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Antibacterial evaluation of 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio) acetonitrile against pathogenic bacterial isolates with special reference to biofilm formation inhibition and anti-adherence properties

Abstract:

Background: Evaluating the effectiveness of a specific quinazoline molecule with antibacterial activity on microorganisms as a potential antibiotic substitution.

Methods: A variety of microorganisms were tested with the designated quinazoline molecule. Differences within two groups were analyzed using the two-tailed Student's t-test,.

Results: Susceptibility tests revealed that the chemical has stronger antibacterial action against S. saprophyticus than other isolates, with just a slight effect on *E. coli* and *M. smegmatis*. The bacterial cells were subjected to varying concentrations of a certain molecule, and the results showed that the inhibition of bacterial adhesion was not consistent. This suggests that the effect of the molecule on bacterial adhesion is dependent on its concentration. After 24 hours of treatment with varying chemical doses, all of the periodontal bacterial strains examined showed considerable inhibition of biofilm formation. It is noticeable that the treatment of the substance at various doses inhibited the development of the bacterial strains.

Conclusions: According to the findings, the chemical molecule quinazoline could be utilized as an alternative therapeutic approach for microorganism-caused infections.

Keywords: Quinazoline, Antibacterial, Microorganisms, Biofilm, EGFR

1. Introduction

The use of antibiotics and the management of treatment control and hospital stays are of interest to healthcare providers. The evolution of antibiotics has served the immune system to eliminate infection rapidly. Antibiotics attack microorganisms through several mechanisms, including inhibition of bacterial cell wall synthesis, blocking deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and protein synthesis [1]. However, the emergence of bacterial resistance to the existing medications has caused numerous challenges for humans and healthcare systems [2]. Misuse and overuse of antibiotics and a lack of alternative approaches established by the pharmaceutical

industry for commercial reasons are among the factors contributing to the antibiotic resistance crisis [3]. Hence, it is imperative to undertake an estimation of the disease and fatalities linked to multidrug-resistant bacteria [4]. However, multidrug-resistant bacteria have prompted scientists to seek alternative treatment strategies that could be as effective as antibiotics but lacks the opportunistic resistance of microbes. Although scientists are now battling pathogens' resistance by manipulating the existing antibiotics, several alternative therapy strategies were designed, that explores the integration of antimicrobial peptides (AMPs), innovative combinatorial therapies and nanoparticles (NPs) [1,5].

The broad pharmacological profile of quinazolines molecules makes their application in medicine broadly accepted [6]. The molecular compound quinazoline has shown promise as an antibacterial, antifungal, and antitumor agent [7,8]. Lapatinib is a highly effective quinazoline derivative that functions as an inhibitor of epidermal growth factor receptors 1 (ErbB1) and 2 (ErbB2) within the human cells, demonstrated antimicrobial efficacy by inhibiting *Staphylococcus aureus* growth and biofilm formation [9]. The EGFR (epidermal growth factor receptor) is known to be involved in cell development, tissue homeostasis, and tumorigenesis. Because of its involvement in many solid tumors, using molecular compounds that inhibit the EGFR pathway is a strategic way to prevent tumor growth and spread [10]. Gefitinib, erlotinib, and afatinib are EGFR tyrosine kinase inhibitors (TKIs) that demonstrated to be an effective threapy for advanced non-small cell lung cancer with EGFR-mutant because of their advantages over palliative platinum-based chemotherapy regarding the rate of response to treatment, the pace of progression, and the quality of life [11]. It has been shown that many EGFR inhibitors, including gefitinib, can block Mycobacterium tuberculosis from replicating in macrophages, indicating the effectiveness of these drugs during infection [12]. A team of scientific collaborators created unique quinazoline derivatives agents. These compounds were evaluated against human cervix cell lines (HELA), human liver cell lines (HEPG2) , and human breast cell lines (MCF-7)and showed promise of anticancer activity [8]. Therefore, in this investigation, we are interested in evaluating one of the designed compounds generated by our collaborators and demonstrating its action against bacteria in vitro.

2. Materials and Methods

2.1 Chemistry

According to a documented process, 2-(6-Chloro-2-p-tolylquinazolin-4-ylthio)acetonitrile was obtained by refluxing 6-chloro-2-p-tolylquinazolin-4(3H)-thione with 2-chloroacetonitrile in pyridine [8].

2.2. Bacterial strains

In this study, a collection of eight microbial strains was utilized, encompassing both Gram positive and Gram negative bacteria. Bacteria such as Enterococcus faecalis, Salmonella spp., Klebsiella pneumoniae, Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes, Pseudomonas aeruginosa, and Staphylococcus saprophyticus were among the strains examined. The different strains were cultivated in lysogenic broth (LB) medium using ideal conditions of 37°C.

2.3. Agar well diffusion assay for determining antibacterial susceptibility

To perform an initial assessment of the susceptibility of the bacteria under study to the compound, the bacterial strains were cultivated until they attend the logarithmic growth phase, the optical density reading at a wavelength of 610 nm (O.D.610) is determined within the range of 0.4 to 0.6. This cultivation was carried out using LB (lysogenic broth) medium. As a result, the experimental bacterial strains were diluted in LB broth to achieve a theoretical optical density at 610 nm of 0.01. To determine the antibacterial efficacy of the compound, agar well diffusion was utilized[13]. To summarize, LB agar wells measuring 6 mm in diameter were generated by employing a sterile syringe tip. The next step was to use the diluted culture to create a bacterial lawn culture on the agar using a sterilized cotton swab. In a Petri dish, 20 µl of a compound with a concentration of 2mg/ml and dimethyl sulfoxide (DMSO) were put into three separate wells. Subsequently, the samples were subjected to aerobic incubation at a temperature of 37°C for a duration of 24 hours. Measurements were taken in millimeters to determine the diameter of the inhibition zone, which included the well diameter. By calculating the difference between the molecule's mean inhibition zone and the DMSO mean inhibition zone, the exact zone of inhibition could be determined..

2.4. Analysis of MBC and MIC Values

The determination of the Minimum Bactericidal Concentration (MBC) and Minimum Inhibitory Concentration (MIC) of the chemical was conducted following standard techniques, albeit with

certain changes [14]. The chemical concentrations used to determine minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) on certain bacterial strains were diluted twice, going from 2 mg/ml to 0.015 mg/ml. To determine the MIC, the bacterial strains were cultivated until they reached the logarithmic phase, as indicated by an optical density (O.D.610) range of 0.4 - 0.6. Subsequently, the bacterial cultures were diluted in LB broth in order to get a theoretical optical density (O.D.610) of 0.01. Consequently, a total of 180µl of bacterial culture was introduced into the wells of polystyrene sterile flat-bottom 96-well plates. Wells were supplied in triplicate with 20 µl of the 2-fold dilution of the compound. As a control, triplicate wells were filled with 20µl of DMSO (5%). Additional aerobic incubation was conducted on the dishes for a period of 24 hours at a temperature of 37°C. Each well was tested hourly for the appearance of a pink color after adding 20µl of alamar blue (Thermo Fisher, USA) following a 24-hour incubation period. The determination of the MIC involved identifying the smallest quantity of the compound that could be detected in a well without inducing a visible alteration in the color of the alamar blue solution to pink.

In order to determine the MBC of the compound against bacterial strains, a subculture consisting of 10 µl was incubated aerobically on LB agar for 24 hours at 37°C using wells that did not exhibit any change in the color of Alamar blue pigment. The MBC of the compound for the organisms under investigation was determined by ascertaining the minimum concentration at which there was no observed growth.

2.5. Adherence assay

The previously elucidated method was successfully executed[15]. In order to conduct this experiment, a total of 96 wells on a plate were filled with 100µl of bacterial cells that were tested and had an optical density at 610nm of 0.01. These bacterial cells were cultured in RPMI 1640 medium and were supplemented with 0.165 M morpholinepropanesulphonic acid buffer at a pH of 7.0. After being exposed to the chemical at a number of different doses (MIC x 0.5, MIC x 1, and MIC x 2), the bacteria were cultured in an aerobic environment at 37 degrees Celsius for a period of six hours. Negative controls were untreated bacterial cells in each investigation. As a result, the medium used for incubation was removed, and the non-adherent bacterial cells that were present in each well were discarded by rinsing them twice with 200 µl of phosphate-buffered saline. After loading 100 µl of alamar blue at a concentration of 5% absolute in RPMI 1640

medium, every well was subjected to aerobic incubation at 37 degrees Celsius for a period of six hours. Consequently, the incubation media was disposed of and bacterial cells that did not adhere were removed by rinsing them twice with 200 µl PBS. Following the loading of 100µl of alamar blue at a 5% absolute concentration in RPMI 1640 media, each well was aerobically incubated at 37°C for a duration of 6 hours. The fluorescence indications were investigated using a Synergy HT microplate reader (BioTek Instruments, WA, USA) with excitation at 555 and emission at 585 nm.

2.6. Biofilm formation

By inoculating bacterial cell suspensions (O.D.610 = 0.01) that were cultivated in RPMI 1640 media was buffered with solution containing 0.165 M Mops at a pH of 7.0, biofilm formation was accomplished. The suspensions that had been inoculated were exposed to aerobic incubation at 37°C for a period of 6 hours in sterile 96-well plates with flat bottoms [16]. After a 6-hour period of adhesion, the media was carefully extracted without inducing any disturbance to the development of the biofilm. Following that, various chemical concentrations (MIC x 0.5, MIC x 1, MIC x 2) were cultured in fresh RPMI 1640 media and subsequently transferred to the designated well. As a negative control, unprocessed bacterial cells were utilized in each experimental cohort. Additional aerobic incubation was performed at 37°C for 24 hours on the 96-well plate. The assessment of the tested compound's effects on biofilm formation was conducted following the methodology specified [17].

2.7. Growth Kinetic assay

To depict the kinetic growth plots, which gave a general idea of the survivability of bacteria, we used the logarithm transformation of the relative population size with respect to time. The compound's antimicrobial activity of the was assessed using these plots. $O.D._{600}$ was utilized to analyze the growth curve throughout sixteen hours of cultivation..

To assess the influence of the compound on the bacterial cells being studied, a volume of 20 µl of the compound was introduced into 180 µl of the bacterial culture, which exhibited an optical density of 0.01at a wavelength of 600 nm at varying concentrations (MIC x 0.5, MIC x 1, MIC x 2). The control group was designated as the culture lacking any chemicals. The aerobic incubation of 96-well plates was performed at a temperature of 37°C. The FLUOstar Omega plate reader, manufactured by BMG Labtech of Allmendgrun, Ortenberg, Germany, was used to detect

absorbance at a 600 nm wavelength at regular 2-hour intervals. The measurements were replicated thrice in order to calculate the average value. A plot was generated with the mean absorbance values plotted against time.

2.8. Statistical analysis

The trials were repeated three times, and each time the mean and standard deviation were obtained. Graphpad Prism, version 6.0, developed by Graphpad Software Inc., was used to conduct the statistical analysis. An analysis of variance was carried out using a Student's t-test (two-tailed) to compare two separate groups. A p-value of less than 0.0001 (***) shows that there is a significant difference between the different groups, meaning that the results are statistically significant.

3. Results

3.1. Antibacterial activity of the compound

To evaluate the antibacterial effectiveness of the chemical, eight different microbial strains were treated (Table 1). The results of the susceptibility studies indicate that the compound exhibits greater antibacterial efficacy against *S. saprophyticus* in comparison to other isolates. Additionally, a modest impact was observed against *E. coli* and *M. smegmatis*. (Table 1). It was determined that a zone size greater than 8 millimetres was indicative of the sensitivity of the bacterial strains to the investigated compound. Bacterial isolates with a zone size greater than 8 millimetres were subjected to various compound concentrations to establish the MIC and MBC.

To ascertain the MBC and MIC, specific bacterial strains were subjected to a predetermined volume of the compound and subsequently incubated for a period of 24 hours. By examining a low concentration of the drug at which the colour of the alamar blue dye remained unaltered, the MIC was established. The results presented in Table 1 demonstrate a significant susceptibility of all bacterial strains to the tested compound. The study found that the bacterial growth was effectively suppressed, with MBC values ranged from 0.125 to 0.5 mg/ml, and MIC values ranged from 0.031 to 0.25 mg/ml. The findings were corroborated by the subsequent increase in the zone of inhibition, which ranged from 12 to 21 mm.

Table 1. Antimycobacterial activity of ***2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile*** against microbial strains.

Organism	Zone of inhibition Mean ± SD (mm)	MIC (mg/ml)	MBC (mg/ml)
P. aeruginosa	14 ± 1.5	0.125	0.5
E. coli	12 ± 1.2	0.25	0.5
K. pneumonae	15 ± 0.75	0.125	0.25
Salmonella	14 ± 1	0.125	0.25
S. aureus	20 ± 1.5	0.062	0.25
S. saprophyticus	21 ± 1.5	0.031	0.125
S. Pyogenes	19 ± 2	0.031	0.25
E. faecalis	18 ± 1	0.062	0.5

3.2. Compound inhibits bacterial adhesion

The impact of the compound on bacterial adherence was evaluated through an adhesion assay utilising the alamar blue dye. The bacterial cells exhibited variable inhibition of bacterial adherence subsequent to exposure to different concentrations of the compound, indicating a concentration-dependent effect (Fig. 1). The compound under investigation exhibited inhibitory effects on bacterial adhesion across all bacterial strains. The degree of inhibition ranged from 25% to 50%, 47% to 71%, and 59% to 86% at concentrations of MIC x 0.5, MIC x 1, and MIC x 2, respectively. The group of bacterial known as the control consisted of strains that did not exhibit any concentration of the compound. The results indicated that the aforementioned compound exhibited significant efficacy in reducing bacterial adhesion as demonstrated by the conducted tests, even when administered at concentrations below the inhibitory threshold.

3.3. Compound inhibits bacterial biofilm formation

It is noteworthy that all the periodontal bacterial strains that were subjected to testing exhibited a substantial reduction in biofilm formation subsequent to exposure to diverse compound concentrations for a duration of 24 hours. Following a 24-hour period, the test entities exhibited

biofilm formation inhibition against all bacterial strains at concentrations of MIC x 0.5, MIC x 1, and MIC x 2. The observed inhibition percentages ranged from 22% to 40%, 47% to 63%, and 67% to 85%, respectively (Fig. 2). The control group consisted of bacterial strains that lacked any concentration of the compound. Additionally, the inhibition rate of biofilm formation by the compound was contingent upon both the duration of treatment and the concentration utilised. The results demonstrated a significant reduction in biofilm formation within the bacterial cells that were tested as a result of the compound.

3.4. Effect on bacterial growth

A real-time study was conducted to examine the impact of the compound on bacterial growth at various time intervals. In order to ascertain the growth kinetics, a bacterial culture measuring 180 µl with an OD610 of 0.01 was subjected to treatment with a compound at concentrations *of MIC x 0.5, MIC x 1, and MIC x 2*, using 20 µl of the aforementioned compound. For every two hours. The bacterial growth rate was recorded, as depicted in Figure 3. The present study indicates that the application of varying concentrations of the compound resulted in a noticeable inhibition of bacterial strain proliferation. The growth kinetics analysis demonstrated that the compound exhibited a bactericidal effect on the tested bacteria in a dose-dependent manner. The data presented indicates a significant level of antibacterial efficacy of the compound when tested against various bacterial strains.

4. Discussion

The use of alternative strategies for infection dissemination is critical due to the overuse and misuse of antimicrobial agents, which leads to drug resistance [18]. It has been demonstrated that several substances, including those based on indole and quinazolines, are efficient for treating microbial activity [19]. Quinazoline, a compound derived from the benzene and pyrimidine ring fusion, exhibited a wide array of biological actions. These included effects against bacteria, cancer, hypertension, analgesia, convulsions, malaria, and tuberculosis[20-47].

Our collaborators created several new classes of quinazoline derivatives molecular compounds tested for tumor cells as therapeutic targets and demonstrated promising antitumor activity [8]. One of the newly designed quinazoline derivative molecular compounds that are effective against

tumor cells is being tested in our study to determine its antimicrobial activities. Our findings indicated that the molecule has good antibacterial activity against various microorganisms.

Compounds containing the Quinazoline moiety have been observed to exhibit a wide range of biological and therapeutic characteristics, including antimicrobial activity. The newly developed quinazoline molecular compound was evaluated for antibacterial activity and shown to be highly effective against specific pathogens. Consequently, the chemical might be utilized as an alternate therapeutic protocol for microbial infection. The viability of using the compound as an antimicrobial agent was confirmed by a number of study methods, as detailed in the result section. The formation of biofilms and adhesion both increase bacterial virulence [49]. Attachment (or adhesion) is the initial stage of bacterial infection, promotingolonization and host cell invasion [50]. Anti-adhesion treatment is thus required to prevent the adhesion step and host cell attachment. Bacteria engage in the formation of biofilms as a means to enhance their survival stratigies. Biofilms are clusters of bacteria embedded in a self-produced matrix composed of numerous elements such as proteins, DNA, and polysaccharides that are linked to a surface and/or each other [51]. According to the findings, the Quinazoline derivative molecule prevented the adhesion and biofilm formation of the tested microorganisms, demonstrating its efficiency as an antimicrobial agent. Additionally, the molecule is an effective inhibitor of microbial growth.

Cancer research has dominated studies involving the epidermal growth factor receptor (EGFR). Epidermal growth factor receptor tyrosine kinase (EGFR-TK) is involved in the regulation of cell development, proliferation, and death, among other cellular processes, in vivo. Overexpression of EGFR-TK may result in uncontrolled cell growth and malignant cell proliferation. As a result, blocking EGFR-TK slows tumor development and proliferation [52]. Targeting EGFR has become a popular research subject, and many generations of quinazoline-based EGFR kinase inhibitors have been developed, including erlotinib and lapatinib [53]. EGFR's role in bacterial infection has been demonstrated in several studies. Infections caused by Haemophilus influenza, Klebsiella pneumonia, Neisseria gonorrhoeae, Pseudomonas aeruginosa, and Helicobacter pylori falls into this category [54]. The molecules developed, namely 4-substituted 6-chloro-2-p-tolylquinazolin, possess a pharmacophore of 4-substituted quinazoline that bears structural similarity to Erlotinib and Lapatinib [8]. As a result, the compound may target microbial growth via EGFR.

It has been proven that the unique quinazoline molecule, as indicated above, is effective as an anticancer therapeutic. Our research showed that the compound is also effective as an antimicrobial therapeutic. The current study indicates using the chemical molecule quinazoline as an alternative therapeutic strategy for treating infectious diseases caused by microorganisms. Even though the molecule's efficiency as an alternative to antibiotics has been demonstrated, further research is still required to understand the molecule's function against infection better. However, the current work provides the basis for future research.

5. Conclusion

The study's findings revealed that 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio) acetonitrile exhibited a growth inhibitory effect on pathogenic bacterial strains in a dose-dependent manner, thereby decreasing their pathogenic potential. The results of this study suggest that 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile exhibits potential as a appropriate contender for the advancement and establishment of effective therapeutics against infections caused by these pathogenic microorganisms, thereby warranting further investigation into its potential as a drug candidate. It is imperative to assess the potential deleterious effects, pharmacokinetic characteristics, and associated adverse reactions. The result of this study indicate that 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile exhibits promising antibacterial properties, making it a viable candidate for incorporation into pharmaceutical formulations.

Conflicts of Interest
None declared.

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References

1. Zaman SB, Muhammed Awlad Hussain, Rachel Nye, Varshil Mehta, Kazi Taib Mamun and Naznin Hossain. A review on antibiotic resistance: Alarm bells are ringing. *Cureus* 9: .

2. van Duin D and David Paterson. Multidrug resistant bacteria in the community: Trends and lessons learned. *Infect Dis Clin North Am* 2016; 30: 377-390.

3. Ventola CL. The antibiotic resistance crisis. *P T* 2015; 40: 277-283.

4. Colomb-Cotinat M, J. Lacoste, C. Brun-Buisson, V. Jarlier, B. Coignard and S. Vaux. Estimating the morbidity and mortality associated with infections due to multidrug-resistant bacteria (MDRB), france, 2012. *Antimicrob Resist Infect Control* 2016; 5: .

5. León-Buitimea A, Cesar R. Garza-Cárdenas, Javier A. Garza-Cervantes, Jordy A. Lerma-Escalera and Jose R. Morones-Ramírez. The demand for new antibiotics: Antimicrobial peptides, nanoparticles, and combinatorial therapies as future strategies in antibacterial agent design. *Front Microbiol* 2020; 11: .

6. Patel H, Atul Shirkhedkar, Sanjay Bari, Kamalkishor Patil, Amit Arambhi, Chandrakantsing Pardeshi, et al. Quinazolino-thiadiazoles as antimicrobial agents. *Bulletin of Faculty of Pharmacy, Cairo University* 2018; 56: 83-90.

7. Rohini R, Kanne Shanker, Puchakayala Muralidhar Reddy, Yen-Peng Ho and Vadde Ravinder. Mono and bis-6-arylbenzimidazo[1,2-c]quinazolines: A new class of antimicrobial agents. *Eur J Med Chem* 2009; 44: 3330-3339.

8. El-Azab AS, Mohamed A. Al-Omar, Alaa A. -M Abdel-Aziz, Naglaa I. Abdel-Aziz, Magda A. -A el-Sayed, Abdulaziz M. Aleisa, et al. Design, synthesis and biological evaluation of novel quinazoline derivatives as potential antitumor agents: Molecular docking study. *Eur J Med Chem* 2010; 45: 4188-4198.

9. Liu Y, Yiyi Shi, Hang Cheng, Junwen Chen, Zhanwen Wang, Qingyin Meng, et al. Lapatinib acts against biofilm formation and the hemolytic activity of staphylococcus aureus. *ACS Omega* 2022; 7: 9004-9014.

10. Dsouza PC and Shiyam Kumar. Role of systemic antibiotics in preventing epidermal growth factor receptor: Tyrosine kinase inhibitors-induced skin toxicities. *Asia Pac J Oncol Nurs* 2017; 4: 323-329.

11. Califano R, N. Tariq, S. Compton, D. A. Fitzgerald, C. A. Harwood, R. Lal, et al. Expert consensus on the management of adverse events from EGFR tyrosine kinase inhibitors in the UK. *Drugs* 2015; 75: 1335-1348.

12. Sogi KM, Katie A. Lien, Jeffrey R. Johnson, Nevan J. Krogan and Sarah A. Stanley. The tyrosine kinase inhibitor gefitinib restricts mycobacterium tuberculosis growth through increased lysosomal biogenesis and modulation of cytokine signaling. *ACS Infect Dis* 2017; 3: 564-574.

13. Magaldi S, S. Mata-Essayag, C. Hartung de Capriles, C. Perez, M. T. Colella, Carolina Olaizola, et al. Well diffusion for antifungal susceptibility testing. *Int J Infect Dis* 2004; 8: 39-45.

14. Wei J, Vidhya Krishnamoorthy, Kenan Murphy, Jee-Hyun Kim, Dirk Schnappinger, Tom Alber, et al. Depletion of antibiotic targets has widely varying effects on growth. *Proc Natl Acad Sci U S A* 2011; 108: 4176-4181.

15. Fazly A, Charu Jain, Amie C. Dehner, Luca Issi, Elizabeth A. Lilly, Akbar Ali, et al. Chemical screening identifies filastatin, a small molecule inhibitor of candida albicans adhesion, morphogenesis, and pathogenesis. *Proc Natl Acad Sci U S A* 2013; 110: 13594-13599.

16. Raut JS, Ravikumar B. Shinde, Nitin M. Chauhan and S. Mohan Karuppayil. Terpenoids of plant origin inhibit morphogenesis, adhesion, and biofilm formation by candida albicans. *Biofouling* 2013; 29: 87-96.

17. Jin Y, Lakshman P. Samaranayake, Yuthika Samaranayake and Hak Kong Yip. Biofilm formation of candida albicans is variably affected by saliva and dietary sugars. *Arch Oral Biol* 2004; 49: 789-798.

18. Leekha S, Christine L. Terrell and Randall S. Edson. General principles of antimicrobial therapy. *Mayo Clin Proc* 2011; 86: 156-167.

19. Nandwana NK, Rajnish Prakash Singh, Om P. S. Patel, Shiv Dhiman, Hitesh Kumar Saini, Prabhat N. Jha, et al. Design and synthesis of imidazo/benzimidazo[1,2-c]quinazoline derivatives and evaluation of their antimicrobial activity. *ACS Omega* 2018; 3: 16338-16346.

20. Gupta T, Ankit Rohilla, Ankita Pathak, Md Jawaid Akhtar, Md Rafi Haider and M. Shahar Yar. Current perspectives on quinazolines with potent biological activities: A review. *Synthetic Communications* 2018; 48: 1099-1127.

21. Abdel-Aziz, A. A.; Abou-Zeid, L. A.; ElTahir, K. E.; Mohamed, M. A.; Abu El-Enin, M. A.; El-Azab, A. S., Design, synthesis of 2,3-disubstitued 4(3H)-quinazolinone derivatives as anti-inflammatory and analgesic agents: COX-1/2 inhibitory activities and molecular docking studies. Bioorganic & medicinal chemistry 2016, 24, (16), 3818-28.

22. Alanazi, A. M.; Abdel-Aziz, A. A.; Al-Suwaidan, I. A.; Abdel-Hamide, S. G.; Shawer, T. Z.; El-Azab, A. S., Design, synthesis and biological evaluation of some novel substituted quinazolines as antitumor agents. European journal of medicinal chemistry 2014, 79, 446-54.

23. Alanazi, A. M.; Al-Suwaidan, I. A.; Alaa, A.-M.; Mohamed, M. A.; El_Morsy, A. M.; El-Azab, A. S., Design, synthesis and biological evaluation of some novel substituted 2-mercapto-3-phenethylquinazolines as antitumor agents. Medicinal Chemistry Research 2013, 22, (11), 5566-5577.

24. Al-Obaid, A. M.; Abdel-Hamide, S. G.; El-Kashef, H. A.; Abdel-Aziz, A. A.; El-Azab, A. S.; Al-Khamees, H. A.; El-Subbagh, H. I., Substituted quinazolines, part 3. Synthesis, in vitro antitumor activity and molecular modeling study of certain 2-thieno-4(3H)-quinazolinone analogs. European journal of medicinal chemistry 2009, 44, (6), 2379-91.

25. Al-Suwaidan, I. A.; Abdel-Aziz, A. A.; Shawer, T. Z.; Ayyad, R. R.; Alanazi, A. M.; El-Morsy, A. M.; Mohamed, M. A.; Abdel-Aziz, N. I.; El-Sayed, M. A.; El-Azab, A. S., Synthesis, antitumor activity and molecular docking study of some novel 3-benzyl-4(3H)quinazolinone analogues. Journal of enzyme inhibition and medicinal chemistry 2016, 31, (1), 78-89.

26. Al-Suwaidan, I. A.; Alanazi, A. M.; Abdel-Aziz, A. A.; Mohamed, M. A.; El-Azab, A. S., Design, synthesis and biological evaluation of 2-mercapto-3-phenethylquinazoline bearing anilide fragments as potential antitumor agents: molecular docking study. Bioorganic & medicinal chemistry letters 2013, 23, (13), 3935-41.

27. El-Azab, A. S.; Abdel-Hamide, S. G.; Sayed-Ahmed, M. M.; Hassan, G. S.; El-Hadiyah, T. M.; Al-Shabanah, O. A.; Al-Deeb, O. A.; El-Subbagh, H. I., Novel 4 (3H)-quinazolinone analogs: synthesis and anticonvulsant activity. Medicinal Chemistry Research 2013, 22, (6), 2815-2827..

28. El-Azab, A. S.; Eltahir, K. E., Synthesis and anticonvulsant evaluation of some new 2,3,8-trisubstituted-4(3H)-quinazoline derivatives. Bioorganic & medicinal chemistry letters 2012, 22, (1), 327-33.

29. El-Azab, A. S.; Abdel-Aziz, A.-M.; Ng, S. W.; Tiekink, E. R., 6-Methyl-3-phenyl-2-sulfanylidene-1, 2, 3, 4-tetrahydroquinazolin-4-one. Acta Crystallographica Section E: Structure Reports Online 2012, 68, (3), o862-o862.

30. El-Azab, A. S.; Abdel-Aziz, A. A.; Bua, S.; Nocentini, A.; El-Gendy, M. A.; Mohamed, M. A.; Shawer, T. Z.; AlSaif, N. A.; Supuran, C. T., Synthesis of benzensulfonamides linked to

quinazoline scaffolds as novel carbonic anhydrase inhibitors. Bioorganic chemistry 2019, 87, 78-90.

31. El-Azab, A. S.; ElTahir, K. E.; Attia, S. M., Synthesis and anticonvulsant evaluation of some novel 4 (3H)-quinazolinones. Monatshefte für Chemie-Chemical Monthly 2011, 142, (8), 837-848.

32. Mohamed, M. A.; Ayyad, R. R.; Shawer, T. Z.; Abdel-Aziz, A. A.; El-Azab, A. S., Synthesis and antitum

r evaluation of trimethoxyanilides based on 4(3H)-quinazolinone scaffolds. European journal of medicinal chemistry 2016, 112, 106-113.

33. Alanazi, A. M.; Abdel-Aziz, A. A.; Shawer, T. Z.; Ayyad, R. R.; Al-Obaid, A. M.; Al-Agamy, M. H.; Maarouf, A. R.; El-Azab, A. S., Synthesis, antitumor and antimicrobial activity of some new 6-methyl-3-phenyl-4(3H)-quinazolinone analogues: in silico studies. Journal of enzyme inhibition and medicinal chemistry 2016, 31, (5), 721-35.

34. El-Azab, A. S.; Al-Dhfyan, A.; Abdel-Aziz, A. A.; Abou-Zeid, L. A.; Alkahtani, H. M.; Al-Obaid, A. M.; Al-Gendy, M. A., Synthesis, anticancer and apoptosis-inducing activities of quinazoline-isatin conjugates: epidermal growth factor receptor-tyrosine kinase assay and molecular docking studies. Journal of enzyme inhibition and medicinal chemistry 2017, 32, (1), 935-944.

35. El-Azab, A. S.; Abdel-Aziz, A. A.; Ghabbour, H. A.; Al-Gendy, M. A., Synthesis, in vitro antitumour activity, and molecular docking study of novel 2-substituted mercapto-3-(3,4,5-trimethoxybenzyl)-4(3H)-quinazolinone analogues. Journal of enzyme inhibition and medicinal chemistry 2017, 32, (1), 1229-1239.

36. Al-Omary, F. A.; Abou-Zeid, L. A.; Nagi, M. N.; Habib, E.-S. E.; Alaa, A.-M.; El-Azab, A. S.; Abdel-Hamide, S. G.; Al-Omar, M. A.; Al-Obaid, A. M.; El-Subbagh, H. I., Non-classical

antifolates. Part 2: synthesis, biological evaluation, and molecular modeling study of some new 2, 6-substituted-quinazolin-4-ones. Bioorganic & medicinal chemistry 2010, 18, (8), 2849-2863.

37. El-Azab, A. S.; ElTahir, K. E., Design and synthesis of novel 7-aminoquinazoline derivatives: antitumor and anticonvulsant activities. Bioorganic & medicinal chemistry letters 2012, 22, (5), 1879-1885.

38. Abdel-Aziz, A. A.; Abou-Zeid, L. A.; ElTahir, K. E. H.; Ayyad, R. R.; El-Sayed, M. A.; El-Azab, A. S., Synthesis, anti-inflammatory, analgesic, COX-1/2 inhibitory activities and molecular docking studies of substituted 2-mercapto-4(3H)-quinazolinones. European journal of medicinal chemistry 2016, 121, 410-421.

39. El-Azab, A. S.; Abdel-Aziz, A. A.; AlSaif, N. A.; Alkahtani, H. M.; Alanazi, M. M.; Obaidullah, A. J.; Eskandrani, R. O.; Alharbi, A., Antitumor activity, multitarget mechanisms, and molecular docking studies of quinazoline derivatives based on a benzenesulfonamide scaffold: Cell cycle analysis. Bioorganic chemistry 2020, 104, 104345.

40. Hamdi, A.; El-Shafey, H. W.; Othman, D. I. A.; El-Azab, A. S.; AlSaif, N. A.; Abdel-Aziz, A. A., Design, synthesis, antitumor, and VEGFR-2 inhibition activities of novel 4-anilino-2-vinyl-quinazolines: Molecular modeling studies. Bioorganic chemistry 2022, 122, 105710.

41. Altamimi, A. S.; El-Azab, A. S.; Abdelhamid, S. G.; Alamri, M. A.; Bayoumi, A. H.; Alqahtani, S. M.; Alabbas, A. B.; Altharawi, A. I.; Alossaimi, M. A.; Mohamed, M. A. J. M., Synthesis, Anticancer Screening of Some Novel Trimethoxy Quinazolines and VEGFR2, EGFR Tyrosine Kinase Inhibitors Assay; Molecular Docking Studies. 2021, 26, (10), 2992.

42. El-Azab, A. S. J. P., Sulfur,; Silicon; Elements, t. R., Synthesis of some new substituted 2-mercaptoquinazoline analogs as potential antimicrobial agents. 2007, 182, (2), 333-348.

43. Aziza, M.; Nassar, M.; AbdelHamide, S.; ElHakim, A.; El-Azab, A., Synthesis and antimicrobial activities of some new 3-heteroaryl-quinazolin-4-ones. Indian Journal of Heterocyclic Chemistry 1996, 6, (1), 25-30.

44. Alafeefy, A. M.; Kadi, A. A.; El-Azab, A. S.; Abdel-Hamide, S. G.; Daba, M. H. Y., Synthesis, Analgesic and Anti-Inflammatory Evaluation of Some New 3H-Quinazolin-4-one Derivatives. Archiv der Pharmazie 2008, 341, (6), 377-385.

45. El-Azab, A. S.; Abdel-Aziz, A. A.; Bua, S.; Nocentini, A.; AlSaif, N. A.; Alanazi, M. M.; El-Gendy, M. A.; Ahmed, H. E. A.; Supuran, C. T., S-substituted 2-mercaptoquinazolin-4(3H)-one and 4-ethylbenzensulfonamides act as potent and selective human carbonic anhydrase IX and XII inhibitors. Journal of enzyme inhibition and medicinal chemistry 2020, 35, (1), 733-743.

46. Alkahtani, H. M.; Abdalla, A. N.; Obaidullah, A. J.; Alanazi, M. M.; Almehizia, A. A.; Alanazi, M. G.; Ahmed, A. Y.; Alwassil, O. I.; Darwish, H. W.; Abdel-Aziz, A. A.; El-Azab, A. S., Synthesis, cytotoxic evaluation, and molecular docking studies of novel quinazoline derivatives with benzenesulfonamide and anilide tails: Dual inhibitors of EGFR/HER2. Bioorganic chemistry 2020, 95, 103461.

47. El-Azab, A. S.; Abdel-Aziz, A. A.; Ahmed, H. E. A.; Bua, S.; Nocentini, A.; AlSaif, N. A.; Obaidullah, A. J.; Hefnawy, M. M.; Supuran, C. T., Exploring structure-activity relationship of S-substituted 2-mercaptoquinazolin-4(3H)-one including 4-ethylbenzenesulfonamides as human carbonic anhydrase inhibitors. Journal of enzyme inhibition and medicinal chemistry 2020, 35, (1), 598-609.

48. Patel AB. Investigation of the antibacterial activity of new quinazoline derivatives against methicillin and quinolone resistant staphylococcus aureus. *Journal of Chemical Research* 2020; 44: 315-321.

49. Dera AA, Irfan Ahmad, Prasanna Rajagopalan, Mesfer Al Shahrani, Ahmed Saif, Mohammad Y. Alshahrani, et al. Synergistic efficacies of thymoquinone and standard antibiotics against multi-drug resistant isolates. *Saudi Med J* 2021; 42: 196-204.

50. Asadi A, Shabnam Razavi, Malihe Talebi and Mehrdad Gholami. A review on anti-adhesion therapies of bacterial diseases. *Infection* 2019; 47: 13-23.

51. Vestby LK, Torstein Grønseth, Roger Simm and Live L. Nesse. Bacterial biofilm and its role in the pathogenesis of disease. *Antibiotics (Basel)* 2020; 9: 59.

52. Mao L, Zhen-Zhen Wang, Qiong Wu, Xiaojie Chen, Jian-Xue Yang, Xin Wang, et al. Design, synthesis, and antitumor activity of erlotinib derivatives. *Front Pharmacol* 2022; 13: 849364.

53. Deng P, Ge Sun, Jie Zhao, Kaitai Yao, Miaomiao Yuan, Lizeng Peng, et al. Synthesis and antitumor activity of erlotinib derivatives linked with 1,2,3-triazole. *Front Pharmacol* 2022; 12: 793905.

54. Yang X, Ji-Yang Fu, Rui-Cheng Yang, Wen-Tong Liu, Tao Zhang, Bo Yang, et al. EGFR transactivation contributes to neuroinflammation in streptococcus suis meningitis. *J Neuroinflammation* 2016; 13: 274.

Figure legend

Figure 1: The inhibition of the microorganism adhesion by 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile. The study employed the Alamar Blue-based polystyrene adhesion assay to assess the impact of 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile on the adherence of different microorganisms. The bacterial strains that were subjected to experimentation were exposed to three different concentrations of the drug, namely MIC x 0.5, MIC x 1, and MIC x 2, for a duration of 6 hours at a temperature of 37 °C. All untreated bacterial isolates are represented by control bars, which represent 0% inhibition. The results of three distinct experiments are presented with means ± SD***$p < 0.0001$

Figure 2: The reduction of biofilm formation by 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile. Under biofilm growth conditions, microorganisms were incubated for 24 hours with 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile at MIC x 0.5, MIC x 1, and MIC x 2 values. All untreated bacterial isolates are represented by control bars, which represent 0% inhibition. The results of three distinct experiments are presented with means ± SD***$p < 0.0001$.

Figure 3: The impact of bacterial growth kinetics by 2-((6-chloro-2-(p-tolyl)quinazolin-4-yl)thio)acetonitrile. Different concentrations of the chemical (MIC x 0.5, MIC x 1, and MIC x 2) were applied to various strains. The strains are as follows: (A) Pseudomonas aeruginosa (B) Escherichia coli (C) Klebsiella pneumoniae (D) Salmonella (E) Staphylococcus aureus (F)

Staphylococcus saprophyticus (G) Streptococcus pyogenes (H) Enterococcus faecalis. The growth cycle of organisms that were not subjected to any treatment was utilized as a control for growth. The absorbance at a wavelength of 610 nm was measured at consistent time intervals of 2 hours. The results of three distinct experiments are presented with means ± SD***$p < 0.0001$.

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