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Synthesis and Biological Evaluation of Some Novel Hybrid Quinazolinone–Piperazine Derivatives as Anti-Microbial Agents

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ABSTRACT

The chemical constituents quinazolinone and piperazine hold significant importance in the realm of organic compounds due to their diverse range of biological and therapeutic attributes. In an effort to investigate the possible uses of these molecules, a team of scientists synthesized a unique set of chemical substances that combined piperazine and quinazolinone structures. This research involved a comprehensive investigation into the antimicrobial properties of a set of derivatives bearing the chemical structure N-(4-oxo-2-(4-(4-(2-(Substituted phenylamino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4H)-yl) benzamide, which was successfully synthesized with high yields. The synthesized compounds were carefully characterized using a range of physical methods, including mass spectrometry, FTIR, and ^{1}H NMR spectroscopy, in addition to physical methods such as melting point measurement and thin-layer chromatography. Subsequently, using the agar well diffusion method, these compounds were evaluated for their antibacterial activity against a panel of microbial strains, including Staphylococcus aureus, Bacillus subtilis, Escherichia coli, and Pseudomonas aeruginosa. The antifungal assay was also conducted against Candida albicans using the same approach. The broth microdilution technique was also used to establish the minimal inhibitory concentration. The synthesized compounds demonstrated impressive antifungal and antibacterial properties against every tested microbe. Notably, among the compounds evaluated, PRP7A6, PRP7A8, and PRP7A11 displayed the most potent antimicrobial effects against the pathogenic strains.

Key words: *Quinazolinone, Piperazine, Amide, Antibacterial activity, Antifungal activity*

INTRODUCTION

Numerous diseases stem from the invasion of pathogenic organisms. In order to combat these ailments, scientists have discovered potent and broad-spectrum antimicrobial agents. While antibiotics are undoubtedly life-saving therapeutic drugs, it's important to note that they can also pose potential risks. These include the development of antibiotic resistance, allergic reactions, anaphylactic reactions, disturbance of the harmless bacterial flora, and specific toxic effects. The emergence of resistance in microorganisms that are normally harmful to humans has been increasing significantly in recent years. This growing resistance has constrained the options for choosing antimicrobial treatments against specific pathogens. Furthermore, considering the scarcity of effective treatments for fungal and mycobacterial infections, there is an urgent need for the development of novel antimicrobials. It is critical to develop new drug classes with shorter half-lives and lesser side effects in order to combat infectious diseases in the future [1].

The quinazolinone ring structure is well known for its broad range of medicinal properties due to its diverse substituents. As a weak base with a variety of biological applications, quinazolinone is regarded as one of the most important heterocyclic compounds and is still of great scientific interest today. These substances are widely used in medicinal and bioorganic chemical science, and they are essential to the drug-discovery process [2]. Quinazoline exhibits a broad spectrum of biological activities, including anticancer, analgesic, diuretic, antibacterial, antihypertensive, antimalarial, sedative, and hypoglycemic effects. These examples clearly demonstrate the potential of quinazoline derivatives as valuable pharmacophores for facilitating the development of new medications [3].

Piperazine is a heterocyclic organic chemical formed by a six-membered ring with two nitrogen atoms, which serve as heteroatoms at the first and fourth positions [4]. The piperazine nucleus has been categorized as a privileged structural element and is commonly encountered in compounds with biological significance. These synthetic compounds exhibit numerous and significant biological actions, including antibacterial, antitubercular, antimalarial, antiviral, antipsychotic, anticonvulsant, antidepressant, anti-inflammatory, cytotoxic, antiarrhythmic, and antioxidant effects [5].

The quinazoline and piperazine nuclei are crucial elements in drug research and development, attracting considerable interest. In the pursuit of uncovering the medicinal potential of hybrids containing quinazoline and piperazine as potential therapeutic agents, we deemed it worthwhile to design and synthesize specific biologically active compounds. The antibacterial and antifungal abilities of these substances were then evaluated *in vitro*.

MATERIALS AND METHODS

Chemistry

We procured all the derivatives of aniline and various other chemicals from commercial sources and utilized them without any additional purification. Prior to commencing the procedures, we ensured that all the equipment had been thoroughly cleaned and sterilized. Using the VEEGO MELTING POINT APPARATUS model VMP-D and the open capillary method, we were able to calculate the melting points of the mentioned analogs. We recorded these temperatures in degrees Celsius with no corrections. The purity of the compounds was confirmed through the application of precoated TLC plates (Merck-TLC Silica gel 60 F254). We subjected all the synthesized analogs to purification via recrystallization using various solvents and characterized them through spectroscopic analysis. Pellets of potassium bromide (KBr) were utilized in Fourier transform infrared spectrophotometry (FTIR-8400S) performed on a Shimadzu, USA, instrument, and proton nuclear magnetic resonance (^{1}H-NMR) spectra of the pure analogs were acquired using a 400 MHz Spectrophotometer by Bruker Advance-II, Japan, with DMSO as the solvent. In reporting, I.R. stretching values were expressed in cm^{-1}, and chemical shift values (δ) were measured in parts per million. Mass spectra of the synthesized compounds were acquired at 70 electron volts using the 2010EV LCMS SHIMADZU apparatus.

Synthesis of targeted analogs

Step 1: Synthesis of 4-chlorobenzoyl chloride

A solution containing 0.01 moles of 4-chloro benzoic acid and 0.015 moles of thionylchloride was subjected to reflux for a duration of 2 hours. Excess thionyl chloride was then removed through distillation, resulting in the formation of the corresponding acid chloride [6]. The synthesis of 4-chlorobenzoyl chloride is depicted in **Figure 1**.

Yield (%): 82.5

B.P. (^{o}C): 206-208

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

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

A given amount of 0.01 moles of 2-aminobenzoic acid was stirred constantly for a duration of three hours at ambient temperature, with 0.01 moles of 4-chlorobenzoyl chloride and pyridine acting as a catalyst. The resulting mixture was subjected to treatment with a 5% solution of sodium bicarbonate, resulting in the precipitation of a solid. This solid was then subjected to filtration, drying, and a subsequent recrystallization process using ethanol [6]. The reaction for the synthesis of 2-(4-chlorophenyl) -4*H*-benzo[d] [1,3] oxazin-4-one was indicated in **Figure 1**.

Yield (%): 70.6 %

M.P. (ºC): 246-250
R_f value: 0.31 (Hexane: Ethyl acetate, 1:1)

Step 3: Synthesis of 3-amino-2-(4-chlorophenyl) quinazolin-4(3H) -one
3-amino-2-(4-chlorophenyl) quinazoline-4 (3H)-one was synthesized by reacting 0.01 mol of 2-(4-chlorophenyl)-*4H*-benzo[d][1,3]oxazin-4-one with 0.02 mol of hydrazine hydrate in the presence of ethanol for five hours. After the reaction was complete, the mixture was allowed to settle, and the solid precipitate was separated by filtration. It was then purified through recrystallization using ethanol [6]. **Figure 1** illustrates the reaction involved in the synthesis of 3-amino-2-(4-chlorophenyl) quinazolin-4(3*H*) -one.
Yield (%): 73.8
M.P. (ºC): 194-196
R_f value: 0.39 (Hexane: Ethyl acetate, 2:1)

Step 4: Synthesis of N-(2-(4-chlorophenyl) -4-oxoquinazolin-3(4H) -yl) benzamide
The reaction associated with the synthesis of N-(2-(4-chlorophenyl) -4-oxoquinazolin-3(4*H*) -yl) benzamide is depicted in **Figure 1**. Stir 0.01 moles of 3-amino-2-(4-chlorophenyl) quinazolin-4(3*H*) -one for 3 hours at room temperature alongside 0.01 moles of Benzoyl Chloride, with a 10% NaOH solution present. Subsequently, dilute the reaction mixture with cold water, perform filtration, wash with cold water, and initiate the crystallization process using ethanol [7-9].
Yield (%): 83.8
M.P. (ºC): 158-164
$R_f$value: 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
The synthetic procedure for N-(4-oxo-2-(4-(piperazin-1-yl) phenyl) quinazolin-3(4*H*) -yl) benzamide is depicted in **Figure 1**. A blend comprising 0.01 moles of piperazine, 0.01 moles of N-(2-(4-chlorophenyl) -4-oxoquinazolin-3(4*H*) -yl) benzamide, and anhydrous potassium carbonate in methanol underwent reflux for a duration of five hours while constantly stirring. The resultant mixture had been cooled to a normal temperature when the reaction was finished. Subsequently, it was immersed in ice-cold water while being continually stirred. The solid that precipitated was subjected to purification through a recrystallization process [10].
Yield (%): 68.00
M.P. (ºC): 172-176
$R_f$value: 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 carried out involving 0.01 moles of N-(4-oxo-2-(4-(piperazin-1-yl) phenyl) quinazolin-3(4*H*) -yl) benzamide and 0.01 moles of chloro acetyl chloride. A few drops of glacial acetic acid were added to absolute ethanol, which served as the reaction medium. The mixture was refluxed for a duration of eight hours. After cooling in ice-cold water, a precipitate formed from the resultant solution. The precipitate was filtered, rinsed with water, allowed to dry, and then crystallized using ethanol [6]. The response to the synthesis of N-(2-(4-(4-(2-chloroacetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4*H*) -yl) benzamide is illustrated in **Figure 1**.
Yield (%): 62.6 %
M.P. (ºC): 242-246
$R_f$value: 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
An amount of 0.01 moles of N-(2-(4-(4-(2-chloroacetyl) piperazin-1-yl) phenyl) -4-oxoquinazolin-3(4*H*) -yl) benzamide was subjected to a reaction with an equimolar quantity of various corresponding anilines. A few drops of glacial acetic acid were used to catalyze this reaction, which occurred in pure ethanol and was refluxed for a period of ten hours. The intended outcome was the synthesis of compounds labeled as (1–11). After the reaction, the mixture was allowed to cool in ice-cold water, resulting in the formation of a precipitate. The precipitate was collected by filtration, rinsed with water, and allowed to dry. It was then recrystallized from ethanol. The synthesis of N-(4-oxo-2-(4-(4-(2-(substituted phenyl amino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4*H*) -yl) benzamide derivatives is depicted in **Figure 1** [6].

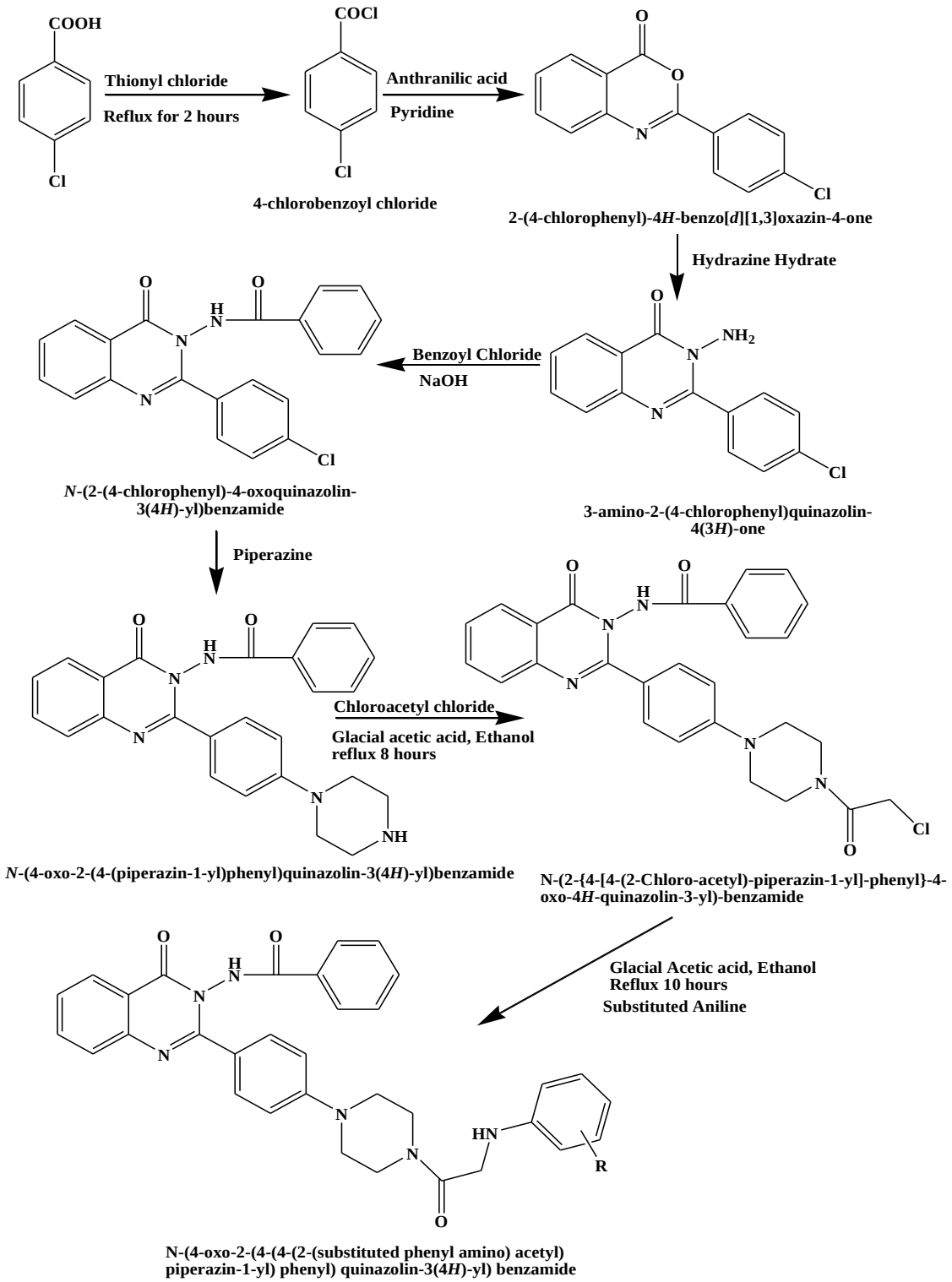

Figure 1. Synthesis of N-(4-oxo-2-(4-(4-(2-(Substituted phenyl amino) acetyl) piperazin-1-yl) phenyl) quinazolin-3(4*H*) -yl) benzamide derivatives

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° $CONH_2$) ;2857 (C-H str., Ar-CH_3) ;1664 (C=O str., Ar-ketone) ; 1597 (C=O str., 2° $CONH_2$) ; 1558 (C=N str., Ar) ; 1384, 1322, 1262 (C–N str.)
^{1}H- NMR (DMSO, δ ppm): 7.71 (s, 1H, N-NH); 6.66-8.05 (m, 18H, ArH); 4.27 (s, 2H, CH_2), 3.99 (s, 1H, -N.H.); 3.46-3.81 (m, 8H, CH_2-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° $CONH_2$) ; 2849 (C-H str., Ar-CH_3) ;1705 (C=O str., Ar-ketone) ;1686 (C=O str., 2° $CONH_2$) ;1644 (C=N str., Ar) ; 1326, 1302, 1263 (C–N str.)
^{1}H- 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, CH_2-Piperazine); 2.35 (t, 3H, CH_3)

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° $CONH_2$) ; 2850 (C-H str., -CO-CH_2) ; 1705 (C=O str., Ar-ketone) ;1686 (C=O str., 2° $CONH_2$) ;1644 (C=N str., Ar) ; 1325, 1302, 1239 (C–N str.) ; 1165 (C-O-C str., Ar-O-CH_3)
^{1}H- NMR (DMSO, δ ppm): 10.5 (s, 1H, N-NH); 7.49-8.45 (m, 17H, ArH); 3.87 (m, 10H, CH_2-Piperazine); 3.39 (s, 1H, -N.H.); 2.50 (m, 3H, Ar-OCH_3)
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° $CONH_2$) ; 2850 (C-H str., -CO-CH_2) ; 1680 (C=O str., Ar-ketone) ; 1590 (C=O str., 2° $CONH_2$) ; 1340, 1300, 1250 (C–N str.) ; 1530 (C=N str., Ar) ; 1110 (C-O-C str., Ar-O-CH_3)
^{1}H- 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, CH_2) ; 3.78 (s, 3H, Ar-OCH_3) ; 3.36-3.57 (m, 8H, CH_2-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° $CONH_2$) ; 2950 (C-H str., -CO-CH_2) ; 1710 (C=O str., Ar-ketone) ;1620 (C=O str., 2° $CONH_2$) ;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° $CONH_2$) ; 2960 (C-H str., -CO-CH_2) ; 1680 (C=O str., Ar-ketone) ;1650 (C=O str., 2° $CONH_2$) ;1590 (C=N str., Ar) ; 1350, 1260, 1190 (C–N str.) ; 750 (C-Cl str., Ar-Cl)
^{1}H- 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, CH_2-Piperazine); 1.22 (d, 2H, CH_2)
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° $CONH_2$) ; 2890 (C-H str., -CO-CH_2) ; 1690 (C=O str., Ar-ketone) ;1650 (C=O str., 2° $CONH_2$) ;1590 (C=N str., Ar) ; 1310, 1290, 1240 (C–N str.)
^{1}H- NMR (DMSO, δ ppm): 6.45-8.06 (m, 17H, Ar-H); 7.73 (s, 1H, N-NH); 3.40-3.75 (m, 8H, CH_2-Piperazine); 4.22 (s, 2H, CH_2); 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° $CONH_2$) ; 2950 (C-H str., -CO-CH_2); 1710 (C=O str., Ar-ketone); 1700 (C=O str., 2° $CONH_2$); 1650 (C=N str., Ar); 1550 (N-O str., Ar-NO_2); 1300, 1280, 1220 (C–N str.)
^{1}H- 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, CH_2); 3.44-3.74 (m, 8H, CH_2-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° $CONH_2$) ; 2980 (C-H str., -CO-CH_2) ; 1750 (C=O str., Ar-ketone) ;1680 (C=O str., 2° $CONH_2$) ;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° $CONH_2$) ; 2840 (C-H str., -CO-CH_2) ; 1680 (C=O str., Ar-ketone) ;1650 (C=O str., 2° $CONH_2$) ;1610 (C=N str., Ar) ; 1300, 1210, 1180 (C–N str.) ; 1150 (C-O-C str., Ar-O-CH_3)

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° $CONH_2$) ; 2960 (C-H str., -CO-CH_2) ; 1700 (C=O str., Ar-ketone) ;1650 (C=O str., 2° $CONH_2$) ;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 technique represents a straightforward and widely employed method for evaluating the antifungal and antibacterial potential of newly synthesized analogs. To initiate the process, one must prepare appropriate culture media for both bacteria, which entails utilizing Mueller Hinton agar medium and fungi, necessitating the use of Sabouraud dextrose agar media. Afterward, the test microorganisms should be added to the prepared media, followed by incubation at 37°C for 24 hours for bacteria and 27°C for 48 hours for fungi. Wells are created in the agar using a sterile cork borer. These wells are subsequently filled with solutions containing the synthesized analogs, specifically PRP7A1 to PRP7A11, utilizing either sterile pipettes or micropipettes. To serve as a negative control, it's essential to include a well filled exclusively with DMSO, which functions as the solvent used to dissolve the antimicrobial agents. Additionally, a positive control should be established by including a well-filled reference drug, such as Ciprofloxacin for antibacterial evaluation and Fluconazole for antifungal assessment. Once the wells are prepared and the control substances are introduced, the agar plates should be re-incubated at the appropriate temperature corresponding to the test microorganisms.

Following this second incubation, the final step involves measuring the diameter of the inhibition zones surrounding each well. The size of these inhibition zones serves as an indicator of the antimicrobial efficacy of the test compound, providing valuable insights into its potential as an antifungal or antibacterial agent [11-13].
The antimicrobial testing encompassed an assessment of various microorganisms. This assessment covered Gram-positive strains, specifically *S. aureus* (MTCC No. 96) and *B. subtilis* (MTCC No. 441), along with Gram-negative strains, which included *E. coli* (MTCC No. 443) and *P. aeruginosa* (MTCC No. 1688). For the examination of antifungal properties, *C. albicans* (MTCC No. 227) was the organism chosen for evaluation.

Minimum inhibitory concentration test (MIC)
The Minimum Inhibitory Concentration (MIC) holds significant importance in the fields of microbiology and clinical practice. It serves as a pivotal resource, furnishing vital data regarding the effectiveness of antimicrobial substances while also playing an indispensable role in the supervision of infectious ailments [14].
A stock solution for each synthesized drug was prepared by diluting it to a concentration of 2000 µg/ml. In the initial screening phase, the synthesized drugs underwent concentration-dependent testing, with concentrations ranging from 125 to 1000 µg/ml. Those drugs displaying activity in the primary screening underwent additional testing. They were further diluted to achieve concentrations of 100, 50, 25, 12.5, and 6.25 µg/ml for evaluation against all microorganisms [15, 16].
An evaluation of the antimicrobial properties of the newly synthesized compounds was conducted through a well-defined procedure. Initially, 1 ml of each highly concentrated solution of these newly synthesized compounds was introduced into a test tube containing 4 ml of nutrient agar, followed by thorough mixing. Subsequently, 0.5 ml of a microbial suspension was incorporated into each test tube and mixed meticulously. The test tubes were subsequently incubated for a total of 48 hours at 27°C for fungus and 24 hours at 37°C for bacteria. Following the designated incubation period, an evaluation of the turbidity within the test tubes was performed, and this data was used to calculate the Minimum Inhibitory Concentration [15, 16].

RESULTS AND DISCUSSION

The objective of this research was to create and assess innovative quinazoline-piperazine hybrids for their potential as antimicrobial substances. The synthesized compounds yielded between 66% and 88%. The physical attributes were determined through measurements of melting points and thin-layer chromatography (TLC), while their spectroscopic characteristics were established via FTIR, proton nuclear magnetic resonance, and mass spectrometry analysis.
The newly synthesized compounds underwent testing on both Gram-positive bacteria (specifically, *Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative bacteria (namely, *Escherichia coli* and *Pseudomonas aeruginosa*). Additionally, they were subjected to testing against the fungus Candida albicans. The results of these tests revealed the compounds' effectiveness in combating all the examined microorganisms. These findings imply that the synthesized compounds hold significant potential as novel antibacterial and antifungal medications. In this study, Ciprofloxacin served as the benchmark antibacterial drug, and Fluconazole was employed as the reference antifungal medication.

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)				
	S. aureus	***B. subtilus***	***E. coli***	***P. aeruginosa***	***C. albicans***
	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)				
	S. aureus	***B. subtilus***	***E. coli***	***P. aeruginosa***	***C. albicans***
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

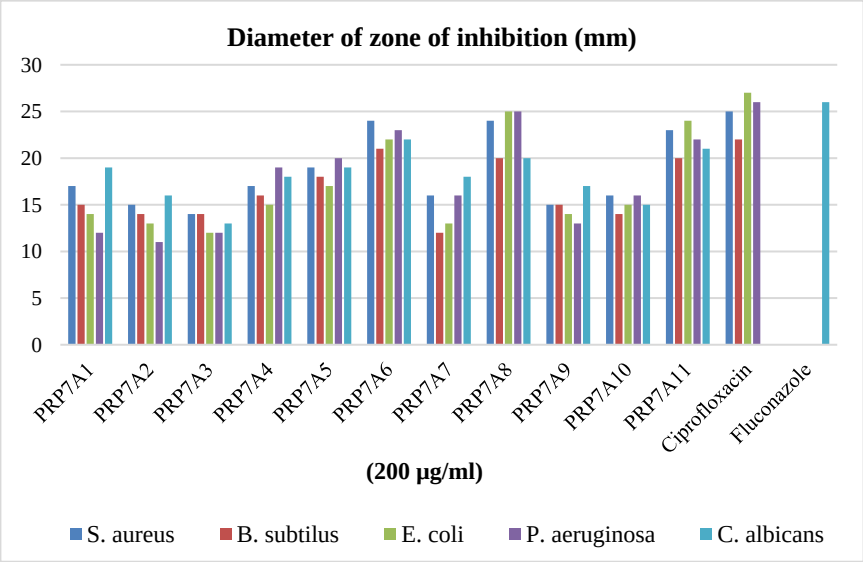

Figure 2. Statistical portrayal of the biological activity of synthesized compounds

PRP7A8 emerges as a stand-out among the synthesized compounds due to its exceptional antibacterial performance. It demonstrates a MIC value of 12.5 µg/ml against S. aureus, 25 µg/ml against B. subtilis, 12.5 µg/ml against *E. coli*, and 12.5 µg/ml against *P. aeruginosa*, surpassing the antibacterial effectiveness of the other synthesized compounds. In addition, both PRP7A6 and PRP7A11 also exhibit significant antibacterial activity,

with MIC values of 12.5 µg/ml against *S. aureus*, 25 µg/ml, and 50 µg/ml against *B. subtilis*, 25 µg/ml against *E. coli*, and 12.5 µg/ml against *P. aeruginosa*. Furthermore, PRP7A8 demonstrates superior antifungal properties, with a MIC value of 25 µg/ml towards the fungus *C. albicans*, distinguishing it from the rest of the synthesized compounds.

In reference to the *in vitro* antibacterial and antifungal qualities, the data presented in **Tables 1 and 2** demonstrate that each produced substance exhibited significant efficacy against strains of bacteria and fungi. **Figure 2** illustrates the statistical representation of the biological efficacy of the synthesized compounds. The introduction of potent electron-withdrawing entities, such as NO_2 and Cl, onto the phenylamino ring of quinazolinone (exemplified by PRP7A8, PRP7A6, and PRP7A11) led to a substantial enhancement in antibacterial and antifungal activity. Conversely, electron-donating groups like CH_3, OCH_3, and O.H. were observed to diminish the antibacterial and antifungal properties. Consistently, substituting groups at the para position yielded higher activity compared to the meta position, and the meta position exhibited greater activity compared to the ortho position. Nevertheless, it should be noted that none of the synthesized compounds displayed activity levels equivalent to those of established standards.

CONCLUSION

An investigation into the antimicrobial characteristics of Quinazolinone derivatives unveiled their pronounced antibacterial and antifungal properties against pathogenic strains. The inclusion of electron-deficient groups such as -NO_2 and -Cl within the compounds' structures was determined to be a key factor contributing to their potent antimicrobial effects. In particular, the compounds PRP7A6, PRP7A8, and PRP7A11 displayed the highest antimicrobial efficacy against the assessed pathogenic strains. These findings indicate the potential for Quinazolinone derivatives to serve as the foundation for a novel category of antimicrobial agents. The substances exhibiting significant biological activity offer a promising avenue for furthering the development of antimicrobial medications with enhanced potency.

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