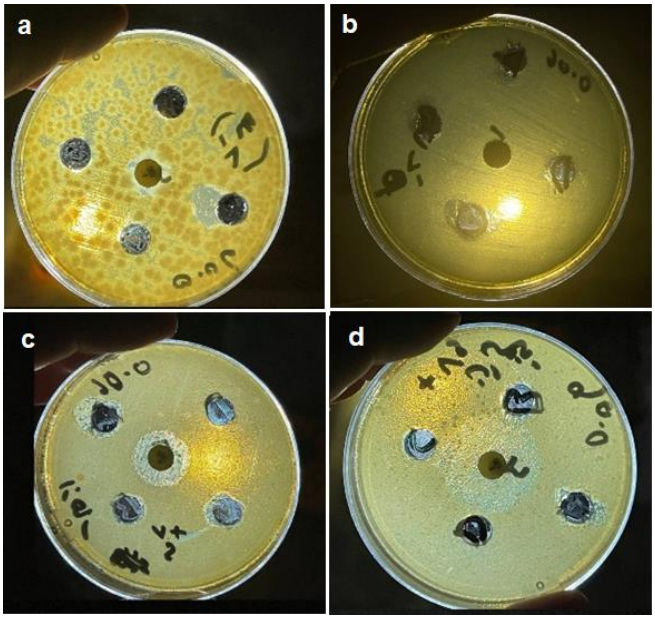

Fig 4: At concentration 0.06, antimicrobial activity against: (a) E. coli, (b) Pseudomonas, (c) Staphylococci, (d) Staphylococci

4. Discussion

4.1. Physical properties of obtained Paracetamol derivatives [P]

The changes in physical properties [the new m.p and color] between the starting compounds and the product is an excellentevidence on in well formation of the novel paracetamol derivative [P] compound.

4.2. Spectroscopy analysis of obtained paracetamol (P) derivative

Several and essential type of spectroscopy diagnosis techniques using UV-Vis, FT-IR, 1H-NMR and 13C-NMR spectroscopies have been utilized in confirmation of prepared paracetamol (P) derivative, as below explained:

4.2.1. UV-Vis analysis

The UV-Visible spectroscopy analysis (Figure 5a) was scanned for the new paracetamol (P) compound at range 200-800 nm. Based on recorded figure 1 analysis, the diagnosis demonstrated absorption bands at (254,359) nm belongs (n-π*) transitions for (N=N) azo group and demonstrate other absorption bands at 225 nm belong to cycle benzene that result for (π-π*) transitions.

4.2.2. FT-IR analysis

The FTIR spectrum (Figure 5b) of the synthesized [P] compound shows several characteristic absorption bands corresponding to the functional groups present in the molecule. The broad and strong absorption band observed in the range 2500–3300 cm^{-1} is attributed to the O–H stretching vibration of the carboxylic acid group, indicating strong hydrogen bonding. Another broad band around 3200–3600 cm^{-1} corresponds to the phenolic O–H stretching, confirming the presence of hydroxyl groups attached to aromatic rings. A medium-intensity band at approximately 3300 cm^{-1} is assigned to the N–H stretching vibration of the amide group. A sharp and strong absorption peak at 1705 cm^{-1} is due to the C=O stretching vibration of the carboxylic group, while another strong band around 1650 cm^{-1} represents the amide carbonyl (C=O) stretching, commonly known as the amide I band. The amide II band, resulting from N–H bending and C–N stretching, appears near 1550 cm^{-1}.

Distinct absorption peaks at 1600 cm^{-1} and 1500 cm^{-1} correspond to C=C stretching vibrations of aromatic rings, confirming the presence of aromatic structures in the compound. The azo group (–N=N–) shows a medium-intensity band near 1450 cm^{-1}, which is consistent with reported values for aromatic azo linkages. Additional absorption in the 1200–1000 cm^{-1} region corresponds to C–O stretching vibrations from both phenolic and carboxylic groups. Finally, the bands observed below 900 cm^{-1} are due to aromatic C–H out-of-plane bending, indicating substitution on the benzene rings.

Overall, the obtained FTIR spectrum confirms the successful synthesis of the compound containing carboxylic acid, phenolic, amide, and azo functional groups. The presence of all expected characteristic absorption peaks provides strong evidence for the proposed molecular structure.

4.2.3. ^{1}H-NMR analysis

The 1H-NMR spectroscopy analysis (Figure 5c) was scanned for the new paracetamol (P) compound at range 0-13 δ ppm. The diagnosis was performed using (400MHz, DMSO-d6, δ ppm). The measurement showed good peaks including: 2.07 (CH_3, 3H, s), phenolic ring = 6.93 (1H, dd, J = 8.5, 0.5 Hz), 7.46-7.72 (4H, 7.52 (dd, J = 8.5, 1.8 Hz), 7.64 (ddd, J = 8.4, 1.7, 0.5 Hz), 7.67 (dd, J = 1.8, 0.5 Hz)), 8.17 (2H, ddd, J = 8.4, 1.6, 0.5 Hz).

4.2.4. ^{13}C-NMR analysis

The 13C-NMR spectroscopy analysis (Figure 5d) was scanned for the new paracetamol (P) compound at range 0-200 δ ppm. The diagnosis was performed using (400MHz, DMSO-d6, δ ppm). The measurement showed good peaks including: 24.0 (CH_3, 1C, s), 114.1, 115.9, 119.0, 136.8, 139.3, 150.3], (phenolic ring, 6C, m), [122.4, 130.8, 131.8, 154.8 (benzoic ring, 6C, m)], 167.1 (CO_2H, 1C, s), 168.9 (amide C=O, 1C, s).

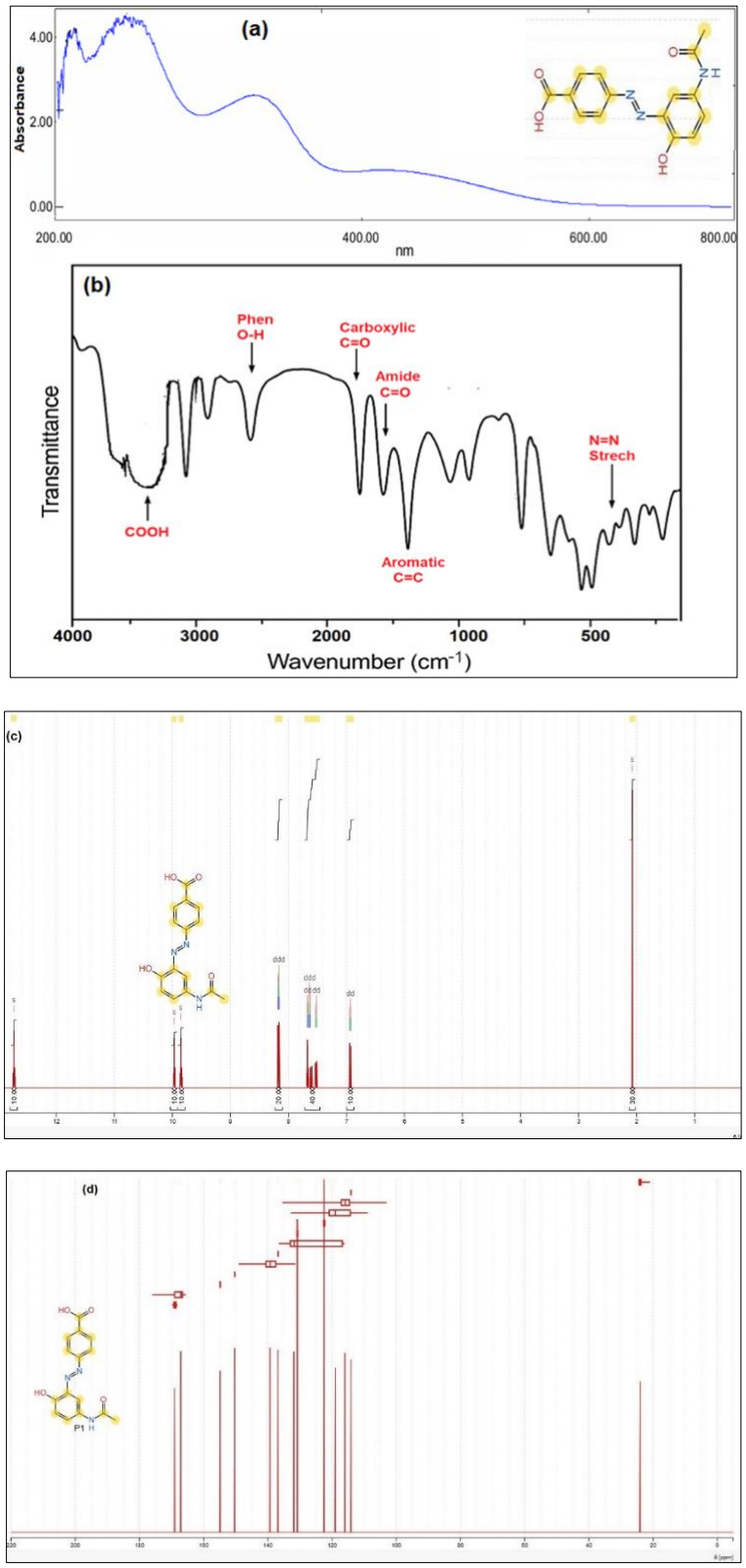

Fig 5: a) UV-Vis; b) FTIR; c) ^{1}H-NMR; ^{13}C-NMR analysis techniques of compound P

4.3. Anti-bacterial activity assay

The antibacterial screening of the tested compounds, namely the derivative drug, para-aminobenzoic acid (PABA), and paracetamol, was performed against both Gram-negative (E. coli and Pseudomonas aeruginosa) and Gram-positive (Staphylococci from different clinical isolates) strains. Ethanol was used as the solvent control, and nitrofurantoin served as the reference standard.

4.3.1. General findings

The tested compounds exhibited variable inhibitory activities depending on the bacterial strain, the compound type, and the concentration used (0.04 g vs. 0.06g). Here, ethanol alone did not exhibit significant antibacterial activity, confirming that the observed effects were due to the tested compounds rather than the solvent. In contrast, the reference standard (nitrofurantoin, 100 µg) consistently inhibited bacterial growth, validating the reliability of the assay.

4.3.2. Activity against gram-negative bacteria

E. coli showed notable sensitivity to both the derivative drug (20 mm at 0.04 g/mL) and PABA (15–16 mm). In contrast, paracetamol displayed only weak inhibition (9 mm at 0.04 g; resistant at 0.06 g/mL). Interestingly, resistance was observed at higher concentration of the derivative drug (0.06 g/mL), which may suggest possible aggregation or solubility issues reducing bioavailability.

Pseudomonas aeruginosa exhibited moderate inhibition by the derivative drug (10–14 mm), but PABA and paracetamol were largely ineffective at higher concentrations. This is consistent with the intrinsic resistance mechanisms of Pseudomonas, such as efflux pumps and reduced membrane permeability, which may restrict compound penetration.

4.3.3. Activity against gram-positive bacteria

Staphylococci (clinical isolates) responded differently depending on the patient source, reflecting possible strain variability.

For the Ghofran isolate, the derivative drug showed modest activity (9 mm at 0.04 g/mL), while PABA showed weak inhibition (8 mm at 0.06 g/mL). Notably, the standard nitrofurantoin achieved 20 mm, indicating higher susceptibility to conventional antibiotics.

For the Azhar isolate, sensitivity was stronger: PABA produced 18 mm inhibition at 0.06 g/mL, which was comparable to the standard (15 mm), suggesting that PABA may exert notable bacteriostatic effects against certain Gram-positive strains. The derivative drug also showed activity (10 mm). Paracetamol remained ineffective, except a marginal effect (4 mm) at higher concentration.

4.3.4. Comparative effectiveness

Derivative drug: Showed broader activity across strains but inconsistently effective at higher concentration, hinting at formulation or solubility-related issues. In contrast, PABA displayed strong and consistent inhibition, particularly against Gram-positive bacteria, aligning with its known role as a precursor in folic acid metabolism, where it may act as a competitive inhibitor to bacterial dihydropteroate synthase. For Paracetamol, exhibited negligible antibacterial activity, suggesting that its chemical structure lacks inherent antibacterial functional groups, and observed minor inhibition could be due to non-specific effects. Standard (Nitrofurantoin): Remained the most effective agent overall, reinforcing its established clinical efficacy.

4.3.5. Possible mechanisms

The significant activity of PABA against Gram-positive bacteria may be attributed to its interference with folate biosynthesis, a pathway more crucial to Gram-positives. The derivative drug, depending on its structural modifications, may possess partial antibacterial functional groups (possibly aromatic or electron-withdrawing substituents) that disrupt bacterial membranes or enzymes. However, reduced activity at higher concentrations suggests that the drug may undergo self-association, lowering effective diffusion. Lack of activity in paracetamol correlates with its analgesic/antipyretic role, as it does not target bacterial metabolic pathways.

4.3.6. Clinical and scientific implications

The results suggest that PABA and the derivative compound could serve as potential scaffolds for antibacterial development, particularly targeting Gram-positive infections. However, their inconsistent activity against Gram-negative bacteria highlights the need for structural optimization or formulation strategies to overcome permeability barriers. The lack of activity of paracetamol reinforces that not all therapeutic drugs possess cross-functional antimicrobial effects.

4.3.7. Limitations and future work

The study was limited to *in vitro* disc diffusion assays, which primarily measure diffusion rather than bactericidal potential. Further studies, including minimum inhibitory concentration (MIC) determination, time-kill kinetics, and synergy testing with existing antibiotics, are recommended. Besides, structural analysis and molecular docking against bacterial enzymes (e.g., dihydropteroate synthase for PABA derivatives) could elucidate precise mechanisms.

5. Conclusion

In Our study, a novel paracetamol derivative, 4-[(4-acetamido-2-hydroxyphenyl) diazenyl] benzoic acid, was successfully synthesized through a diazotization and coupling reaction involving para-aminobenzoic acid and paracetamol. The structure of the synthesized compound was comprehensively confirmed using physicochemical and spectroscopic techniques, including FTIR, UV–Vis, and NMR analyses, which validated the presence of the expected functional groups and molecular framework.

The antimicrobial evaluation demonstrated that the synthesized derivative exhibited enhanced antibacterial activity compared to paracetamol and para-aminobenzoic acid alone. Notably, the compound showed promising inhibitory effects against both Gram-positive and Gram-negative bacterial strains, although its efficacy varied depending on the bacterial species and concentration. The derivative displayed broader-spectrum activity, while para-aminobenzoic acid showed relatively strong and consistent effects, particularly against Gram-positive bacteria. In contrast, paracetamol alone exhibited negligible antibacterial activity, confirming that its parent structure lacks intrinsic antimicrobial properties.

Despite these encouraging findings, certain limitations were observed, including reduced activity at higher concentrations, possibly due to solubility or aggregation issues. Additionally, the study was limited to *in vitro* assays, which primarily assess diffusion-based antibacterial effects rather than precise bactericidal activity.

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