Palestinian Medical and Pharmaceutical Journal

New Non-B-Lactam P-Dicarboxybenzene Derivatives: Insilco Design, Synthesis and Assessment of Their Antimicrobial Activities

Mohammed Saarti[1,*], Ahmed A. J. Mahmood[2], Safaa P. Bahnam[2] & Musab M Khalaf[1]

Type: Full Article. Received: 13rd June. 2025, Accepted: 19th Jul. 2025 Published: ××××, DOI: ××××

Abstract: Infectious diseases were a leading cause of death worldwide, and antimicrobial resistance was consistently reported across the globe. Therefore, these challenges highlighted the need to explore new treatments with antimicrobial properties. This study focused on designing, synthesizing, and assessing the antimicrobial activity of new p-dicarboxybenzene (terephthalic acid) amide compounds. The new compounds were chosen based on docking study results and were synthesized by reacting freshly prepared acid chlorides with the prepared p-dicarboxybenzene hydrazide amines to produce the new amides. Their structures were confirmed using physical and spectral data. The antimicrobial activity was tested by measuring inhibition zones using the disk diffusion method. The results showed that all the synthesized compounds exhibited antimicrobial activity against the tested pathogenic microbes. The most powerful were T1, T3, and T4. T1 demonstrated inhibition zones of 28 mm, 26 mm, and 25 mm for Staphylococcus aureus, Streptococcus pneumoniae, and Escherichia coli, respectively, while T3 and T4 showed lower activity. These activities appeared to be weaker than those of standard antimicrobial agents. This indicated that the new amides were able to inhibit the penicillin-binding proteins in the microbial strains tested. Some findings were concluded concerning the structure-activity relationship of the synthesized compounds: the combination of nitro and halogen groups exhibited stronger antibacterial activity than either group used alone. Regarding antifungal activity, only T6 demonstrated a moderate effect (24 mm), which might have been due to the presence of ether or additional amide groups in those compounds. Overall, the synthesized compounds showed weak antimicrobial activity against all tested pathogenic microbes, implying that higher concentrations of these new amides were necessary to effectively inhibit PBPs in the microbial strains tested. The results also highlighted that combining nitro and halogen substituents produced stronger antibacterial effects than either group alone.

Keywords: P-Dicarboxybenzene, Hydrazides, Terephthalic Acid, Docking, Antimicrobial, Amide.

Introduction

Antimicrobial resistance (AMR) is a grave global health threat, projected to cause millions of deaths and trillions in economic losses. Bacteria are increasingly able to withstand antibiotics, making common infections difficult, if not impossible, to treat. Research is crucial to develop new drugs and alternative therapies, as the current pipeline is insufficient [1]. This escalating crisis is driven by a complex interplay of factors, including the overuse and misuse of antimicrobials in human medicine, veterinary practices, and agriculture, as well as inadequate infection prevention and control strategies. The consequences are dire: prolonged illnesses, increased mortality rates, greater healthcare costs, and a looming threat of a "post-antibiotic era" where common infections could once again become life-threatening [2]. We need novel therapeutic agents with new ways of attacking bacteria. Understanding resistance mechanisms and implementing "One Health" approaches are vital to combat this escalating crisis [1,2]. Despite extensive research efforts over the years, the discovery of new, effective, safe, and selective antioxidant and antimicrobial agents remains a significant challenge [2,3].

Many existing and new antibiotics target penicillin-binding proteins (PBPs). These bacterial enzymes are vital for building and maintaining the bacterial cell wall, a crucial protective layer. PBPs, specifically transpeptidases are responsible for the final stages of peptidoglycan synthesis and regulating its recycling [4]. Since the advent of β-lactam antibiotics in the 1940s, PBPs have emerged as one of the most important and clinically relevant targets for antibacterial drugs [3]. The inhibition of PBP activity disrupts cell wall synthesis, leading to structural defects, aberrant cell morphology (e.g., filamentation or spheroplast formation), and eventually cell death. This makes PBPs excellent drug targets [4]. The shape and integrity of bacterial cells are maintained by their rigid outer layer, the cell wall. A key component unique to prokaryotes within this protective layer is peptidoglycan (PG), a heteropolymer composed of sugar chains cross-linked by peptide subunits [4]. The specific composition and structure of PG significantly influence the cell wall's thickness and strength, which in turn dictates the bacteria's susceptibility to antibiotics targeting the cell wall and to the host's immune defenses [5]. Bacteria are categorized by their cell wall structure, which dictates how susceptible they are to antibiotics:

Gram-Positive Bacteria: These have a thick peptidoglycan layer and no outer membrane. This makes them generally more vulnerable to antibiotics that target the cell wall, as these drugs can easily reach their PBP targets [4,5]. Gram-Negative Bacteria: These possess a thin peptidoglycan layer protected by

1 Department of pharmacology and toxicology, College of Pharmacy, University of Mosul, Mosul, Iraq
* Corresponding author email: mohammedpharma79@uomosul.edu.iq
2 Department of Pharmaceutical Chemistry, College of Pharmacy, University of Mosul, Mosul, Iraq.

an outer membrane. This outer membrane acts as a formidable barrier, making Gram-negative bacteria inherently more resistant to many antibiotics. Drugs must navigate through specialized channels (porins) or be actively transported, and efflux pumps can even expel them [3-5].

This structural intricacy often makes Gram-negative bacteria intrinsically more resistant to a broader range of antibiotics compared to Gram-positive bacteria, posing a greater challenge for therapeutic intervention [1,3].The search for new antimicrobials often involves exploring established chemical structures. These diverse structures allow for chemical modifications that can fine-tune their activity [2]. Previous research highlights several relevant compound classes:

- Heterocyclic Compounds: These are widespread in medicinal chemistry and include structures like pyrazoles, imidazoles, triazoles, thiazoles, quinolines, and pyridines. Many have demonstrated antibacterial and antifungal properties, some even targeting specific bacterial enzymes like PBP3 [1,4].

- Aromatic/Biphenyl/Benzene Scaffolds: While not always p-dicarboxybenzene, studies on biphenyl and dibenzofuran derivatives have shown potent antibacterial activities, emphasizing the potential of such aromatic systems as core structures for antimicrobial agents [3,5].

Notably, Terephthalic acid dihydrazide (TPAD), a compound derived from terephthalic acid, and its derivatives have demonstrated a range of biological activities, including antimicrobial, antioxidant, and anticancer effects, showing promise for pharmaceutical and healthcare applications [6-8].

Building upon this favorable pharmacological profile of TPAD, the current study aimed to synthesize new non-β-lactam antimicrobial derivatives. These compounds, featuring two identical amidic arms, were designed in silico, and their antimicrobial activities were subsequently evaluated after confirming their structural conformations.

Methodology:

Chemicals and materials

In this study, all the materials utilized were obtained from reputable commercial suppliers, ensuring their quality and consistency. The implied chemical are p-dicarboxybenzene (Fluka, Switzerland), 1,4-di nitro benzoic acid (Fluka, Switzerland), 3-Chloro benzoic acid (Fluka, Switzerland), 4-Chloro phenoxy acetic acid (Fluka, Switzerland), 4-florolbenzoic acid (Thomas Baker,India), 2-florolbenzoic acid (Thomas Baker,India), 2-Cl, 5-nitrobenzoic acid (Thomas Baker,India), Absolute Ethanol (Scharlau,Spain), Dichloroethane (Scharlau, Spain), Dimethylsulfoxide (DMSO) (Fluka, Switzerland), Naphthaldehyde (Scharlau, Spain), P-nitro benzaldehyde (Fluka,Switzerland), Pyridine (Fluka, Switzerland), Thionyl chloride (Alpha, India). The melting points of the synthesized compounds were determined using open capillaries.

Instruments and tools:

The FTIR spectra were recorded using a PerkinElmer infrared spectrophotometer. Additionally, ^{1}H NMR and ^{13}C NMR spectra were recorded in DMSO-d_6 using a Bruker Avance DPX 400 MHz spectrometer. Tetramethylsilane was used as an internal reference in these NMR experiments to standardize the chemical shift values. Molecular structures of all synthesized and depicted chemical compounds were generated using ChemDraw software (version 16.0.0.82(68) from PerkinElmer), then visualized and analyzed (for docking) using BIOVIA Discovery Studio Visualizer v20.1.0.19295.

Docking Study;

The docking procedure was performed using the online platform Mcule (https://mcule.com/apps/1-click-docking/) [18]. The Penicillin-binding protein (transpeptidase) 2X (PDB ID: 1PYY) was utilized as a template to design an antibacterial compounds. To select the most effective compounds, we considered both the docking scores of the binding energies (using Amoxicillin, Ampicillin, Ceftriaxone, Cefotaxime as models) and an estimation of the geometric shape fitness within the active binding site of the (1PYY) enzyme [19].

The structure of the Penicillin-binding protein (transpeptidase) 2X (PDB ID: 1PYY) was retrieved from the Protein Data Bank (PDB). The docking scores, expressed in Kcal/mol, were selected based on the lowest negative score, with geometric shape complementarity visualized by BIOVIA Discovery Studio Visualizer v20.1.0.19295 [18,19].

Synthetic section:

The General procedures for chemical synthesis is represented in the figure1

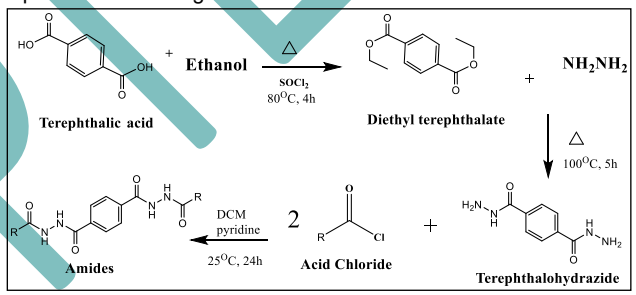


Figure (1): General scheme for chemical synthesis

Preparation of the diethyl terephthalate ester;

To an ice-cooled mixture of 0.09 moles of terephthalic acid (p-benzenedicarboxylic acid) in 20 mL of absolute ethanol, 0.09 moles of $SOCl_2$ was added dropwise, and the mixture was refluxed for 2 hours under a fume hood. Excess $SOCl_2$ was removed under reduced pressure by distillation, and the residue was cooled in an ice bath. The resulting white precipitate was filtered and recrystallized from ethanol, yielding white crystals of diethyl terephthalate ester in 80% yield [9]. The product had a molecular weight of 222.24 and a melting point of 48–52°C.

Preparation of terephthalic dihydrazide;

A mixture of 0.07 moles of diethyl terephthalate in 18 mL of absolute ethanol was combined with 50 mL of 80% hydrazine hydrate. This mixture was refluxed for 5 hours. After evaporation, the resulting product was collected and recrystallized from distilled water [10]. The terephthalic dihydrazide was obtained as a white powder with a 60% yield, a molecular weight of 194.19, and a melting point of 196–199ºC.

Standard protocol for the synthesis of acid chlorides;

A mixture of 2–3 moles of carboxylic acid and 15 mL of thionyl chloride ($SOCl_2$) was refluxed in a fume hood for 30 minutes. Excess $SOCl_2$ was then removed by distillation under reduced pressure. After cooling, the resulting benzoyl chloride was used directly in the next step without further purification [11].

In a cautiously controlled experiment, the acid chloride (5 × 10^{-3} moles) was added dropwise to a mixture of terephthalic dihydrazide and pyridine, each at 2.5 × 10^{-3} moles, dissolved in 5 mL of dichloromethane. The addition was performed at 0°C to maintain optimal reaction conditions. The mixture was then stirred overnight at room temperature to allow the reaction to complete. After solvent evaporation, the crude product was obtained [12]. To purify it, the product was washed 2–3 times with cold ethanol to remove impurities and byproducts, ensuring a high-quality, contaminant-free final compound [13]

Antimicrobial Activity;

The synthesized compounds were tested for antimicrobial activity using the disk diffusion method to measure zones of inhibition. The tests involved the pathogenic bacteria Staphylococcus aureus and Streptococcus pneumoniae (Gram-positive), Escherichia coli (Gram-negative), and the fungus Candida albicans. Each compound, including standards, was prepared as 5 µL/disc with a series of five two-fold dilutions starting from 2000 µg/mL. These discs were placed on Mueller–Hinton agar plates that had been inoculated with the tested microbial strains using sterile cotton swabs. After incubation at 37°C for 24 hours (bacteria) or 48 hours (fungi), the diameters of the inhibition zones—areas where microbial growth was suppressed around the discs—were measured and recorded [15,16]. Discs containing amoxicillin, ceftriaxone, and fluconazole served as controls. Dimethyl sulfoxide (DMSO) was used as the solvent for the tested substances, with its final concentration kept below 2% to avoid inhibiting bacterial growth [16].

Physicochemical properties and ADME prediction;

The drug-likeness of a compound can be assessed based on its physicochemical propertiesby ADME (absorbion, disribution, metabolisim and excreation) prediction . SwissADME, an online tool, was used to analyze and predict the pharmacokinetic properties of the top compounds selected from the docking study. First, the 2D structures of the new compounds were drawn using ChemDraw software, then each structure was uploaded to the Swiss ADME web server (https://www.swissadme.ch). Finally, the ADME data for each synthesized compound were generated and reported [17].

Results and Discussion

Molecular Docking Study:

Docking analysis was performed using the Mcule website [18]. Docking studies for Amoxicillin, Ampicillin, Ceftriaxone, Cefotaxime, and the designed amides were carried out against the Penicillin-binding protein (transpeptidase) 2X (PDB ID: 1PYY) [19]. The chemical structures of the designed amide substituents are presented in Table 1, while the docking results are summarized in Table 2. The binding affinity scores for the standard antibiotics were: Amoxicillin (-6.0), Ampicillin (-6.4), Ceftriaxone (-7.5), and Cefotaxime (-6.3).

The selection of the best docking scores involved two stages. Step 1 consisted of visually evaluating the top four poses for each amide to identify the optimal binding conformation within the active pocket of the PBP, comparing these to the standard inhibitors. The scoring data from the best poses were then recorded in the table 2. This visual assessment was performed using DS Visualizer v20.1.0.19295 [20, 21]. Step 2 involved selecting the amides with the best poses with the enzyme, as shown in the table 2. The final choice of compounds for synthesis was based on the highest docking scores reflecting binding energies and geometric shape complementarity [21, 22].

Table 1. The designed structures of the amide substituents.

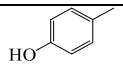
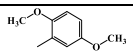
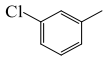
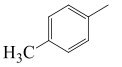
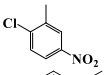
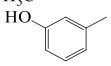
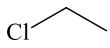
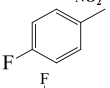
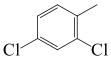
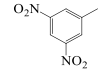
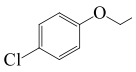
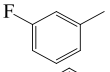
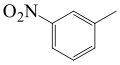
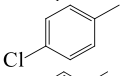
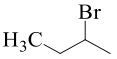
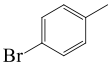
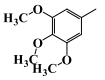
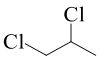
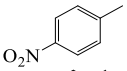
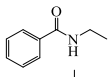
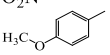
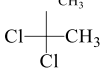
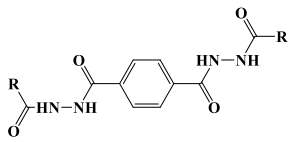
Item	K	Item	K	Item	K
K1		K11		K21	
K2		K12		K22	
K3		K13		K23	
K4		K14		K24	
K5		K15		K25	
K6		K16		K26	
K7		K17		K27	
K8		K18		K28	
K9		K19		K29	
K10		K20		K30	

Table 2: The docking results for terephthalic dihydrazide amides with the Penicillin-binding protein 2X (1pyy) enzyme are expressed in kcal/mol.



No.	Docking scores	No.	Docking scores	No.	Docking scores
K1	-6.2	K11	-7.0	K21	7.0
K2	-6.8	K12	-6.9	K22	-6.8
K3	-7.8	K13	7.0	K23	-6.2
K4	-7.5	K14	-6.8	K24	7.0
K5	-7.6	K15	-7.3	K25	-7.5
K6	-6.8	K16	7.0	K26	7.0
K7	-7.6	K17	-6.8	K27	-6.2
K8	-6.2	K18	-6.2	K28	7.0
K9	-5.8	K19	-6.8	K29	-7.0
K10	-6.8	K20	-6.2	K30	-6.9

The standard inhibitor docking was performed not only to use their scores as reference controls but also to identify the binding poses that define the active site amino acids of the enzyme. The interaction between the standard inhibitors and the 1PYY enzyme revealed nine amino acid residues that are essential for the binding of the standard inhibitor within the active site. Among these, four standard inhibitors interact with TRP231 and ARG229, while only two inhibitors engage with THR227 and TYR418. Additionally, five other residues—GLY421, ASP232, GLN409, TRP420, and GLN304—are contacted exclusively by either Ceftriaxone or Cefotaxime (see Figure 1).

Regarding the interaction of the terephthalic dihydrazide amide compounds with the enzyme (Table 2), the results showed good performance of the amides. Specifically, six compounds with various substituents (K3, K4, K5, K7, K15, and K25) were found to bind with 5 to 6 of the 9 amino acids that characterize the binding site of the 1PYY enzyme. Additionally, each compound interacted with 3 to 5 other amino acids that help enhance the overall binding affinity with the enzyme.

The selected amide compounds demonstrate promising outcomes, achieving equal or higher docking scores compared to the standard inhibitor when tested with the enzyme. Based on the data presented, it can be concluded that aromatic halogenated groups (whether positioned ortho or para) and nitro substituents are favored at the enzyme's active site, as they exhibit the strongest binding scores and affinities toward the active binding pocket [23], Figure 2.

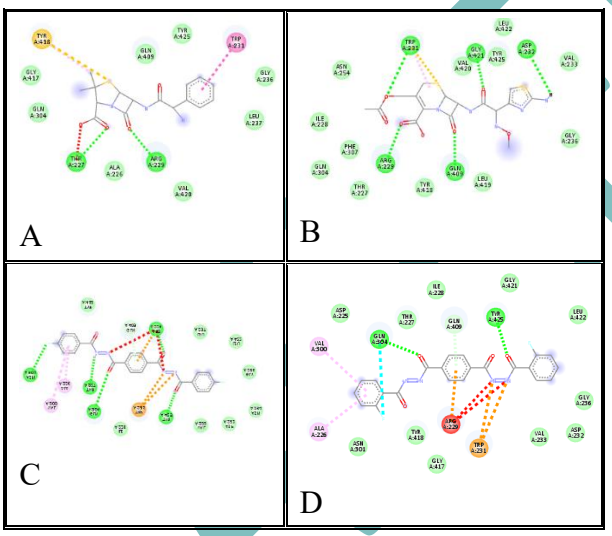
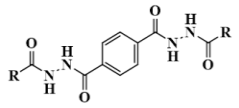


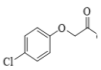
Figure 2: 2D chemical structure for selected ampicillin (A) cefotaxime (B) K4 (C) K5 (D) with the 1pyy enzyme.

Synthesis of Amides (T1-T6)

The physical properties and the key IR absorption bands (expressed as ν in cm^{-1}) of the FTIR spectra for the amide compounds (T1–T6) are presented in Table 3.

Table 3: Physical properties and the most characteristic FT-IR absorption bands (ν cm^{-1}) for the amide compounds (T1–T6).



No.	R	M wt.	m.p(° C)	Color	Yield %	N-H amide	C-H	C=O Amide	N-O	C-X
T1		561.29	400>	Light brown	75%	w 3173	w 3016 2952	S 1663 1597	m 1346	C-Cl m 648
T2		438.39	400>	White	62%	w 3191	w 3010 2942	S 1662 1590	-----	C-F m 839
T3		438.39	400>	Off-white	74%	w 3179	w 3006 2848	S 1662 1584	-----	C-F m 752
T4		471.29	400>	Off-white	58%	w 3177	w 3015 2914	S 1663 1588	-----	C-Cl m 667
T5		582.40	400>	white	83%	w 3204	w 3095 2980	S 1663 1601	m 1346	-----
T6		531.35	400>	White	67%	w 3205	w 3027 2930	S 1693 1576	-----	C-Cl m 678

The FTIR spectra confirm the formation of amide bonds, evidenced by the disappearance of the N–H peak at 3292 cm^{-1}, which corresponds to the primary amine group of terephthalic dihydrazide, and its replacement by amide N–H peaks in the range of 3173–3205 cm^{-1} and amide C=O peaks at 1663–1698 cm^{-1}, verifying amide formation. Additionally, other characteristic peaks specific to the substituents of the acid chlorides used are also observed for each compound (see Table 3).

The ^{1}H-NMR spectra of the amides show signals for one proton corresponding to the NH group (N14) in compounds T1–T6, appearing between 10.03 and 11.13 ppm. Meanwhile, the ^{13}C-NMR spectra display peaks at 162.25–169.21 ppm, indicating the presence of the amide carbonyl carbon (C17) in compounds T1–T6, and peaks at 162.38–165.35 ppm, corresponding to carbonyl carbons C15 and C17 in T1–T6. Additional chemical shifts observed in both the ^{1}H-NMR and ^{13}C-NMR spectra correspond to the substituents from the amines and acid chlorides used in the synthesis of each individual compound.

Chemical names and spectral characterization of the new amides;

T1[N'1,N'4-bis(2-chloro-5-nitrobenzoyl)terephthalohydrazide]. **The ^{1}H NMR of T1** (δ, ppm) DMSO/*d6*: 9.45 (s, 1H, N 11,14) 8.39 (d, 1H, J = 2.1 Hz, C 21,23), 8.34 (dd, 1H, J = 8.6, 2.2 Hz, C 19,31), 8.09 (s, 2H, C 3,4,7,8), 7.93 (d, 1H, J = 8.6 Hz, C 18,30). The 13C NMR of T1 (δ, ppm) DMSO/d6: 165.46 (C1,6), 164.33 (C15,17), 146.39 (C21,28), 138.12 (C2,5), 135.55 (C24,31), 132.23 (C19,26), 128.29 (C23,30), 127.79 (C3,4,7,8), 126.82 (C22,29), 124.57 (C20,27).

T2[N'1,N'4-bis(4-fluorobenzoyl)terephthalohydrazide]. **The ^{1}H NMR of T2** (δ, ppm) DMSO/*d6*: 9.13 (s, 1H, N 11,14), 10.64-7.97 (d, 1H, C 17,21,28,30), 7.81-7.72 (m, 1H, C 3,4,7,8), 7.36-7.40 (t, 1H, C 18,20,27,29). **The ^{13}C NMR of T2** (δ, ppm) DMSO/*d6*: 165.62 (C1,6), 165.35 (C15,17), 163.48 (C22,28), 135.42 (C2,5), 130.69 (C19,25), 130.69(C19,25), 129.66 (C20,24,26,30), 128.19 (C3,4,7,8), 116.04 (C21,23,27,29).

T3[N'1,N'4-bis(2-fluorobenzoyl)terephthalohydrazide]. **The ^{1}H NMR of T3** (δ, ppm) DMSO/*d6*: 9.87 (d, J = 6.4 Hz, 1H, N11,14), 7.90 (s, 2H, C3,8), 7.84 (m, J = 9.0, 5.0, 1.6 Hz, 1H, C21,30),

7.51 (m, J = 8.6, 7.9, 4.9, 1.6 Hz, 1H, C19,28), 7.34 – 7.27 (m, 1H, 20,29), 7.19 (td, J = 7.9, 1.5 Hz, 1H, C18,27). **The ^{13}C NMR of T3** (δ, ppm) DMSO/*d6*: 165.62 (C1,6), 165.35 (C15,17), 163.48 (C22,28), 135.42 (C2,5), 130.69 (C19,25), 130.69(C19,25), 129.66 (C20,24,26,30), 128.19 (C3,4,7,8), 116.04 (C21,23,27,29).

T4[N'1,N'4-bis(3-chlorobenzoyl)terephthalohydrazide]. **The ^{1}H NMR of T4** (δ, ppm) DMSO/*d6*: 10.03 (s, 1H, N 11,14), 7.91 (d, *J* = 2.7 Hz, 3H, C 21,25), 7.80 (m, *J* = 8.1, 2.1, 1.2 Hz, 1H, C 17,29), 7.56 (m, *J* = 8.0, 2.1, 1.2 Hz, 1H, C 19,27), 7.47 (t, *J* = 8.1 Hz, 1H, C 18,28).**The ^{13}C NMR of T4** (δ, ppm) DMSO/*d6*: 165.58 (C6), 165.46 (C1), 165.39 (C18), 165.26 (C15), 137.24 (C28), 136.51 (C5), 135.82 (C2), 134.43 (C24), 133.57 (C17), 131.66 (C20), 129.91 (C23), 129.55 (C22), 129.15 (C27,29), 128.55 (C26,30), 128.27 (C3,4,7,8), 128.20 (C25), 124.25 (C21).

T5[N'1,N'4-bis(3,5-dinitrobenzoyl)terephthalohydrazide]. **The ^{1}H NMR of T5** (δ, ppm) DMSO/*d6*: 10.03 (s, 1H, N 11,14), 9.03-9.32 (m, q, J = 1.6 Hz, 2H, C19,33,17,21,31,35), 8.11 (s, 1H, C 3,4,7,8). **The ^{13}C NMR of T5** (δ, ppm) DMSO/*d6*: 165.51 (C1,6), 162.38 (C15,17), 148.87 (C21,23,27,29), 135.68 (C2,5), 135.25 (C19,25), 128.16 (C3,4,7,8), 127.44 (C20,24,26,30), 122.05 (C22,28).

T6[N'1,N'4-bis(2-(4-chlorophenoxy)acetyl)terephthalohydrazide]. **The ^{1}H NMR of T6** (δ, ppm) DMSO/*d6*: 10.03 (s, 1H, N 11,14), 9.77 – 9.70 (m, 1H, 10,11,13,14), 7.90 (s, 1H, 3,4,7,8), 7.29 – 7.23 (m, 1H, 21,23,32,34), 7.01 – 6.94 (m, 1H, 20,24,31,35), 4.56 (s, 1H, 17,28). **The ^{13}C NMR of T6** (δ, ppm) DMSO/*d6*: 165.48 (C24,26), 165.30 (C10,15), 157.06 (C3,30), 135.02 (C11,14), 129.70 (C5,7,32,34), 128.15 (C6,33), 125.47 (C12,13,16,17), 117.07 (C4,8,31,35), 66.81 (C2,29).

Antimicrobial activities determination;

The synthesized compounds were assessed for their antimicrobial activity using the disk diffusion method to measure the zones of inhibition [24]. The tests were conducted against pathogenic bacterial isolates, including Staphylococcus aureus and Streptococcus pneumoniae (Gram-positive), Escherichia coli (Gram-negative), as well as the fungus Candida albicans. All microbial strains were obtained from pathogenic isolates collected from the community [25].

Each tested compound, including both synthesized and standard ones, was evaluated individually by preparing disks (5 µL per disk) at a concentration of 20 mg/mL, with a series of five two-fold dilutions starting from 2000 µg/mL. The diameters of the inhibition zones were measured by observing the areas where microbial growth was suppressed around the disks after incubation—24 hours for bacteria and 48 hours for fungi—at 37 °C [25]. Disks containing amoxicillin, ceftriaxone, and fluconazole served as controls, and the results are summarized in Table 4.[≤10 very weak activities; 10-10 moderate activities; ≥20 strong activities]

Table 4: Antimicrobial activity results expressed as inhibition zone diameters.

The inhibition zone (IZ) (mm) ± SD (n = 3)																
Comp.	**Grame +ve**								**Grame -ve**				**Fungus**			
	Staph. Aureus				***Strep. Pneumoni***				***E. coli***				***C. abicals***			
	A	**B**	**C**	**D**	**A**	**A**	**C**	**D**	**A**	**B**	**C**	**D**	**A**	**B**	**C**	**D**
T1	**28**	**15**	**----**	**----**	**26**	**18**	**----**	**----**	**25**	**16**	**----**	**----**	**----**	**----**	**----**	**----**
T2	**20**	**14**	**----**	**----**	**----**	**----**	**----**	**----**	**17**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
T3	**23**	**10**	**----**	**----**	**----**	**----**	**----**	**----**	**20**	**13**	**8**	**----**	**----**	**----**	**----**	**----**
T4	**25**	**16**	**----**	**----**	**15**	**----**	**----**	**----**	**20**	**14**	**----**	**----**	**----**	**----**	**----**	**----**
T5	**25**	**10**	**----**	**----**	**12**	**10**	**9**	**----**	**16**	**----**	**----**	**----**	**----**	**----**	**----**	**----**
T6	**23**	**17**	**----**	**----**	**20**	**14**	**----**	**----**	**19**	**13**	**----**	**----**	**24**	**14**	**----**	**----**
Amoxicillin	**29**	**25**	**23**	**14**	**27**	**23**	**20**	**10**	**30**	**27**	**25**	**20**	**----**	**-----**	**-----**	**-----**
Ceftriaxone	**32**	**30**	**27**	**23**	**31**	**30**	**25**	**24**	**32**	**28**	**24**	**24**	**----**	**-----**	**-----**	**-----**
Fluconazole	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**-----**	**32**	**30**	**25**	**20**

A= the concentration is 2000 mg/mL
B= the concentration is 1000 mg/mL
C= the concentration is 500 mg/mL
D= the concentration is 250 mg/mL
(-----): very low activity (IZ > 5 mm).

Overall, the results show that all the synthesized compounds exhibited antimicrobial activity against the tested pathogenic microbes (the most powerful T1, T3 and T4) although their effects were weaker compared to standard antimicrobial agents. This suggests that the newly synthesized amides were able to inhibit the penicillin-binding proteins (PBPs) in the microbial strains used, but only at relatively high concentrations. These findings are consistent with the docking studies, which support the idea that performing docking prior to synthesis can help save time, effort, and costs by identifying promising candidates with good binding affinity to the target enzymes. The requirement for high concentrations to achieve antimicrobial effects may be due to differences between the enzyme models used in the docking study and the actual enzymes present in the tested bacterial strains, which may even belong to different families. Nonetheless, testing the synthesized compounds against

bacterial strains with lower resistance could potentially demonstrate higher antimicrobial activity and lead to the identification of more effective antibacterial agents.

The structure-activity relationship (SAR) for the new compounds reveals that antimicrobial activity is primarily driven by specific electron-withdrawing substituents and their placement. Compounds with both nitro and halogen groups (e.g., T1) showed potent antibacterial effects, aligning with PBP active site preferences. Halogens at ortho, meta, or para positions (T2, T3, T4) contributed significantly to activity, with meta/para positions enhancing PBP binding. The presence of multiple nitro groups (T5) also conferred activity but often at the cost of drug-likeness. Uniquely, compound T6, featuring an ether linkage and an additional phenyl ring with a halogen, displayed moderate antifungal activity, suggesting that specific linker modifications can expand the biological spectrum. However, the results indicate that the combination of nitro and halogen substituents produces stronger antibacterial activity than either group alone. Additionally, halogens contribute to antibacterial effects regardless of their position on the compound—whether at the para, meta, or ortho positions. Halogenated substituents in the meta or para positions, or elsewhere on the molecule, appear to enhance binding affinity to penicillin-binding proteins (PBPs). Incorporating an amide group into either a halogenated or hydrophobic substituent further improves interaction with PBPs by promoting additional hydrogen bonding with amino acid residues in the enzyme's binding site, thereby enhancing antibacterial activity.

Regarding antifungal activity, only T6 demonstrated moderate effects, which may be attributed to the presence of ether or additional amide groups within those substituents.

The results of the ADME study:

The six new compounds that showed the best results in the docking studies were further assessed for their pharmacokinetic properties and drug-likeness. According to Lipinski's Rule of Five, an orally active drug-like compound should generally violate no more than one of the following criteria: hydrogen bond acceptors (HBA) ≤ 10, hydrogen bond donors (HBD) ≤ 5, molecular weight (MW) under 500 Da, octanol-water partition coefficient (LogP) ≤ 5, and an ideal LogS (solubility) value of ≥ -4 [27, 28].

As detailed in Table 5, all six compounds complied with the HBD and LogP requirements. However, their water solubility (LogS) values fell below the accepted threshold, indicating relatively poor aqueous solubility, which might pose challenges for dissolution. Typically, increased hydrophobicity favors better diffusion through the gastrointestinal (GI) tract. Despite this, the compounds showed low blood-brain barrier (BBB) penetration and generally good GI absorption—except for compounds T1 and T5. These properties suggest that if developed further as drugs, these compounds are unlikely to cross into the central nervous system and will primarily remain in the bloodstream.

The molecular weights (MW) of these compounds generally fall within the acceptable range, except for T1, T5, and T6. Additionally, only compound T5 exceeds the standard limit for hydrogen bond acceptors (HBA). Compounds T1 and T6 show potential to inhibit liver CYP450 enzymes, suggesting they could act as enzyme inhibitors if developed as drugs, whereas the others do not exhibit this effect. According to Lipinski's Rule of Five, T1 and T5 each violate more than one criterion, while the remaining synthesized compounds comply with the accepted parameters. A key trade-off emerged: while larger, highly substituted compounds like T1 and T5 were active, they often violated Lipinski's Rule of Five, indicating potential bioavailability issues compared to smaller, more drug-like compounds (T2, T3, T4) that maintained good activity.

Table 5: The pharmacokinetic data of the tested synthesized products projected by SwissADME.

	Physicochemical Properties			Lipophilic activity	Aguas Solubility	Drugs kinetics			Drug- likeness
Comp. No.	Molecular weight g/mol	No. of HBA	No. of HBD	Log Po/w	Log S	GI availability	BBB penetration	CYP 450	Lipinski
T1	561	8	4	1.6	-5.22	Low	No	Yes	No
T2	438	6	4	2.82	-4.21	High	No	No	Yes
T3	438	6	4	2.75	-4.22	High	No	No	Yes
T4	471	4	4	3.34	-5.08	High	No	No	Yes
T5	582	12	4	-0.61	-4.19	Low	No	No	No
T6	531	6	4	3.17	-5.19	High	No	Yes	Yes

Conclusion

A series of amide compounds, designed based on docking results, were synthesized as new penicillin-binding protein (PBP) inhibitors. Their antimicrobial activity and ability to inhibit PBPs were evaluated in vitro. The docking data revealed that the binding scores of tested compounds increased proportionally with the number of nitrated or halogenated substitutions, indicating a preference of the PBP binding pocket for these groups. Overall, the synthesized compounds showed weak antimicrobial activity against all tested pathogenic microbes compared to standard antimicrobial agents, implying that higher concentrations of these new amides are necessary to effectively inhibit PBPs in the microbial strains tested. The results also highlight that combining nitro and halogen substituents produces stronger antibacterial effects than either group alone. Regarding antifungal activity, only one substituent exhibited measurable effects, potentially due to the presence of ether or additional amide groups within those compounds.

This research successfully synthesized novel compounds demonstrating antimicrobial activity, particularly T1, T3, and T4, although less potent than standard agents. The findings align with docking studies, suggesting penicillin-binding protein (PBP) inhibition, which could guide future synthesis efforts. While most compounds adhered to Lipinski's Rule of Five, poor aqueous solubility and elevated molecular weights for some, alongside potential CYP450 inhibition for T1 and T6, highlight areas for further optimization in developing these promising new antibacterial candidates.

Disclosure Statement

- **Ethics of Study:** not applicable (there is no need for Ethical approval)
- **Conflicts of interest:** The authors declare that there is no conflict of interest regarding the publication of this article
- **Author's contribution:** All authors reviewed the results and approved the final version of the manuscript.
- **Funding:** Self funding
- **Acknowledgements:** The authors acknowledge [University of Mosul/ college of pharmacy] for providing necessary facilities to carry out this research.

References:

1] Pal C, Bengtsson-Palme J, Kristiansson E, Larsson DGJ. The structure and diversity of human, animal and environmental resistomes. *Microbiome*. 2016;4:54.

2] Mahmood A AJ. Synthesis, antioxidant and antimicrobial activities for new 4,4'-methylenedianiline amide compounds. *Egypt J Chem*. 2021;64(12):6999–7005.

3] Baquero F, Tedim AP, Coque TM. Antibiotic resistance shaping multi-level population biology of bacteria. *Front Microbiol*. 2013;4:15.

4] Zervosen A, Sauvage E, Frère J, Charlier P, Luxen A. Development of new drugs for an old target—the penicillin binding proteins. *Molecules*. 2012;17:12478–12505.

5] Rakesh KP, Wang SM, Leng J, Ravindar L, Asiri AM, Marwani HM. Recent development of sulfonyl or sulfonamide hybrids as potential anticancer agents: a key review. *Anti Cancer Agents Med Chem*. 2018;8:488–505.

6] Fahim AM, Farag AM, Nawwar GAM, Yakout ESMA, Ragab EA. Chemistry of terephthalate derivatives: a review. *Int J Environ Waste Manag*. 2019;24(3):273–301.

7] Hussain EM, Baqir RK, Tomma JH. Synthesis, characterization and study the biological activity of some new heterocyclic compounds derived from terephthalic acid. *J Chem Health Risks*. 2023;13(4):635–645.

8] Hussain EM. Synthesis, characterization and study the biological activity of some new heterocyclic compounds derived from terephthalic acid. *J Chem Health Risks*. 2023;13(4):623–634.

9] Srivastava KA, Verma S, Shubhra S. Synthesis and biological activity of some novel chalcone derivatives containing [1,3,4] oxadiazole-2(3H)-thione. *Res J Recent Sci*. 2015;4:74–79.

10] Galge R, Raju A, Degani MS. Synthesis and in vitro antimicrobial activity of 1,3,4-oxadiazole-2-thiol and its analogs. *J Heterocycl Chem*. 2014;52(2).

11] Mahmood AAJ. Synthesis, antimicrobial and antioxidant activities evaluation for new amide compounds. *AIP Conf Proc*. 2024;3219(1):050001. https://doi.org/10.1063/5.0236446

12] Saleh AA, Mahmood AAJ. New p-aminodiphenylamine amide compounds: design, synthesis and anti β-lactamases activity evaluation. *Chem Probl*. 2024;(1):UDC 547-327. ISSN 2221-8688.

13] Mahmood AAJ. Synthesis, antioxidant and antimicrobial activities for new 4,4'-methylenedianiline amide compounds. *Egypt J Chem*. 2021;64(12):6999–7005.

14] Feng M, Tang B, Liang SH, Jiang X. Sulfur-containing scaffolds in drugs: synthesis and application in medicinal chemistry. *Curr Top Med Chem*. 2016;16:1200–1216.

15] Mahmood AAJ, Al-Iraqi MA, Abachi FT. Design, synthesis and anti-β-lactamase activity for new monobactam compounds. *Mater Today Proc*. 2021;42:1860–1866.

16] Mahmood AAJ, Al-Iraqi MA, Abachi FT. New 4,4'-methylenedianiline monobactam compounds: synthesis, antioxidant and antimicrobial activities evaluation. *AIP Conf Proc*. 2022;2660:020024.

17] Artunç T, Cetinkaya Y, Menzek A. Investigation of cholinesterase and α-glucosidase enzyme activities, and molecular docking and DFT studies for 1,2-disubstituted cyclopentane derivatives with phenyl and benzyl units. *Mol Divers*. 2024:1–17. https://doi.org/10.1007/s11030-024-10911-y

18] SM. Computational calculations of molecular properties and molecular docking of new and reference cephalosporins on penicillin binding proteins and various β-lactamases. *J Pharm Pharmacol*. 2016;4:212–225.

19] Rafiq Z, Saranya S, Vaidyanathan R. Computational docking and in silico analysis of potential efflux pump inhibitor punigratane. *Int J Pharm Pharm Sci*. 2018;10(3).

20] Lin X, Li X, Lin X. A review on applications of computational methods in drug screening and design. *Molecules*. 2020;25:1375. https://doi.org/10.3390/molecules25061375

21] Bahnam S, Jasim M, Mahmood AAJ. In silico study of new Schiff base- and amide-boronic acid derivatives as potential inhibitors of β-lactamases. Indian Drugs. 2023 Sep 28. https://doi.org/10.53879/id.60.09.13937

22] Saleh A, Mahmood A. Docking study for new 7-aminocephalosporinic acid derivatives as potential inhibitors for β-lactamases. Iraqi J Pharm. 2023;20:23–32. https://doi.org/10.33899/iphr.2023.138503.01025

23] Wang F, Shen L, Zhou H, Wang S, Wang X, Tao P. Machine learning classification model for functional binding modes of TEM-1 β-lactamase. Front Mol Biosci. 2019;6:47. https://doi.org/10.3389/fmolb.2019.00047

24] Pitucha M, Wos M, Miazga-Karska M, Klimek K, Mirosław B. Synthesis, antibacterial and antiproliferative potential of some new 1-pyridinecarbonyl-4-substituted thiosemicarbazide derivatives. Med Chem Res. 2016;25:1666–1677

25] Saleh AA, Mahmood AAJ. New p-aminodiphenylamine amide compounds: design, synthesis and anti β-lactamase

activity evaluation. Chem Probl. 2024;(1):UDC 547-327. ISSN 2221-8688.

26] Arun K, Sharma L, Kaura M. Synthesis and comparative antimicrobial study of beta-lactam derivatives. Int J Pharm Chem Sci. 2013;2(2):Apr–Jun. ISSN: 2277–5005.

27] Ertl P, Schuffenhauer A. Estimation of synthetic accessibility score of drug-like molecules based on molecular complexity and fragment contributions. J Cheminform. 2009;1(1):1.

28] Bashir M, Oglah M, Mahmood A. In silico study of NSAIDs conjugated with coumarins as new anti-inflammatory compounds. Indian Drugs. 2024;61:20–29. https://doi.org/10.53879/id.61.11.14808