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 exhibited inhibitory zone diameters of 28 mm, 26 mm, and 25 mm against Staphylococcus aureus, Streptococcus pneumoniae and Escherichia coli, respectively and T3 and T4 exhibited weak activity. These actives seemed to be less strong than the controled antimicrobials. This implied that the new amides can bind and inhibit the penicillin-binding proteins in the microbial strains employed. A few conclusions could be drawn from the structure-activity relationship of the synthesized compounds: nitro and halogen-substitution together showed enhanced antibacterial activity than the two groups alone. With respect to antifungal activity only T6 showed a mild activity (24 mm) that may be attributed to the presence of ether or more amide groups in the two compounds tested. In general the new amides of this work however all showed poor antimicrobial activity against all tested pathogenic microbes which suggested that a considerably higher concentration of these new amides was required to effectively inhibit PBPs in the microbial strains. The results also revealed that a combination of nitro and halogen substitutent had better antibacterial activity than either group alone.

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

Introduction

Antimicrobial resistance (AMR) is a serious global health threat that could result in millions of deaths and trillions of economic losses. Bacteria are routinely resisting antibiotics and simple infections are becoming increasingly hard to treat, and in some cases treatable. Research is needed to develop new drugs and alternative treatments because the current pipeline is inadequate [1]. This emerging crisis is the result of complex interrelated factors including overuse and misuse of antimicrobials in human, veterinary and agricultural domains, and poor infection prevention and control. The implications are dire: longer illnesses, higher death rates, more healthcare costs, and an impending specter of a "post-antibiotic era" in which everyday infections could once more become fatal [2]. We need novel therapeutic agents with novel mechanisms of bacterial attack. [1,2] For this growing problem, understandings of the mechanisms mediating resistance and "One Health" solutions are imperative to turn the tide. Although several studies have been conducted in the field, the identification of new effective, safe and selective antioxidant and antimicrobial compounds is still a challenge [2,3].

A number of known and novel antibiotics act on penicillin-binding proteins (PBPs). These enzyme of bacteria play an essential role in survival of bacteria through synthesis and maintenance of bacterial cell wall which is an important protective layer. PBPs, in particular transpeptidases, undertake the last steps of the peptidoglycan biosynthesis and control its recycling [4]. Since the introduction of β-lactam antibiotics in the 1940s, PBPs have become one of the most effective and targetable class of antibacterial drug targets [3].The activity of PBPs is inhibited, which interferes with the cell wall synthesis and causes morphological distortions, such as filamentation and spheroplast formation, and ultimately cell lysis. Therefore, PBPs are ideal drug targets [4]. Bacterial cells are shaped and maintained by their rigid exterior, the cell wall. A vindicative element exclusive to prokaryotes of this barrier layer is PG, a sugar-based heteropolymer joined together by peptides [4]. The unique nature of the PG, in terms of its composition and structure, strongly affects the thickness and strength of the cell wall, which also adapts the susceptibility of the bacteria to cell wall active antibiotics and host immune components [5]. The cell wall defines how bacteria are classified and how resistant they are to antibiotics:

They have thick peptidoglycan layers and lack an outer membrane. This means they are also more susceptible to drugs that attack the cell wall and can reach their targets PBP easily [4,5]. Gram-Negative Bacteria: They include an outer membrane

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.

surrounding a thin peptidoglycan layer. This outer membrane serves as a very effective barrier, rendering many antibiotics ineffective against Gram-negative bacteria. Drugs must pass through specialized channels (porins) or be actively taken up, and efflux pumps can even expel them [3-5].

This complexity in structure is partly the reason why Gram-negative bacteria are generally considered to be inherently more resistant to a wider variety of antibiotics than Gram-positive bacteria, which makes therapeutic targeting of them more difficult [1,3].In a quest for new antimicrobials, it is not uncommon to start from known chemical scaffolds. Such diverse structures enable chemical modifications to adjust their activities [2]. Relevant compound classes as derived from previous studies of interest are:

- Heterocyclic Compounds: These are increasingly important in medicinal chemistry and consist of pyrazoles, imidazoles, triazoles, thiazoles, quinolines, pyridines etc. Many have shown antibacterial and antifungal activity, some even against bacterial enzymes such as PBP3 [1,4].
- Aromatic/Biphenyl/Benzene Scaffolds: Although not always p-dicarboxybenzene, biphenyl and dibenzofuran derivatives have also been investigated and tested good antibacterials, demonstrating that such aromatic systems can be promising core scaffolds as antimicrobial agents [3,5].

Terephthalic acid dihydrazide (TPAD), a derivative of terephthalic acid, and its derivatives have been found to possess diverse biological activities, such as antimicrobial, antioxidant and anticancer activities, and therefore have potential for use in pharmaceutical and healthcare related applications [6–8].

Based on this positive pharmacological profile of TPAD, the present study was to design and synthesize novel non-β-lactam antimicrobial derivatives. These are molecules bearing two identical amidic arms, which have been conceived in silico, and the antimicrobial properties of some of them have been also tested once their structural conformations have been experimentally confirmed.

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

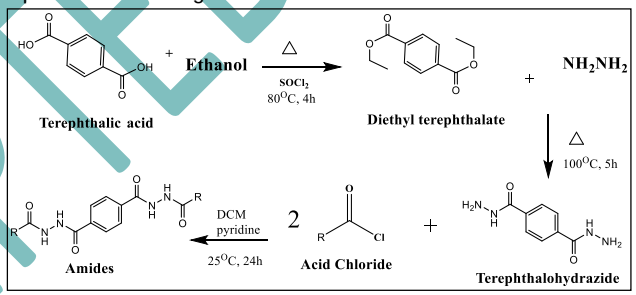


Figure (1): General scheme for chemical synthesis

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 solution of 0.07 g of diethyl terephthalate in 18 mL of absolute ethanol was mixed with 50 mL of 80% hydrazine hydrate. This preparation was refluxed for 5h. Following evaporation the crude product was collected and the crystallized from d H2O “10]. The terephthalic dihydrazide was obtained with 60% yield as a white powder, its molecular weight was 194.19, and melting point was 196-199ºC.

Generaläsynthesis procedure for acid chlorides;

An assembly of 2-3 moles carboxylic acid and 15 mL thionyl chloride ($SOCl_2$) was refluxedprofith the fume hood for 30 minutes. Excess $SOCl_2$ was then distilled off under vacuum. The solid acid chloride was mixed with distillative hydrogenation in the room temperature. Upon cooling, the benzoyl chloride

thus obtained was used in the next step as is, without any further purification [11].

In a tightly controlled experiment, a solution of terephthalic dihydrazide and pyridine (2.5 × 10-³ moles each) in 5 mL of dichloromethane was cooled to 0°C and the acid chloride (5 × 10⁻³ moles) added dropwise over the course of 10 min. The addition was done at 0°C to have good reaction. The mixture was then stirred at room temperature overnight for completion reaction. After evaporating the solvent, the raw product was obtained [12].According to dry the product Washed several times with cold ethanol to remove impurities and byproduct at the end of the Reaction to afford a good final, byproduct free Compound [13]

Antimicrobial Activity;

The synthesized compounds were screened for their antimicrobial activities by the disc diffusion method in terms of zones of inhibition. The study was conducted using the pathogenic bacteria Staphylococcus aureus, Streptococcus pneumoniae (Gram-positive), Escherichia coli (Gram-negative), and the fungus Candida albicans. All the compounds including standards were dissolved as 5 µL/disc in 5 two-fold dilutions from 2000 µg/mL. The discs were then applied onto the plates of Muller- Hinton agar, swabbed with the microbial strains under test with the help of sterile cotton swabs. After 24 h at 37°C for bacteria, 48 h for fungi, the inhibition zones (clear, non-growth zone surrounding the disc impacted by the antimicrobial [15,16]) were measured and recorded. Discs of amoxicillin, ceftriaxone and fluconazole were included as controls. The tested compounds were dissolved in the dimethyl sulfoxide (DMSO) as the solvent, and the final concentration of DMSO was less than 2% so as to not inhibit 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 choosing of the top docking scores was composed of two rounds. Step 1 was a visual analysis of the four best scoring poses per amide for finding the best binder in the active pocket of the PBP and comparing these to that of 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.

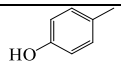
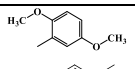
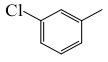
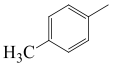
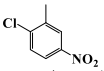
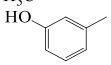
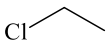
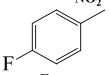
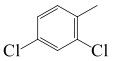
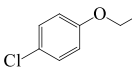
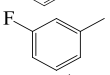
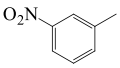
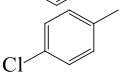
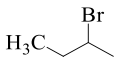
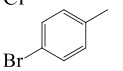
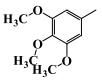
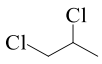
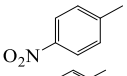
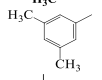
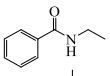
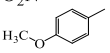
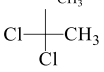
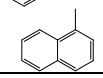
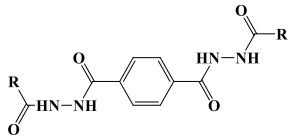
Item	K	Item	K	Item	K
K1		K11		K21	
K2		K12		K22	
K3		K13		K23	
K4		K14		K24	
K5		K15		K25	
K6		K16		K26	
K7		K17		K27	
K8		K18		K28	
K9		K19		K29	
K10		K20		K30	

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.

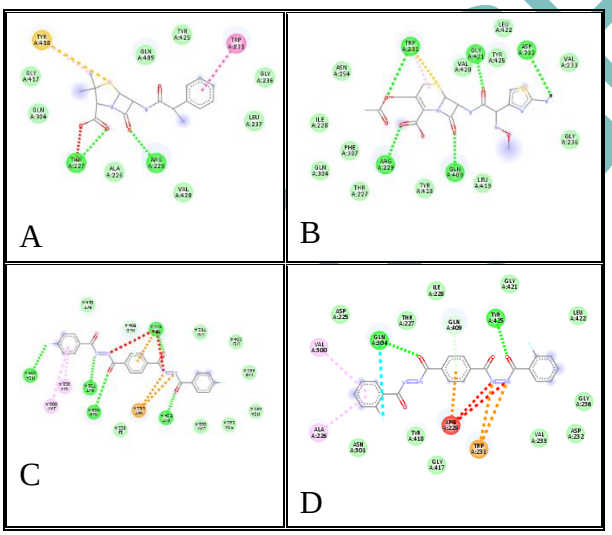
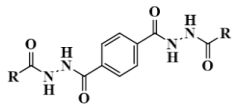


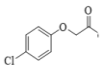
Figure 2: 2D chemical structure for selected ampicillin (A) cefotaxime (B) K4 (C) K5 (D) with the 1pyy enzyme.

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. However, it is possible that the evaluated synthesized compounds could present higher activity when tested against bacterial strains with lower resistance, which may potentially result in the discovery of more efficient antibacterial agents.

The SAR of the novel compounds shows that the antimicrobal activity is favored by some specific electron-withdrawing substituents and their positions. Compounds bearing both nitro and halogen moieties (such as T1) exhibited strong antibacterial activities consistent with PBP active site recognition. Halogens at ortho, meta, and para positions (T2, T3, T4) promoted activity greatly, while meta/para also improved PBP binding. The compound with multiple nitro groups (T5) was also active but frequently that was to the detriment of drug-likeness. Interestingly, T6 introduced an ether linkage and an additional phenyl ring bearing a halogen and showed moderate antifungal activity, indicating that certain linker moieties could diversify the biological profile. The findings, however, suggest that the nitro and halogen substituents together exhibit a better antibacterial activity than as individual substituents. Moreover, halogens are important for antibacterial activity regardless of which part of the compound they are at be it the para, meta or the ortho positions. Halogenated groups at the meta or para positions or any position on the structure confer increased binding affinity to PBPs. The introduction of an amide moiety to either the halogenated or hydrophobic group enhances binding to PBPs through extra H bonds with amino acid residues in the binding pocket of the enzyme, and thus increases the antibacterial activity.

As for antifungal activity, only T6 exerted moderate effect, which can be explained by the fact that these substituents contain ether or additional amide moieties.

The results of the ADME study:

The six compounds that displayed the highest affinity in docking studies were selected for further pharmacokinetic and drug-likeness analysis. A compound that is drug-like and likely to be orally active has the following properties according to Lipinski's rule of five for drug-likeness: no more than 1 violation of the following drug-like properties - HBA ≤ 10, HBD ≤ 5, MW ≤ 500 Da, LogP ≤ 5, and a recommended LogS value of≥ − 4 [27, 28].

As shown/rummarized in Table 5 all 6 complied with the HBD and LogP criteria. Yet, their LogS (aqueous solubility) was below the recommended value meaning that these compounds are classified as poorly water soluble, and this could be an issue when it comes to dissolution. Generally, more hydrophobicity contributes to more effective passage through the gastrointestinal (GI) tract. Yet the compounds were characterized by low blood-brain barrier (BBB) permeability and by generally good GI absorption—except for compounds T1 and T5. When combined, these traits imply that if these compounds are ever taken forward as drugs, they will most likely not cross into the central nervous system, but remain largely confined to the vasculature.

Generally, the molecular weights (MW) of these compounds are within the permitted range except for the T1, T5 and T6. Moreover, compound T5 was the only one that violated the maximum threshold of HBA. The T1 and T6 compounds have the potential to inhibit liver CYP450 enzymes, indicating that they could serve as enzyme inhibitors should they be developed as drugs, but the other compounds do not show this effect. T1 and T5 violate more than one criterion of the Lipinski's Rule of Five, while the other synthesized compounds are within the acceptable values. A clear trade-off became apparent: The larger, more extremely substituted compounds such as T1 and T5 were active, but typically violated Lipinski's Rule of Five with potential bioavailability problems when compared to the smaller, more drug-like molecules (T2, T3, T4) which nevertheless showed 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 amides, based on docking results, were prepared as new inhibitors of the penicillin-binding protein (PBP). In vitro, their antimicrobial potential and PBP inhibitory activity were assessed. The docking results demonstrated that the binding scores of the tested compounds were positively correlated with the number of nitrated or halogenated substituents, for which the PBP binding pocket has some affinity. In general, the compounds synthesized were found to be weakly active against all the pathogenic microbes under test when compared to standard antimicrobial agents, indicating that a higher concentration of these novel amides is required for an effective inhibition of PBPs in tested microbial strains. The results also suggest that the combination of nitro and halogen substituents have a greater antibacterial activity than either disubstituent group. As for the antifungal activity, only one substituent showed some activity that could be related to the presence ether or additional amide substituents on those molecules.

This studies successfully designed and synthesized novel compounds showing antimicrobial effect especially T1, T3 and T4, albeit with a lower potency when compared to standard agents. These results are consistent with docking studies, which may indicate penicillin-binding protein (PBP) inhibition, and could lead the way for subsequent synthesis. Although most of the molecules followed Lipinski's Rule of Five, poor aqueous solubility, high molecular weights for some of them, and potential CYP450 inhibition for T1 and T6, suggest that there is room for

improvements in terms of 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