

Synthesis and Biological Assessment of Novel Quinazolinone–Piperazine Hybrid Derivatives as Antimicrobial Agents

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ABSTRACT

The chemical entities quinazolinone and piperazine are highly valued in organic chemistry due to their broad spectrum of therapeutic and biological activities. To explore their potential applications, researchers developed a novel set of compounds combining quinazolinone and piperazine structures. These compounds, featuring the N-(4-oxo-2-(4-(4-(2-(substituted phenylamino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide framework, were synthesized in high yields. Characterization of the newly synthesized derivatives was carried out using mass spectrometry, FTIR, ¹H NMR spectroscopy, melting point analysis, and thin-layer chromatography. The antimicrobial properties of these derivatives were evaluated using the agar well diffusion method against a variety of microbial strains, including *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*. The compounds were also tested for antifungal activity against *Candida albicans*. The broth microdilution technique was used to determine the minimum inhibitory concentration. The results showed that the synthesized compounds exhibited significant antimicrobial and antifungal activities against all tested strains. Among the tested compounds, PRP7A6, PRP7A8, and PRP7A11 were found to exhibit the strongest antimicrobial effect against pathogenic microorganisms.

Keywords: Antifungal activity, Quinazolinone, Amide, Piperazine, Antibacterial activity

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Introduction

Pathogenic microorganisms are responsible for many diseases, prompting the development of effective antimicrobial agents. While antibiotics play a crucial role in treating infections, they also present certain risks, such as the potential for resistance, allergic reactions, anaphylactic responses, disturbances in beneficial bacteria, and specific toxic effects. Resistance to antibiotics in microorganisms that affect humans is becoming more prevalent, making it harder to find effective treatment options. Moreover, the scarcity of effective treatments for fungal and mycobacterial infections underscores the urgent need for novel antimicrobial drugs. Future drug development must focus on creating new classes of drugs that offer shorter half-lives and fewer side effects to address the rising challenges of infectious diseases [1].

The quinazolinone structure is well-known for its broad therapeutic potential, attributed to its diverse substituents. Quinazolinone, a weak base with various biological effects, is recognized as one of the most valuable heterocyclic compounds, and it continues to be an area of significant scientific interest. These compounds play a vital role in medicinal and bioorganic chemistry and are crucial for drug development [2]. Quinazoline derivatives demonstrate a wide spectrum of biological activities, such as anticancer, analgesic, diuretic, antibacterial, antihypertensive, antimalarial, sedative, and hypoglycemic effects. This versatility makes quinazoline derivatives promising pharmacophores for the creation of new medications [3].

Piperazine, a heterocyclic compound, consists of a six-membered ring with nitrogen atoms positioned at the first and fourth locations [4]. The piperazine structure is classified as a privileged scaffold and is commonly found in biologically active compounds. These molecules exhibit numerous therapeutic effects, including antibacterial, antimalarial, antitubercular, antiviral, antipsychotic, antidepressant, anticonvulsant, anti-inflammatory, cytotoxic, antiarrhythmic, and antioxidant properties [5].

Both quinazoline and piperazine structures are essential in drug design, garnering significant research interest. In our study, we aimed to explore the therapeutic potential of hybrid molecules containing both quinazoline and piperazine. We synthesized specific compounds and evaluated their antibacterial and antifungal properties *in vitro*.

Materials and Methods

Chemistry

All the aniline derivatives and other chemicals used in this study were sourced from commercial suppliers and were used without further purification. Before initiating the procedures, we made sure to clean and sterilize all equipment thoroughly. The melting points of the analogs were determined using the Veego Melting Point Apparatus (model VMP-D) with the open capillary technique, and the temperatures were recorded in degrees Celsius without any adjustments. The purity of the compounds was verified through thin-layer chromatography (TLC) on precoated silica gel plates (Merck-TLC Silica Gel 60 F254). All synthesized analogs underwent recrystallization from various solvents for purification and were characterized using different spectroscopic techniques. Potassium bromide (KBr) pellets were used in Fourier transform infrared (FTIR) spectroscopy, conducted on a Shimadzu FTIR-8400S instrument. Proton nuclear magnetic resonance (¹H-NMR) spectra were recorded on a 400 MHz Bruker Advance-II spectrometer (Japan), using DMSO as the solvent. Infrared stretching frequencies were reported in cm⁻¹, while chemical shifts (δ) were presented in parts per million (ppm). Mass spectrometry for the synthesized compounds was carried out using the 2010EV LCMS SHIMADZU apparatus, operating at seventy electron volts.

Synthesis of targeted analogs

Step 1: synthesis of 4-chlorobenzoyl chloride

A reaction mixture containing 0.01 moles of 4-chloro benzoic acid and 0.015 moles of thionyl chloride was heated to reflux for two hours. After the reaction, the surplus thionyl chloride was eliminated by distillation, yielding the corresponding acid chloride [6]. The synthesis of 4-chlorobenzoyl chloride is shown in **Figure 1**.

Yield (%): 82.5

B.P. (°C): 206-208

Rf value: 0.35 (Hexane: Ethyl acetate, 2:1)

Step 2: synthesis of 2-(4-chlorophenyl)-4H-benzo[d][1,3]oxazin-4-one

0.01 moles of 2-aminobenzoic acid were combined with 0.01 moles of 4-chlorobenzoyl chloride and pyridine, and the mixture was stirred for three hours at room temperature. Following this, a 5% sodium bicarbonate solution was added, which led to the formation of a precipitate. The precipitate was then filtered, dried, and purified through recrystallization with ethanol [6]. The process for synthesizing 2-(4-chlorophenyl)-4H-benzo[d][1,3]oxazin-4-one is shown in **Figure 1**.

Yield (%): 70.6 %

M.P. (°C): 246-250

Rf value: 0.31 (Hexane: Ethyl acetate, 1:1)

Step 3: synthesis of 3-amino-2-(4-chlorophenyl)quinazolin-4(3H)-one

A reaction was conducted by combining 0.01 mol of 2-(4-chlorophenyl)-4H-benzo[d][1,3]oxazin-4-one with 0.02 mol of hydrazine hydrate in ethanol, and the mixture was stirred for five hours. Upon completion of the reaction, the mixture was left to stand, and the resulting solid was isolated by filtration. The solid was then purified through recrystallization with ethanol [6]. The reaction pathway for synthesizing 3-amino-2-(4-chlorophenyl)quinazolin-4(3H)-one is shown in **Figure 1**.

Yield (%): 73.8

M.P. (°C): 194-196

Rf value: 0.39 (Hexane: Ethyl acetate, 2:1)

Step 4: synthesis of N-(2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl) benzamide

The preparation of N-(2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl) benzamide is shown in **Figure 1**. To synthesize it, 0.01 moles of 3-amino-2-(4-chlorophenyl) quinazolin-4(3H)-one are mixed with 0.01 moles of benzoyl chloride in the presence of 10% sodium hydroxide solution and stirred at ambient temperature for three hours. After the reaction, the mixture is diluted with chilled water, filtered, and rinsed with cold water, and the resulting compound is recrystallized from ethanol [7-9].

Yield (%): 83.8

M.P. (°C): 158-164

Rfvalue: 0.43 (Hexane: Ethyl acetate, 2:1)

Step 5: synthesis of N-(4-oxo-2-(4-(piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide

Figure 1 outlines the synthesis of N-(4-oxo-2-(4-(piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide. To prepare this compound, 0.01 moles of piperazine, 0.01 moles of N-(2-(4-chlorophenyl)-4-oxoquinazolin-3(4H)-yl) benzamide, and anhydrous potassium carbonate were combined in methanol and heated under reflux for five hours with continuous stirring. Once the reaction was complete, the mixture was allowed to cool to room temperature. It was then added to ice-cold water while stirring, and the precipitate formed was purified by recrystallization [10].

Yield (%): 68.00

M.P. (°C): 172-176

Rfvalue: 0.36 (Chloroform: Methanol (8:2))

Step 6: synthesis of N-(2-(4-(4-(2-chloroacetyl) piperazin-1-yl) phenyl)-4-oxoquinazolin-3(4H)-yl) benzamide

A reaction was conducted by combining 0.01 moles of N-(4-oxo-2-(4-(piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide with 0.01 moles of chloroacetyl chloride in an ethanol medium. To facilitate the process, a few drops of glacial acetic acid were added, and the mixture was subjected to reflux for eight hours. After the reaction, the solution was cooled in ice water, allowing a precipitate to form. The precipitate was collected by filtration, washed with water, dried, and purified through recrystallization with ethanol [6]. The process for synthesizing N-(2-(4-(4-(2-chloroacetyl) piperazin-1-yl) phenyl)-4-oxoquinazolin-3(4H)-yl) benzamide is shown in **Figure 1**.

Yield (%): 62.6 %

M.P. (°C): 242-246

Rfvalue: 0.34 (Chloroform: Methanol (8:2))

Step 7: synthesis of N-(4-oxo-2-(4-(4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide derivatives

A mixture of 0.01 moles of N-(2-(4-(4-(2-chloroacetyl) piperazin-1-yl) phenyl)-4-oxoquinazolin-3(4H)-yl) benzamide and an equal amount of different aniline derivatives was prepared. The reaction was catalyzed by adding a few drops of glacial acetic acid, and the reaction medium was pure ethanol. The mixture was then heated under reflux for ten hours. Upon completion, the solution was cooled with ice-cold water, causing a precipitate to form. The precipitate was isolated via filtration, washed thoroughly with water, allowed to dry, and purified by recrystallization from ethanol. The overall synthesis of N-(4-oxo-2-(4-(4-(2-(substituted phenylamino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide derivatives is outlined in **Figure 1** [6].

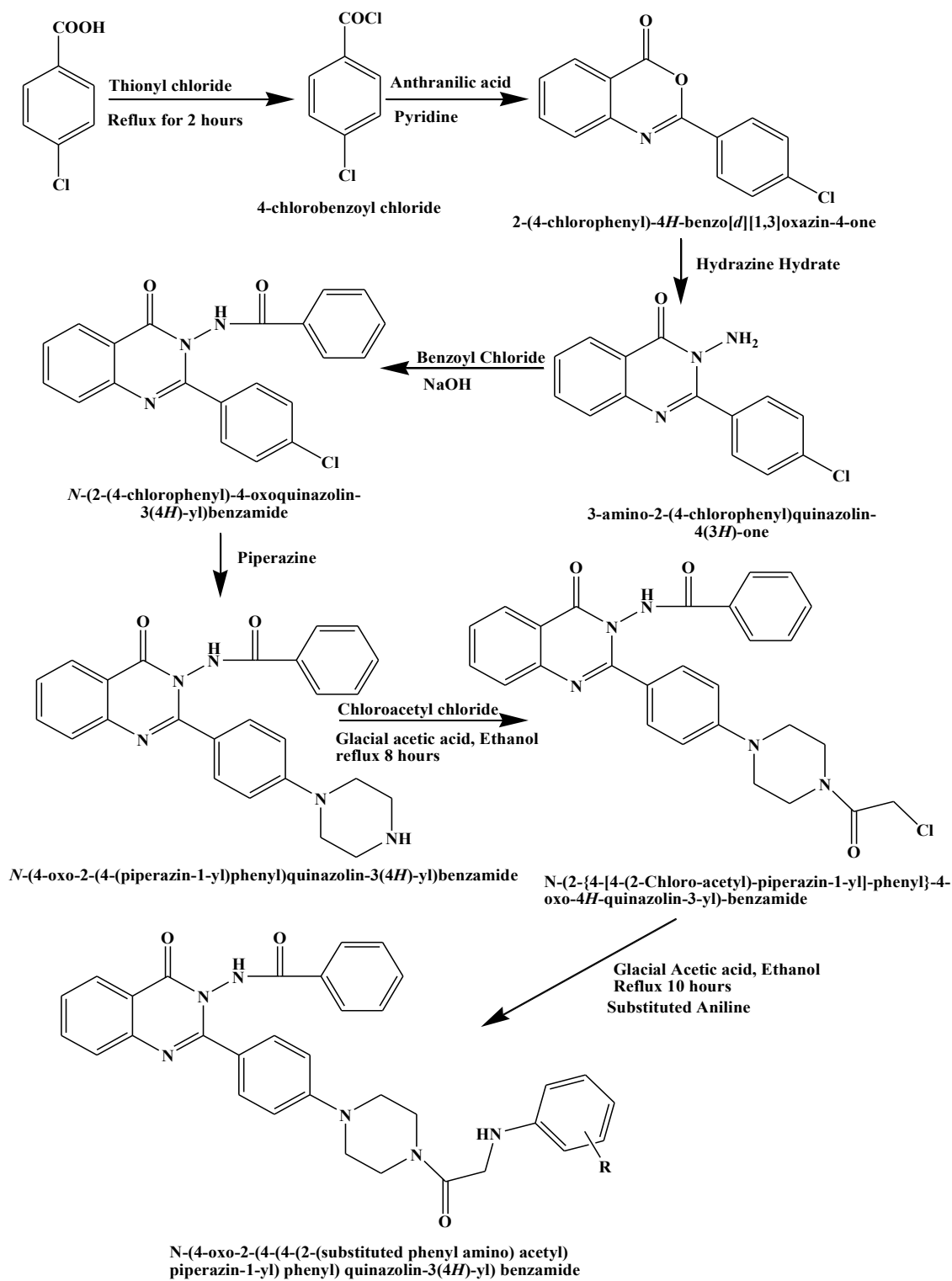


Figure 1. Synthesis of N-(4-oxo-2-(4-(4-(2-(Substituted phenylamino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide derivatives

N-(4-oxo-2-(4-(4-(2-(phenylamino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide [PRP7A1]

Yield (%): 76.6

M.P. (°C): 222-226

R_f value: 0.38 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3296 (N–H str., 2° CONH₂); 2857 (C–H str., Ar–CH₃); 1664 (C=O str., Ar–ketone); 1597 (C=O str., 2° CONH₂); 1558 (C=N str., Ar); 1384, 1322, 1262 (C–N str.)

¹H-NMR (DMSO, δ ppm): 7.71 (s, 1H, N-NH); 6.66-8.05 (m, 18H, ArH); 4.27 (s, 2H, CH₂), 3.99 (s, 1H, -N.H.); 3.46-3.81 (m, 8H, CH₂-Piperazine)
Mass spectra (m/z) : 558.43 (M⁺)

N-(2-(4-(4-(2-(*o*-toluidino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A2]

Yield (%): 88.4

M.P. (°C): 224-228

R_f value: 0.57 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3300 (N–H str., 2° CONH₂); 2849 (C–H str., Ar-CH₃); 1705 (C=O str., Ar-ketone); 1686 (C=O str., 2° CONH₂); 1644 (C=N str., Ar); 1326, 1302, 1263 (C–N str.)

¹H-NMR (DMSO, δ ppm): 8.034 (s, 1H, N-NH); 7.4-7.75 (m, 17H, ArH); 4.3 (s, 1H, -N.H.); 3.34, 3.88 (m, 10H, CH₂-Piperazine); 2.35 (t, 3H, CH₃)

N-(2-(4-(4-(2-(2-methoxyphenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A3]

Yield (%): 78.9

M.P. (°C): 218-222

R_f value: 0.44 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3300 (N–H str., 2° CONH₂); 2850 (C–H str., -CO-CH₂); 1705 (C=O str., Ar-ketone); 1686 (C=O str., 2° CONH₂); 1644 (C=N str., Ar); 1325, 1302, 1239 (C–N str.); 1165 (C–O–C str., Ar–O–CH₃)

¹H- NMR (DMSO, δ ppm): 10.5 (s, 1H, N-NH); 7.49-8.45 (m, 17H, ArH); 3.87 (m, 10H, CH₂-Piperazine); 3.39 (s, 1H, -N.H.); 2.50 (m, 3H, Ar-OCH₃)

Mass spectra (m/z) : 588.25 (M⁺)

N-(2-(4-(4-(2-(4-methoxyphenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A4]

Yield (%): 75.2

M.P. (°C): 182-186

R_f value: 0.53 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3310 (N–H str., 2° CONH₂); 2850 (C–H str., -CO-CH₂); 1680 (C=O str., Ar-ketone); 1590 (C=O str., 2° CONH₂); 1340, 1300, 1250 (C–N str.); 1530 (C=N str., Ar); 1110 (C–O–C str., Ar–O–CH₃)

¹H- NMR (DMSO, δ ppm): 10.51 (s, 1H, N-NH); 7.53-8.44 (m, 17H, ArH), 4.34 (s, 1H, -NH); 3.87 (d, 2H, CH₂); 3.78 (s, 3H, Ar-OCH₃); 3.36-3.57 (m, 8H, CH₂-Piperazine)

N-(2-(4-(4-(2-(*p*-toluidino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A5]

Yield (%): 72.7

M.P. (°C): 194-198

R_f value: 0.57 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3290 (N–H str., 2° CONH₂); 2950 (C–H str., -CO-CH₂); 1710 (C=O str., Ar-ketone); 1620 (C=O str., 2° CONH₂); 1590 (C=N str., Ar); 1320, 1300, 1280 (C–N str.)

N-(2-(4-(4-(2-(4-chlorophenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A6]

Yield (%): 66.6

M.P. (°C): 212-216

R_f value: 0.35 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3250 (N–H str., 2° CONH₂); 2960 (C–H str., -CO-CH₂); 1680 (C=O str., Ar-ketone); 1650 (C=O str., 2° CONH₂); 1590 (C=N str., Ar); 1350, 1260, 1190 (C–N str.); 750 (C–Cl str., Ar–Cl)

¹H-NMR (DMSO, δ ppm): 10.52 (s, 1H, N-NH); 7.47-8.45 (m, 17H, ArH); 3.36 (s, 1H, -N.H.); 2.50 (m, 8H, CH₂-Piperazine); 1.22 (d, 2H, CH₂)

Mass spectra (m/z) : 592.89 (M⁺)

N-(2-(4-(4-(2-(4-hydroxyphenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A7]

Yield (%): 74.6

M.P. (°C): 176-180

R_f value: 0.51 (Chloroform: Methanol, 8:2)

IR (KBr, cm⁻¹): 3350 (OH str., Ar); 3240 (N–H str., 2° CONH₂); 2890 (C–H str., -CO-CH₂); 1690 (C=O str., Ar-ketone); 1650 (C=O str., 2° CONH₂); 1590 (C=N str., Ar); 1310, 1290, 1240 (C–N str.)

¹H-NMR (DMSO, δ ppm): 6.45-8.06 (m, 17H, Ar-H); 7.73 (s, 1H, N-NH); 3.40-3.75 (m, 8H, CH₂-Piperazine); 4.22 (s, 2H, CH₂); 3.71 (s, 1H, O.H.); 4.05 (s, 1H, N.H.)

Mass spectra (m/z) : 574.11 (M⁺)

N-(2-(4-(4-(2-(4-nitrophenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A8]

Yield (%): 74.9

M.P. (°C): 204-208

R_f value: 0.66 (Chloroform: Methanol, 8:2)

IR (KBr, cm⁻¹): 3300 (N–H str., 2° CONH₂) ; 2950 (C–H str., -CO-CH₂); 1710 (C=O str., Ar-ketone); 1700 (C=O str., 2° CONH₂); 1650 (C=N str., Ar); 1550 (N–O str., Ar-NO₂); 1300, 1280, 1220 (C–N str.)

¹H- NMR (DMSO, δ ppm): 6.51-8.14 (m, 17H, ArH); 7.07 (s, 1H, N-NH); 4.76 (s, 1H, -N.H.); 4.13-4.17 (d, 2H, CH₂); 3.44-3.74 (m, 8H, CH₂-Piperazine)

Mass spectra (m/z): 603.23 (M⁺)

N-(2-(4-(4-(2-(*m*-toluidino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A9]

Yield (%): 82.2

M.P. (°C): 212-216

R_f value: 0.46 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3250 (N–H str., 2° CONH₂); 2980 (C–H str., -CO-CH₂); 1750 (C=O str., Ar-ketone); 1680 (C=O str., 2° CONH₂); 1650 (C=N str., Ar); 1350, 1300, 1260 (C–N str.)

N-(2-(4-(4-(2-(3-methoxyphenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A10]

Yield (%): 72.2

M.P. (°C): 190-194

R_f value: 0.57 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3300 (N–H str., 2° CONH₂); 2840 (C–H str., -CO-CH₂); 1680 (C=O str., Ar-ketone); 1650 (C=O str., 2° CONH₂); 1610 (C=N str., Ar); 1300, 1210, 1180 (C–N str.); 1150 (C–O–C str., Ar–O–CH₃)

N-(2-(4-(4-(2-(2-chlorophenylamino) acetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4H) -yl) benzamide [PRP7A11]

Yield (%): 80.0

M.P. (°C): 206-210

R_f value: 0.42 (Hexane: Ethyl acetate, 8:2)

IR (KBr, cm⁻¹): 3300 (N–H str., 2° CONH₂); 2960 (C–H str., -CO-CH₂); 1700 (C=O str., Ar-ketone); 1650 (C=O str., 2° CONH₂); 1600 (C=N str., Ar); 1350, 1290, 1240 (C–N str.) ; 700 (C–Cl str., Ar–Cl)

Assess the effectiveness of antimicrobial agents against microorganisms

Agar well diffusion assay

The agar well diffusion method is a widely used and simple approach to test the antibacterial and antifungal properties of newly synthesized compounds. To start, suitable culture media must be prepared for the bacteria, which includes Mueller Hinton agar, and for fungi, which requires Sabouraud dextrose agar. Once the media are set, the microorganisms to be tested are introduced, and incubation is carried out at 37 °C for 24 hours for bacteria and 27 °C for 48 hours for fungi. Wells are then created in the agar using a sterile cork borer, and these wells are filled with solutions of the synthesized analogs (PRP7A1 to PRP7A11), using sterile pipettes. A well containing

only DMSO serves as a negative control, and a positive control is also included, typically using Ciprofloxacin for bacterial testing and fluconazole for antifungal testing. After setting the wells and adding the control substances, the plates are re-incubated at the respective temperatures for each microorganism. The final step is to measure the inhibition zones around each well, which indicates the antimicrobial activity of the tested compound and provides valuable data about its effectiveness as an antibacterial or antifungal agent [11-13].

The antimicrobial testing was conducted against several microorganisms, which included gram-positive bacteria such as *S. aureus* (MTCC No. 96) and *B. subtilis* (MTCC No. 441), gram-negative bacteria including *E. coli* (MTCC No. 443) and *P. aeruginosa* (MTCC No. 1688), and the fungus *C. albicans* (MTCC No. 227).

Minimum inhibitory concentration test (MIC)

The minimum inhibitory concentration (MIC) is a critical metric used in microbiology and clinical medicine to assess the effectiveness of antimicrobial agents. It provides essential data that helps in understanding the potency of these substances and is indispensable in the treatment and monitoring of infections [14].

To prepare the stock solutions, each of the synthesized drugs was initially diluted to a concentration of two thousand µg/ml. In the first phase of testing, a range of concentrations from 125 to 1000 µg/ml was used to evaluate the compounds. Those that exhibited antimicrobial activity during this preliminary testing were selected for further concentration testing, using dilutions of 100, 50, 25, 12.5, and 6.25 µg/ml to assess their efficacy against a variety of microorganisms [15, 16].

The antimicrobial activity of the synthesized compounds was assessed following a structured procedure. Initially, 1 ml of each high-concentration solution was added to 4 ml of nutrient agar in a test tube, and the contents were thoroughly mixed. A 0.5 ml microbial suspension was then introduced into each tube and mixed carefully. The tubes were incubated at 27 °C for 48 hours for fungal evaluation and at 37 °C for 24 hours for bacterial testing. After the incubation period, the turbidity in the tubes was observed, and the MIC was determined based on these results [15, 16].

Results and Discussion

This study aimed to synthesize and evaluate new quinazoline-piperazine hybrid compounds for their antimicrobial properties. The yield of these compounds ranged from 66% to 88%. Physical characterization was done by measuring the melting points and performing thin-layer chromatography (TLC), while their structural features were confirmed through FTIR, proton NMR, and mass spectrometry.

These newly synthesized compounds were tested against a range of microorganisms, including gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and the fungus *Candida albicans*. The results indicated that the compounds exhibited activity against all tested microorganisms, suggesting their potential as effective antibacterial and antifungal agents. Ciprofloxacin and fluconazole were used as reference standards for antibacterial and antifungal activities, respectively.

Table 1. Investigating the antibacterial and antifungal effectiveness of synthesized substances through the assessment of their zone of inhibition

Compounds	Diameter of zone of inhibition (mm)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
	200 µg/ml	200 µg/ml	200 µg/ml	200 µg/ml	200 µg/ml
PRP7A1	17	15	14	12	19
PRP7A2	15	14	13	11	16
PRP7A3	14	14	12	12	13
PRP7A4	17	16	15	19	18
PRP7A5	19	18	17	20	19
PRP7A6	24	21	22	23	22
PRP7A7	16	12	13	16	18
PRP7A8	24	20	25	25	20

PRP7A9	15	15	14	13	17
PRP7A10	16	14	15	16	15
PRP7A11	23	20	24	22	21
Ciprofloxacin	25	22	27	26	-
Fluconazole	-	-	-	-	26
Control (DMSO)	-	-	-	-	-

“(–)” signifies the absence of an inhibition zone, indicating no observable activity.

Table 2. The minimal inhibitory concentration of the synthesized substances for both bacterial and fungal strains is being determined

Compounds	Minimum inhibitory concentration (µg/ml)				
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>C. albicans</i>
PRP7A1	25	50	25	25	125
PRP7A2	50	100	125	50	125
PRP7A3	100	125	125	100	125
PRP7A4	25	50	25	25	50
PRP7A5	25	50	50	25	50
PRP7A6	12.5	25	25	12.5	25
PRP7A7	50	100	100	50	125
PRP7A8	12.5	25	12.5	12.5	25
PRP7A9	50	50	25	25	125
PRP7A10	25	100	100	25	125
PRP7A11	12.5	50	25	12.5	50
Ciprofloxacin	< 3	< 3	< 3	< 3	-
Fluconazole	-	-	-	-	< 8

(-) indicates not applicable

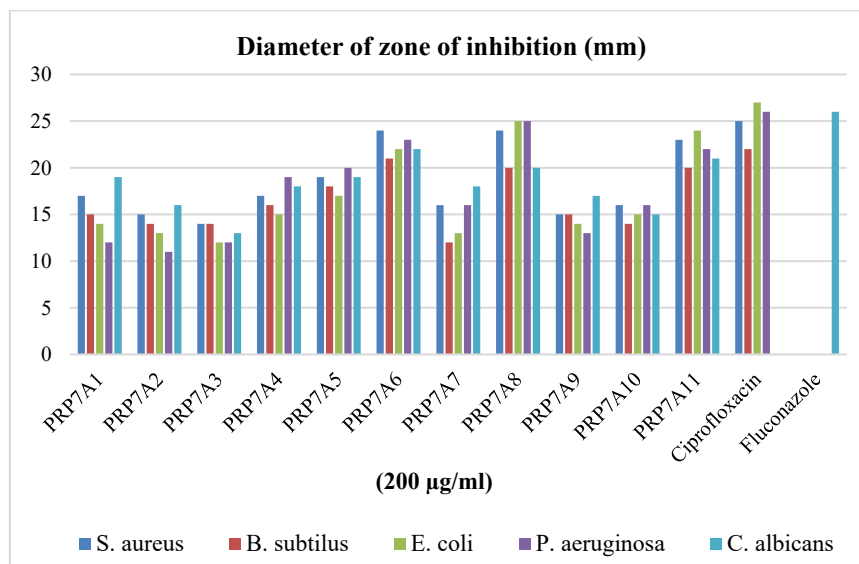


Figure 2. Statistical portrayal of the biological activity of synthesized compounds

PRP7A8 emerged as the most effective compound among those synthesized, showing excellent antibacterial results. It achieved MIC values of 12.5 micrograms per milliliter against *Staphylococcus aureus*, 25 micrograms per milliliter against *Bacillus subtilis*, 12.5 micrograms per milliliter against *Escherichia coli*, and 12.5 micrograms per milliliter against *Pseudomonas aeruginosa*, outperforming the other compounds. In addition,

PRP7A8 demonstrated strong antifungal properties, with a MIC of 25 µg/ml against *Candida albicans*, making it the most effective in this category as well.

PRP7A6 and PRP7A11 also exhibited significant antibacterial activity, with MICs of 12.5 micrograms per milliliter against *S. aureus*, 25 micrograms per milliliter, and 50 micrograms per milliliter against *B. subtilis*, 25 micrograms per milliliter against *E. coli*, and 12.5 micrograms per milliliter against *P. aeruginosa*. The antimicrobial assessment, detailed in **Tables 1 and 2**, confirms that all tested compounds displayed notable activity against a variety of microbial strains. **Figure 2** provides a graphical representation of the biological efficacy of these compounds.

The results indicated that introducing electron-withdrawing groups like NO₂ and Cl to the phenylamino ring of quinazolinone (as seen in PRP7A8, PRP7A6, and PRP7A11) enhanced their antibacterial and antifungal activities. Conversely, electron-donating groups such as CH₃, OCH₃, and OH were found to decrease the compounds' effectiveness. Additionally, substituents positioned at the para location resulted in stronger activity than those placed at the meta position, and the meta position was more potent than the ortho position. Despite these promising results, the synthesized compounds did not perform as well as the reference drugs.

Conclusion

An exploration of the antimicrobial properties of quinazolinone derivatives revealed their strong antibacterial and antifungal activities against various pathogenic strains. The presence of electron-withdrawing groups like -NO₂ and -Cl in the compound structures was identified as a major contributor to their robust antimicrobial effects. Notably, the compounds PRP7A6, PRP7A8, and PRP7A11 exhibited the highest level of antimicrobial effectiveness against the tested pathogens. These results highlight the potential of quinazolinone derivatives as a basis for a new class of antimicrobial agents. Compounds that demonstrate significant biological activity open up promising prospects for the development of more potent antimicrobial drugs.

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Conflict of Interest: None

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Ethics Statement: None

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