Int. J. Pharm. Investigation, 2023; 13(1):62-73.
www.jpionline.org

Original Article

Evaluation of DHFR Inhibition and Antimicrobial Activity of Some Newly Synthesized Quinazolin-4(3H)-one Scaffold Coupled with Benzylidene and Ethylidene Amino Motifs

Sunil Harer[1,*], Manish Bhatia[2], Priyanka Sonar[3]

[1]Department of Pharmaceutical Chemistry, Dattakala College of Pharmacy, Swami Chincholi, Pune, Maharashtra, INDIA.
[2]Bharati Vidyapeeth College of Pharmacy, Kolhapur, Maharashtra, INDIA.
[3]Progressive Education Society's Modern College of Pharmacy, Nigdi, Pune, Maharashtra, INDIA.

ABSTRACT

Objectives: Substituted quinazolin-4(3H)-ones at position-3 with phenyl ring, heterocycles and aliphatic moieties, were reported to impart antimicrobial activities. In light of this, we have attempted to prepare a novel series of 2-pheny-3-substituted quinazolin-4(3H)-ones fused with an azomethine (-CH=N-) connection to Benzylidene and ethylidene motifs. Each of these motifs underwent testing to determine whether it could inhibit *in-vitro* microbial DHFR and the subsequent antimicrobial action. **Materials and Methods:** The synthesized 2-phenyl-3-substituted quinazolin-4(3H)-ones were characterized by FT-IR, ^{1}H-NMR, ^{13}C-NMR, ESI-MS and elemental (C, H, N, O and X=halogen) analysis. Evaluated results of *in-vitro* microbial DHFR inhibition are compared with the reported drug trimethoprim. Agar disc diffusion method was used for *in-vitro* antimicrobial activity, performed against pathogenic Gram-positive and Gram-negative bacteria like *Staphylococcus aureus*, *Bacillus subtilis,* and *Escherichia coli, Pseudomonas aeruginosa* respectively, and fungi like *Candida albicans,* and *Aspergillus niger.* **Results:** Docking analysis of ligands with DHFR (PDB=2W3M) has shown strong hydrophobic binding interaction and confirmed a perfect fit into the active domain of the target protein. Possible antimicrobial activity was induced from microbial DHFR inhibition. The results of the tests are compared with gentamycin, ciprofloxacin, and clotrimazole. Compounds with potent antibacterial activity were QI-j, and QII-f (MIC=0.1-0.2µg/mL), and moderately active compounds were QIa-d, QII-m, QIII-d, and QIIIe-f (MIC=0.5-2.0µg/mL). Compounds exhibited potent antifungal activity were QI-c, QII-b, and QIII-f (MIC=0.1-0.2µg/mL), moderately active compounds were QIc-e, QI-g, QIm-n, QII-d, QIII-b, and QIII-e (MIC=0.5-2.0µg/mL). **Conclusion:** Particularly test compounds have produced DHFR inhibition in a range of 4-24µM as compared with trimethoprim (IC_{50}=10 µM). Benzylidene and ethylidene moieties attached to the quinazolin-4(3H)-one had contributed to this activity. Present series of substituted quinazolin-4(3H)-ones provide a path for the design and development of newer antimicrobial agents in the treatment of deadly pathogenic infections.

Keywords: Quinazolin-4(3H)-ones, Antimicrobial activity, MIC, DHFR, IC_{50}, Docking analysis.

Correspondence
Dr. Sunil Harer
Department of Pharmaceutical Chemistry, Dattakala College of Pharmacy, Swami Chincholi, Pune, Maharashtra, INDIA.
Email id: sunil.harer5@gmail.com
ORCID ID 0000-0003-2916-727X

Received: 16-08-2022;
Revised: 18-09-2022;
Accepted: 12-10-2022.

INTRODUCTION

Dihydrofolate reductase (DHFR) is essential for the production of nucleic acids by microbes. It catalyses the NADPH reduction of 7,8-dihydrofolate to 5,6,7,8-tetrahydrofolate in close association with thymidylate synthase. Thymidylate synthase, the main enzyme, catalyses the reductive methylation of deoxyuridine monophosphate (dUMP) to deoxythymidine monophosphate by using N5, N10-methylene-tetrahydrofolate as a cofactor (dTMP). Co-factors play a crucial role in the one-carbon transfer processes that lead to the biosynthesis of pyrimidine, purine, and amino acids. Thymidylate synthase or DHFR suppression may lead to a lack of tetrahydrofolate co-factor. Finally, a decrease in tetrahydrofolate levels causes a decrease in the production of methionine, thymidylate, and glycine to serine, which stops DNA replication.[1,2] The interference with nucleotide production ultimately causes the death of microbial cells.[3] As a result, we can consider DHFR as a focused target for the development of novel drugs for the treatment of parasite and microbial diseases that are drug-resistant, as well as chemotherapeutic treatments for the treatment of various cancers.[4]

A proven therapeutic target for chemotherapy and the management of bacterial and parasitic diseases is DHFR.[5] There are two types of antifolates: traditional and unconventional DHFR inhibitors. Classical antifolates feature an embedded p-aminobenzoyl

DOI: 10.5530/223097131749

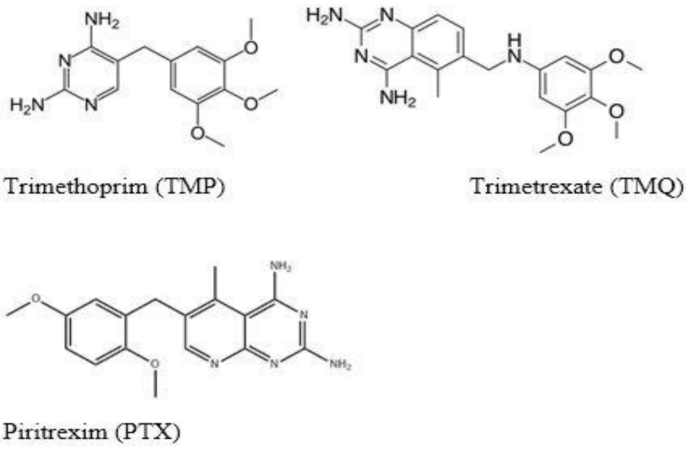

Figure 1: Reported quinazoline-containing antifolate compounds.

glutamate side-chain in contrast to non-classical antifolates, which substitute a lipophilic side-chain for the glutamate moiety. Numerous non-classical antifolates, including those from the quinazoline heterocycle and trimethoprim (TMP), trimetrexate (TMQ), piritrexim (PTX), have been reported (Figure 1).[6-9] Quinazolinone derivatives are widely sought-after antibacterial agents because of their wide spectrum of chemotherapeutic *in-vitro* and *in-vivo* activities.[10] Antibacterial quinazolin-4(3H)-ones have been reported, including those having bridging phenyl rings, heterocyclic rings, and aliphatic systems at position-3.[11-15] On the other hand, Schiff bases having an azomethine group (-CH=N-) in their structure have gained increased importance as a result of their biological and pharmacological properties as antibacterial, antifungal, anticancer, and antiviral medicines.[16,17] Due to the fact that the quinazolinone moiety appears to be a potential pharmacophore in a variety of pharmacologically active agents, we decided to synthesize quinazolin-4(3H)-ones functionality coupled with benzylidene amino and ethylidene amino moieties as potential antimicrobial agents that could provide better antimicrobial results.

In view of this, we have developed a unique family of 2-pheny-3 substituted quinazolin-4(3H)-ones (Scheme 1, 2 and 3). The phenyl moiety of every chemical created is substituted with highly active functional groups via an azomethine (-CH=N-) link (Figure 2, 3). All of these themes were examined in our laboratories for their capacity to obstruct *in-vitro* microbial DHFR utilizing trimethoprim (TMP) as a reference substance. All of these generated compounds were examined for *in-vitro* antibacterial activity against a range of pathogenic microbes using the agar disc diffusion method. Nearly all drugs have demonstrated strong to moderate anti-bacterial and anti-fungal activities when compared to common antibiotics as gentamycin, ciprofloxacin, and clotrimazole (Scheme 1). When this family of compounds was used in molecular modelling and docking studies, it was discovered that the active pocket of the DHFR target protein can bind pharmacophoric groups with a variety of

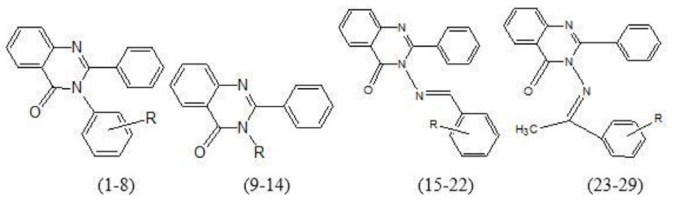

Figure 2: General framework of compounds with the quinazolin-4(3H)-one scaffold.

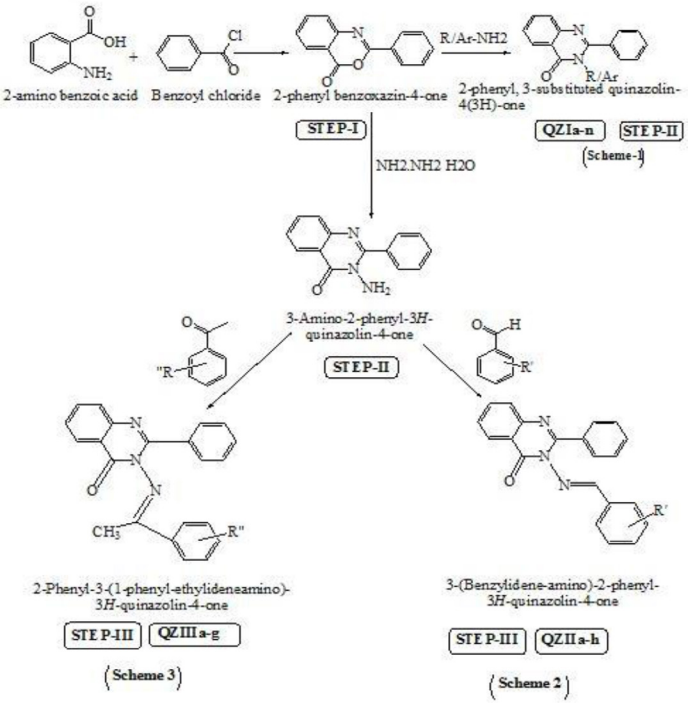

Figure 3: Synthesis pathway of the title compounds 2-phenyl-3-substituted quinazolin-4(3H)-ones (Scheme 1, 2 and 3).

forms of binding, strengths, and capacities. N-acetyl sulfonamide, thio-formamide, thio-formic acid hydrazide, 4-phenylamine, 4-methoxy benzene, 2-methylamine, 3-hydroxy-4-methoxy benzene, and 3,4-dihydroxy benzene are just a few of the implicated pharmacophore groups. Their significance has been shown. The relative spatial separation between the ligands and the protein's active pocket determines the strength of the binding interaction. The spatial considerations and ligand substitution pattern were critical for DHFR with respect to the quinazolin-4(3H)-one nucleus.[18,19]

Our current effort aims to establish efficient, repeatable synthesis and to identify novel synthetic lead compound(s) that target the folate pathway in relation to antibacterial activity. Phenyl ring bearing groups like hydroxy, amino, methoxy, sulfonyl-acetamide, and benzene sulfonamide bonded at position-3 of quinazolin-4(3H)-one nucleus are more efficient at inhibiting microbial DHFR. Several quinazoline analogues with different

groups at position-3 N-atom, such as carbo-thioamide, carbo-amide, carbo-hydrazide, and carbo-thihydrazide, also contributed to the inhibition of the DHFR target protein.

MATERIALS AND METHODS

Chemicals, Reagents and Equipments

Melting points (°C) were determined using a Fischer-Jones melting point apparatus and were found uncorrected. Microanalyses (C, H, N, O and X=halogen) were performed at the micro-analytical center, University of Pune using Rapid analyzer. Fourier Transform Infrared spectra (FT-IR, KBr cm^{-1}), were run on JASCO 401 FT-IR spectrometer. ^{1}H NMR and ^{13}C NMR spectra were recorded on BRUKER AVANCE II FT-NMR (400MHz) using TMS as an internal standard (chemical shifts in δ, ppm), s=singlet, d=doublet, m=multiplet, bs=broad singlet. The relative integrals of peak areas agreed with those expected for the assigned structures. Mass spectra were recorded on WATERS Q-TOF Micromass, performed at SAIF, Punjab University, Chandigarh, India. TLC analysis was carried out on silica gel pre-coated aluminum sheets (Type 60 F_{254}, Merck) and the spots were detected under UV-Lamp at short wavelength λ=254nm and longer wavelength λ=364nm.

Experimental

Synthesis of Quinazolin-4(3H)-one Scaffold

General procedure: Synthesis of 2-phenyl-3-substituted 3H-quinazoline-4-one (Scheme-1)

The reaction was started by dissolving anthranilic acid (0.029mole) in dry pyridine at room temperature. At 0°C, slowly pouring benzoyl chloride and whirling it formed a pasty material. The resulting reaction mixture was precipitated with 10% aqueous sodium bicarbonate until the effervescence subsided. The precipitate was washed with cold water and recrystallized from diluted ethanol to produce 2-Phenyl-benzo[d][1,3]oxazin-4-one. After drying, it was immediately put to use for the subsequent action. Equimolar (0.01mole) amounts of 2-phenyl-benzo[d][1,3]oxazin-4-one (step-1) are refluxed in ethanol with various substituted amines for three hours at 85°C. Acetic acid, which was extremely cold, was present. The reaction mixture was finished and placed over crushed ice to produce 2-phenyl-3-aryl quinazolin-4(3H)-one (Scheme-1, QIa-n).

3-(4-Chloro-phenyl)-2-phenyl-3H-quinazolin-4-one: (QIa)

It was obtained from 4-chloro aniline according to the general procedure for Scheme 1 as off-white solid. Yield 65.00%; m.p 111-114°C. The crude mass was purified by recrystallization using ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (d, 5H, *J*=8.40 Hz, Ar-H), 7.35 (d, 4H, *J*=6.67 Hz, Ar-H), 7.25 (d, 4H, *J*=4.45 Hz, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 134, 122, 129, 127. Electro-spray Ionization (ESI)-MS: m/z=333.07 $[M+H]^+$(100 %). Analysis calculated for $C_{20}H_{13}ClN_2O$ (332.7); C, 72.19; H, 5.14; N, 8.1; O, 4.8; Cl, 10.6 %. Found: C, 72.20; H, 5.16; N, 8.11; O, 4.75; Cl, 10.45%.

3-(4-nitrophenyl)-2-phenylquinazolin-4(3H)-one: (QIb)

It was obtained from 4-nitro aniline according to the general procedure for Scheme 1 as a yellow solid. Yield 75.00%; m.p 130-132°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (d, 4H, *J*=5.40 Hz, Ar-H), 7.6 (d, 5H, *J*=6.45 Hz, Ar-H), 7.4 (d, 4H, *J*=4.45Hz, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 144, 125, 123. Electro-spray Ionization (ESI)-MS: *m/z*=344.02$[M+H]^+$(100%). Analysis calculated for $C_{20}H_{13}N_3O_3$ (343.3); C, 69.9; H, 3.78; N, 12.00; O, 13.00%. Found: C, 69.86; H, 3.69; N, 12.10; O, 13.06%.

3-(4-bromophenyl)-2-phenylquinazolin-4(3H)-one: (QIc)

It was obtained from 4-bromo aniline according to the general procedure for Scheme 1 as a faint-yellow solid. Yield 80.00%; m.p 100-102°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (d, 4H, *J*=6.5, Ar-H), 7.6 (d, 5H, *J*=6.24, Ar-H), 7.4 (d, 4H, *J*=6.6, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 144, 125, 118. Electro-spray Ionization (ESI)-MS: m/z=377.02$[M+H]^+$(100%). Analysis calculated for $C_{20}H_{13}BrN_2O$ (377.2); C, 63.06; H, 3.44; N, 7.4; O, 4.2, Br, 21.1 %. Found: C, 63.08; H, 3.46; N, 7.41; O, 4.30; Br, 21.00%.

4-(4-oxo-2-phenylquinazolin-3(4H)-yl)benzoic acid: (QId)

It was obtained from 4-amino benzoic acid according to the general procedure for Scheme 1 as a white solid. Yield 69.00%; m.p 120-122°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 11.00 (s, 1H, OH), 8.1 (d, 4H, *J*=6.5 Hz, Ar-H,), 7.6 (d, 5H, *J*=6.55 Hz, Ar-H), 7.4 (d, 4H, *J*=4.5 Hz, Ar-H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 172, 164, 152, 133, 126, 125. Electro-spray Ionization (ESI)-MS: m/z=343.10$[M+H]^+$(100%). Analysis calculated for $C_{21}H_{14}N_2O_3$ (342.3); C, 73.6; H, 4.08; N, 8.1; O, 14.0%. Found: C, 73.59; H, 4.09; N, 8.10; O, 14.06%.

3-(2-aminophenyl)-2-phenylquinazolin-4(3H)-one: (QIe)

It was obtained from 2-amino aniline according to the general procedure for Scheme 1 as a white solid. Yield 68.00%; m.p 140-142°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 4H, *J*= 5.45 Hz, Ar-H), 7.6 (d, 5H, *J*=4.63 Hz, Ar-H), 7.4 (d, 4H, *J*=6.25 Hz, Ar-H), 4.0 (s, 2H, NH_2). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 138, 133, 125, 122, 115. Electro-spray Ionization (ESI)-MS: m/z=314.12$[M+H]^+$(100%). Analysis calculated for $C_{20}H_{15}N_3O$ (313.3); C, 76.06; H, 4.78; N, 13.0; O, 5.1 %. Found: C, 76.10; H, 4.74; N, 13.00; O, 5.01%.

3-(4-aminophenyl)-2-phenylquinazolin-4(3H)-one: (QIf)

It was obtained from 4-amino aniline according to the general procedure for Scheme 1 as a white solid. Yield 65.00%; m.p

128-131°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 4H), 7.6 (s, 5H), 7.4 (s, 4H), 4.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 142, 133, 125, 122, 115. Electro-spray Ionization (ESI)-MS: *m/z*=314.12 $[M+H]^+$(100%). Analysis calculated for $C_{20}H_{15}N_3O$ (313.3); C, 76.06; H, 4.78; N, 13.4; O, 5.1%. Found: C, 76.09; H, 4.75; N, 13.35; O, 5.08%.

N-{[4-(4-oxo-2-phenylquinazolin-3(4H)-yl)phenyl] sulfonyl}acetamide: (QIg)

It was obtained from *N*-[(4-aminophenyl)sulfonyl]acetamide according to the general procedure for Scheme 1 as a white solid. Yield 78.00%; m.p 164-167°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 10.0 (s, 1H, NH), 7.9 (d, 4H, Ar-H), 7.4 (d, 4H, Ar-H), 7.29 (d, 5H, Ar-H), 2.0 (s, 3H, CH_3). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 142, 134, 129, 127, 121, 115. Electro-spray Ionization (ESI)-MS: *m/z*= 420.10 $[M+H]^+$ (100%). Analysis calculated for $C_{22}H_{17}N_3O_4S$ (419.4); C, 62.9; H, 4.05; N, 10.0; O, 15.00, S, 7.62 %. Found: C, 62.76; H, 4.10; N, 10.01; O, 15.04, S, 7.57%.

4-(4-oxo-2-phenylquinazolin-3(4H)-yl) benzenesulfonamide: (QIh)

It was obtained from 4-aminobenzene sulfonamide according to the general procedure for Scheme 1 as a gray solid. Yield 66.00%; m.p 114-117°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 4H), 7.29 (s, 5H), 2.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 173, 164, 152, 134, 127, 16.6 (s, 3H). Electro-spray Ionization (ESI)-MS: *m/z*=378.09$[M+H]^+$(100%). Analysis calculated for $C_{20}H_{15}N_3O_3S$ (377.4); C, 63.06; H, 3.97; N, 11.1; O, 12.00, S, 8.47%. Found: C, 63.12; H, 3.87; N, 11.11; O, 12.05, S, 8.45%.

4-oxo-2-phenylquinazoline-3(4H)-carboxamide: (QIi)

It was obtained from urea according to the general procedure for Scheme 1 as gray solid. Yield 74.00%; m.p 140-142°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (s, 4H), 7.29 (s, 5H), 6.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 134, 132, 129, 125. Electro-spray Ionization (ESI)-MS: m/z=266.09$[M+H]^+$(100%). Analysis calculated for $C_{15}H_{11}N_3O_2$ (265.26); C, 67.9; H, 4.14; N, 15.8; O, 12.06%. Found: C, 67.85; H, 4.11; N, 15.75; O, 12.09%.

4-oxo-2-phenylquinazoline-3(4H)-carbothioamide: (QIj)

It was obtained from thio-urea according to the general procedure for Scheme 1 as a black color solid. Yield 60.00%; m.p 139-141°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (s, 4H), 7.29 (s, 5H), 2.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 159, 152, 134, 132, 125. Electro-spray Ionization (ESI)-MS: *m/z*=282.06$[M+H]^+$(100%). Analysis calculated for $C_{15}H_{11}N_3OS$ (281.3); C, 64.0; H, 3.91; N, 14.9; O, 5.6; S, 11.37%. Found: C, 64.1; H, 3.87; N, 14.89; O, 5.54; S, 11.42%.

4-oxo-2-phenylquinazoline-3(4H)-carbohydrazide: (QIk)

It was obtained from hydrazine carboxamide according to the general procedure for Scheme 1 as faint yellow solid. Yield 69.00%; m.p 123-126°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (s, 4H), 7.29 (s, 5H), 6.0 (1H), 2.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 157, 152, 134, 125. Electro-spray Ionization (ESI)-MS: m/z=281.10$[M+H]^+$(100%). Analysis calculated for $C_{15}H_{12}N_4O_2$ (280.28); C, 64.2; H, 4.28; N, 19.9; O, 11.42%. Found: C, 64.13; H, 4.25; N, 19.88; O, 11.41%.

4-oxo-2-phenylquinazoline-3(4H)-carbothiohydrazide: (QIl)

It was obtained from hydrazine carbo-thioamide according to the general procedure for Scheme 1 as a faint black solid. Yield 54.00%; m.p 118-121°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (s, 4H), 7.29 (s, 5H), 6.0 (1H), 2.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 183, 164, 157, 152, 134, 125. Electro-spray Ionization (ESI)-MS: m/z= 297.08$[M+H]^+$(100%). Analysis calculated for $C_{15}H_{12}N_4OS$ (296.3); C, 60.7; H, 4.04; N, 18.89; O, 5.3; S, 10.79%. Found: C, 60.67; H, 4.03; N, 18.90; O, 5.25; S, 10.80%.

2-phenyl-3-(phenylamino)quinazolin-4(3H)-one: (QIm)

It was obtained from phenylhydrazine according to the general procedure for Scheme 1 as a white solid. Yield 77.00%; m.p 169-171°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (s, 4H), 7.29 (s, 5H), 7.1 (s, 5H), 4.0 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 142, 129, 114. Electro-spray Ionization (ESI)-MS: m/z=314.12 (100%). Analysis calculated for $C_{20}H_{15}N_3O$ (313.3); C, 49.06; H, 5.57; N, 15.59; O, 29.71%. Found: C, 49.08; H, 5.65; N, 15.60; O, 29.69%.

3-[(2,5-dinitrophenyl)amino]-2-phenylquinazolin-4(3H)-one: (QIn)

It was obtained from 2,4-dinitro phenyl hydrazine according to the general procedure for Scheme 1 as a yellow solid. Yield 57.00%; m.p 177-180°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 3H), 7.5 (s, 4H), 7.29 (s, 5H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 164, 152, 133, 129, 128, 108. Electro-spray Ionization (ESI)-MS: *m/z*=404.09$[M+H]^+$(100%). Analysis calculated for $C_{20}H_{13}N_5O_5$ (403.3); C, 59.5; H, 3.22; N, 1.23; O, 19.83%. Found: C, 59.46; H, 3.21; N, 1.24; O, 19.86%.

General procedure: synthesis of 2 phenyl 3-benzylideneamino 3(H) quinazolin-4-ones (Scheme-2)

The drop wise addition of a solution of 2-Phenyl-benzo[d] [1,3]oxazin-4-one (Step 1, Scheme 1) (0.01mole) in 20mL of

anhydrous pyridine was performed while continuously stirring hydrazine hydrate (0.02mole, 5.4mL) in anhydrous pyridine. After all of the ingredients had been added, the reaction mixture was vigorously agitated for 30min at room temperature before being heated under reflux for 6 hrs under anhydrous reaction conditions. Crushed ice is used to precipitate the reaction mixture, producing crude 3-amino-2-phenyl-3H-quinazolin-4-one. The finished product underwent additional recrystallization in diluted ethanol. In the presence of glacial acetic acid, an equimolar amount of 2-phenyl-3-amino-3H-quinazoline-4-one (0.02mole) and different substituted aldehydes (Scheme-2) were heated to reflux for 1.5 to 2 hrs. The crude 2-phenyl 3-benzylidene amino-3H-quinazoline-4-one product was further refined *via* recrystallization after precipitation in crushed ice using ethanol.

3-{[(E)-(4-hydroxyphenyl)methylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIa)

It was obtained from 4-hydroxy benzaldehyde according to the general procedure for Scheme 2 as a yellow solid. Yield 83.00%; m.p 137-139°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.45 (s, 4H), 7.29 (s, 5H), 5.0 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170, 164, 159, 157, 133, 129, 125. Electro-spray Ionization (ESI)-MS: m/z=342.12 $[M+H]^+$(100%). Analysis calculated for $C_{21}H_{15}N_3O_2$ (341.3); C, 73.8; H, 4.39; N, 12.30; O, 9.30%. Found: C, 73.75; H, 4.41; N, 12.33; O, 9.29%.

3-{[(E)-(2-hydroxyphenyl)methylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIb)

It was obtained from 2-hydroxy benzaldehyde according to the general procedure for Scheme 2 as off white solid. Yield 82.00%; m.p 112-115°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.45 (s, 4H), 7.29 (s, 5H), 5.0 (s, 1H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170, 164, 159, 157, 133, 129, 125. Electro-spray Ionization (ESI)-MS: 342.12$[M+H]^+$(100%). Analysis calculated for $C_{21}H_{15}N_3O_2$ (341.3); C, 73.8; H, 4.39; N, 12.30; O, 9.3%. Found: C, 73.78; H, 4.41; N, 12.36; O, 9.35%.

3-{[(E)-(3,4-dihydroxyphenyl)methylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIc)

It was obtained from 3,4-di hydroxyl benzaldehyde according to the general procedure for Scheme 2 as yellow solid. Yield 81.00%; m.p 132-136°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.29 (s, 5H), 6.9 (s, 3H), 5.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170, 164, 159, 157, 146,144, 133, 129, 125. Electro-spray Ionization (ESI)-MS: 358.11$[M+H]^+$(100%). Analysis calculated for $C_{21}H_{15}N_3O_3$ (357.3); C, 70.58; H, 4.19; N, 11.75; O, 13.43%. Found: C, 70.55; H, 4.20; N, 11.71; O, 13.45%.

3-({(E)-[4-(dimethylamino)phenyl]methylidene}amino)-2-phenylquinazolin-4(3H)-one: (QIId)

It was obtained from 4-amino benzaldehyde according to the general procedure for Scheme 2 as a yellow solid. Yield 69.00%; m.p 120-122°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.29 (s, 5H), 6.6 (s, 4H), 2.85 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$) δ: 170, 164, 154, 146, 133, 125, 113, 43.6. Electro-spray Ionization (ESI)-MS: 369.15; found 369.17 $[M+H]^+$(100%). Analysis calculated for $C_{23}H_{20}N_4O$ (368.4); C, 74.98; H, 5.4; N, 15.2; O, 4.3%. Found: C, 74.95; H, 5.43; N, 15.24; O, 4.32%.

3-{[(Z)-(4-methoxyphenyl)methylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIe)

It was obtained from 4-methoxy benzaldehyde according to the general procedure for Scheme 2 as white solid. Yield 75.00%; m.p 128-131°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.5 (s,2H), 7.29 (s, 5H), 6.8 (s, 2H), 3.73 (s, 3H).^{13}C NMR (400 MHz, $CDCl_3$) δ: 170, 164, 154, 133, 132, 113, 56. Electro-spray Ionization (ESI)-MS: 356.13 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{17}N_3O_2$ (355.28); C, 74.36; H, 4.7; N, 11.8; O, 9.0%. Found: C, 74.27; H, 4.65; N, 11.75; O, 9.01%.

3-{[(E)-(4-hydroxy-3-methoxyphenyl)methylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIf

It was obtained from 3-methoxy, 4-hydroxy benzaldehyde according to the general procedure for Scheme 2 as a brown solid. Yield 58.00%; 165-167°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.5 (s, 2H), 7.29 (s, 5H), 7.0 (s, 3H), 3.73 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 152, 149, 145, 134, 132, 127, 124, 116, 56. 3. Electro-spray Ionization (ESI)-MS: 372.13 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{17}N_3O_3$ (371.3); C, 71.16; H, 4.57; N, 1131; O, 12.92%. Found: C, 71.13; H, 4.55; N, 11.34; O, 12.90%.

3-{[(E)-(2-chlorophenyl)methylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIg)

It was obtained from 2-chloro benzaldehyde according to the general procedure for Scheme 2 as a pale yellow solid. Yield 62.00%; m.p 117-120°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.5 (s, 2H), 7.29 (s, 5H), 7.2 (s, 4H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 152, 134, 133, 128, 122. Electro-spray Ionization (ESI)-MS: 360.08 $[M+H]^+$(100%). Analysis calculated for $C_{21}H_{14}ClN_3O$ (359.8); C, 70.09; H, 3.89; N, 11.67; O, 4.44; Cl, 9.8%. Found: C, 70.10; H, 3.87; N, 11.65; O, 4.41; Cl, 9.78%.

3-{[(Z)-furan-2-ylmethylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIh)

It was obtained from furfuraldehyde according to the general procedure for Scheme-2 as a white solid. Yield 62.00%; m.p 117-120°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 8.1 (s, 1H), 7.9 (s, 4H), 7.5 (s, 2H), 7.29 (s, 5H), 6.3 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 154, 152, 143, 133, 129, 125, 122, 110. Electro-spray Ionization (ESI)-MS: 316.10 $[M+H]^+$(100%). Analysis calculated for $C_{19}H_{13}N_3O_2$ (315.3); C, 72.37; H, 4.12; N, 13.32; O, 10.14%. Found: C, 72.33; H, 4.14; N, 13.31; O, 10.15%.

General procedure: synthesis of 2-phenyl-3-ethylidene amino 3H-quinazoline-4-one (Scheme-3)

Synthesis of 2 phenyl 3-ethylidene amino-quinazoline-4(3H)-one

The equimolar amounts of various substituted ketones (Scheme 3) and 3-amino-2-phenyl-3H-quinazolin-4-one (from Step II of Scheme 2) were dissolved in ethanol, and the reaction mixture was heated to reflux for 1.5–2 hrs. As a catalyst, glacial acetic acid was used during the reaction. By dumping the reaction liquid onto crushed ice, a precipitate of crude 2-phenyl-3-ethylidene amino 3H-quinazoline-4-one was produced. The item was dried and refined using ethanol recrystallization (Scheme-3, QIIIa-g).

3-{[(1E)-1-(4-hydroxyphenyl)ethylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIIa)

It was obtained from 4-hydroxy acetophenone according to the general procedure for Scheme-3 as a yellow solid. Yield 55.00%; m.p 145-147°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 5H), 7.29 (s, 5H), 6.8 (s, 4H), 5.0 (s, 1H), 0.9 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 159, 157, 146,144, 133, 129, 125. Electro-spray Ionization (ESI)-MS: 356.13 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{17}N_3O_2$ (355.3); C, 49.06; H, 5.57; N, 15.59; O, 29.71%. Found: C, 49.10; H, 5.54; N, 15.53; O, 29.69%.

3-{[(1E)-1-(4-chlorophenyl)ethylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIIb)

It was obtained from 4-chloro acetophenone according to the general procedure for Scheme 3 as a brown yellow solid. Yield 59.00%; m.p149-151°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 5H), 7.29 (s, 5H), 7.6 (s, 4H), 0.9 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 159, 155, 133, 130, 128, 125, 11.7. Electro-spray Ionization (ESI)-MS: 374.10 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{16}ClN_3O$ (373.8); C, 74.36; H, 4.78; N, 11.82; O, 9.0%. Found: C, 74.39; H, 4.75; N, 11.81; O, 9.10%.

3-{[(1E)-1-(3-aminophenyl)ethylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIIc)

It was obtained from 3-amino acetophenone according to the general procedure for Scheme 3 as 66.00 %, as a white solid, m.p 168-169°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 5H), 7.29 (s, 5H), 7.0 (s, 4H), 0.9 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 155, 152, 146, 129, 115, 11.7. Electro-spray Ionization (ESI)-MS: 355.15 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{18}N_4O$ (354.4); C, 74.55; H, 5.07; N, 15.80; O, 4.51%. Found: C, 74.51; H, 5.10; N, 15.81; O, 4.50%.

3-{[(1E)-1-(2,4-dihydroxyphenyl)ethylidene]amino}-2-phenylquinazolin-4(3H)-one: (QIIId)

It was obtained from 2,4-dihydroxy acetophenone according to the general procedure for Scheme 3 as off white solid. Yield 79.00%; m.p 119-121°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 5H), 7.29 (s, 5H), 6.3 (s, 4H), 5.0 (s, 1H), 0.9 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 155, 132, 129, 125, 111, 108, 12. Electro-spray Ionization (ESI)-MS: 372.13 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{17}N_3O_3$ (371.3); C, 71.16; H, 4.5; N, 11.31; O, 12.92%. Found: C, 71.11; H, 4.45; N, 11.30; O, 12.88%.

3-({(1E)-1-[2-(aminomethyl)-3,4-dihydroxyphenyl] ethylidene}amino)-2-phenylquinazolin-4(3H)-one: (QIIIe)

It was obtained from amino methyl 3,4-dihydroxy acetophenone according to the general procedure for Scheme 3 as gray solid. Yield 79.00%; m.p 119-121°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.5 (s, 4H), 7.29 (s, 5H), 6.9 (s, 2H), 5.0 (s, 1H), 3.9 (s, 2H), 2.0 (s, 2H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 155, 146, 143, 132, 129, 12. Electro-spray Ionization (ESI)-MS: 390.10 $[M+H]^+$(100%). Analysis calculated for $C_{23}H_{20}N_4O_3$ (400.4); C, 68.98; H, 5.0; N, 14.0; O, 11.98%. Found: C, 68.95; H, 5.04; N, 14.10; O, 11.97%.

3-{[(1E)-1-(4-chloro-2-hydroxyphenyl)ethylidene] amino}-2-phenylquinazolin-4(3H)-one: (QIIIf)

It was obtained from 4-chloro, 2-hydroxy acetophenone according to the general procedure for Scheme 3 as a faint yellow solid. Yield 64.00%; m.p 140-143°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 5H), 7.29 (s, 5H), 6.3 (s, 4H), 5.0 (s, 1H), 0.9 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 159, 152, 137, 133 129, 125, 116, 12. Electro-spray Ionization (ESI)-MS: 404.09 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{16}ClN_3O_2$ (389.8); C, 68.28; H, 4.38; N, 1084; O, 16.52%. Found: C, 68.27; H, 4.34; N, 10.78; O, 16.45%.

2-phenyl-3-{[(1E)-1-(2,3,4-trihydroxyphenyl)ethylidene] amino}quinazolin-4(3H)-one: (QIIIg)

It was obtained from 2,3,4-tri hydroxyl acetophenone according to the general procedure for Scheme 3 as faint yellow solid. Yield 63.00%; m.p 122-125°C. The crude product was recrystallized from ethanol. ^{1}H-NMR (400MHz, $CDCl_3$) δ: 7.9 (s, 4H), 7.6 (s, 5H), 7.29 (s, 5H), 6.3 (s, 4H), 5.0 (s, 1H), 0.9 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$, in ppm): 170, 164, 155, 152, 147, 132, 127, 124, 109, 12. Electro-spray Ionization (ESI)-MS: 388.12 $[M+H]^+$(100%). Analysis calculated for $C_{22}H_{17}N_3O_4$ (387.3); C, 49.06; H, 5.57; N, 15.59; O, 29.71%. Found: C, 49.08; H, 5.55; N, 15.49; O, 29.73%.

In-vitro DHFR Inhibition Assay

Following the described approach, all produced compounds were assessed as human DHFR inhibitors. The assay mixture included 0.02U of human DHFR in a final volume of 2.0mL, 50μM Tris-HCl buffer (pH 7.4), 50μM NADPH, 20μL of DMSO, or the same volume of DMSO solution containing the test chemicals to a final concentration of 10^{-11} - 10^{-5} mole. The reaction was started by adding 25mM dihydrofolic acid to the mixture, which was then incubated at room temperature for 2.0 min. The change in absorbance (DA/min) was then measured at 340 nm. For 10min, the activity was linear under these circumstances. Results are expressed as a percentage inhibition of enzyme activity determined using the formula below.

$$\% \text{ inhibition} = \left(1 - \frac{\Delta A/\min_{test}}{\Delta A/\min_{D\ MSO}}\right) \times 100$$

The percentage of inhibition was plotted against drug concentration (log scale). Each compound's 50% inhibitory concentration (IC_{50}) was discovered. Trimethoprim (TMP) inhibition plot was also created, displaying an IC_{50} value of 10mole.

In-vitro anti-microbial activity

Using a Muller-Hinton agar medium, the agar disc-diffusion method was used to conduct the primary screening. Gentamicin, ciprofloxacin, and clotrimazole, three antibacterial and antifungal medications, were carefully placed on the agar cultures plates that had been previously inoculated separately with the micro-organisms. Sterile filter paper discs (8mm diameter) were moistened with the compound solution in dimethyl sulphoxide of a specific concentration of 200μg/disc. The plates were incubated at 37°C for 24hr for bacteria and 48hrs for fungi before measuring the diameter (mm) of the growth inhibition zones. Using the micro-dilution susceptibility method in Muller-Hinton Broth, the minimum inhibitory concentrations (MIC) for the compounds against the same bacteria employed in the main screening were determined.[20] was dissolved in test substances and standards such gentamicin, ciprofloxacin, and clotrimazole were dissolved in dimethyl sulphoxide at a concentration of 64μg/mL. The solution was made in two-fold dilutions (64, 32,..., 0.5, 0.25, and 0.125μg/mL). The corresponding wells were infected with the microbe suspensions at a concentration of 106 CFU/mL (colony forming units/mL). The plates were incubated for 24 hrs at 37°C. The MIC values were established as the lowest concentrations that entirely prevented the microorganism from growing in a way that could be seen by unaided eyes.

Docking and molecular modeling study

Using the Vlife MDS 4.3 suite, the docking investigation of quinazolin-4(3H)-ones was carried out. The 2, 3-disubstituted quinazolin4-(3H)-one derivative's neutral three-dimensional structures were created after 2D structures were converted to 3D utilizing the Vlife engine module tools option and saved in a 3D-output folder. Force field batch minimization was used to optimize 3D molecules, and the results were saved in a separate folder while running in Vlife engine compute mode. From www.rscpdb.org, the target protein PDB and reference ligand were downloaded. The Monte Carlo approach produced conformers of the compound. The Conformers were all then saved in a separate folder after being energetically minimized up to the rms gradient of 0.001. Each of the novel analogues was docked into the DHFR enzyme-binding domain in its lowest energy conformation. The enzyme structure underwent a procedure of refinement in which the restrictions placed on the enzyme were gradually relaxed and minimized until the rms gradient was 0.01 kcal/mol. A 10.0 radius was used to define the enzyme's active region around TMP. The difference between the complex and individual energies of the enzyme and ligands was used to compute the energy of binding.[21,22]

RESULTS

Chemistry of synthesis of title compounds

Introduction of groups such as 4-chloro phenyl, 4-bromo phenyl, 4-nitro phenyl, 4-carboxy phenyl, 2-aminophenyl, and 4-aminophenyl connected to position-3 N-atom of quinazolin-4(3H)-one nucleus in the synthesis of compounds in the Scheme 1 series. Additional groups such as 4-phenyl sulfonyl-acetamide, 4-benzene sulfonamide, carboxamide, carbo-thioamide, carbo-hydrazide, phenyl amino, 2,4-dinitro phenyl amino, and carbo-thiohydrazide attachment at the same position in the same series of compounds at the similar position-3, N-atom of quinazolin-4(3H)-one nucleus (Scheme 1, QIa-n).

The second series of quinazolin-4(3H)-one analogue (QII) comprise an aromatic moiety connected with substantial functional groups and a benzylidene amino-linkage between the position-3N-atom of the quinazolin-4(3H)-one nucleus. The 4-hydroxy phenyl, 2-hydroxy phenyl, 3, 4-dihydroxy phenyl, 4-dimethyl amino benzene, 4-methoxy phenyl, 3-methoxy and 4-hydroxy phenyl, 2-chloro phenyl, and furyl moiety were the

Table 1: Physicochemical characteristics of quinazolin-4(3H)-one derivative.

Sl. No	Compound Code	Substituents R/Ar, R', R"	Product Yield (%)	Melting Point (°C)	Molecular Formula (Molecular Weight)
1	QIa	4-Cl	65	111-114	$C_{20}H_{13}ClN_2O$ (332.78)
2	QIb	4-NO_2	75	130-132	$C_{20}H_{13}N_3O_3$ (343.33)
3	QIc	4-Br	80	100-102	$C_{20}H_{13}BrN_2O$ (377.23)
4	QId	4-COOH	69	120-122	$C_{21}H_{14}N_2O_3$ (342.34)
5	QIe	2-NH_2	68	140-142	$C_{20}H_{15}N_3O$ (313.35)
6	QIf	4-NH_2	65	128-131	$C_{20}H_{15}N_3O$ (313.35)
7	QIg	4-SO_2-NH-$COCH_3$	78	164-167	$C_{22}H_{17}N_3O_4S$ (419.45)
8	QIh	4-SO_2-NH_2	66	114-117	$C_{20}H_{15}N_3O_3S$ (377.41)
9	QIi	CO-NH_2	74	140-142	$C_{15}H_{11}N_3O_2$ (265.26)
10	QIj	CS-NH_2	60	139-141	$C_{15}H_{11}N_3OS$ (281.33)
11	QIk	CONH-NH_2	59	123-126	$C_{15}H_{12}N_4O_2$ (280.28)
12	QIl	CS-NH-NH_2	54	118-120	$C_{15}H_{12}N_4OS$ (296.34)
13	QIm	NH-C_6H_5	77	169-171	$C_{20}H_{15}N_3O$ (313.35)
14	QIn	NH-C_6H_4-(2, 4-NO_2)	57	177-180	$C_{20}H_{13}N_5O_5$ (403.34)
15	QIIa	4-OH	83	137-139	$C_{21}H_{15}N_3O_2$ (341.36)
16	QIIb	2-OH	82	112-115	$C_{21}H_{15}N_3O_2$ (341.36)
17	QIIc	3,4-OH	81	132-136	$C_{21}H_{15}N_3O_3$ (357.36)
18	QIId	4-$N(CH_3)_2$	69	120-122	$C_{23}H_{20}N_4O$ (368.43)
19	QIIe	4-OCH_3	75	128-131	$C_{22}H_{17}N_3O_2$ (355.38)
20	QIIf	3-OH,4-OCH_3	58	165-167	$C_{22}H_{17}N_3O_3$ (371.38)
21	QIIg	2-Cl	62	117-120	$C_{21}H_{14}ClN_3O$ (359.80)
22	QIIh	Furyl	59	124-127	$C_{19}H_{13}N_3O_2$ (315.32)
23	QIIIa	4-OH	55	145-147	$C_{22}H_{17}N_3O_2$ (355.38)
24	QIIIb	4-Cl	59	149-151	$C_{22}H_{16}ClN_3O$ (373.83)
24	QIIIc	3-NH_2	68	166-169	$C_{22}H_{18}N_4O$ (354.40)
25	QIIId	2,4-OH	79	119-122	$C_{22}H_{17}N_3O3$ (371.38)
26	QIIIe	2-CH2-NH_2, 3-OH, 4-OH	68	184-186	$C_{23}H_{20}N_4O_3$ (400.42)
27	QIIIf	2-OH, 4-Cl	64	140-143	$C_{22}H_{16}ClN_3O_2$ (389.83)
28	QIIIg	2,3,4-OH	63	122-125	$C_{22}H_{17}N_3O_4$ (387.38)

groups connected to the methylidene amino moiety (Scheme-2, QIIa-h).

Another set of quinazolin-4(3H)-one analogue (QIII) have a substantial functional group at the site of the 3N-atom of the quinazolin-4(3H)-one nucleus linked to the phenyl moiety. The groups that were connected to the ethylidene bridge included 4-hydroxy, 4-chloro, 3-amino, 2, 4-dihydroxy, 2-aminomethyl-3,4-dihydroxy, and 2,3,4-trihydroxy phenyl moieties (Scheme 3, QIIIa-g). Table 1 provides physicochemical information for the compounds that were obtained. More potent compounds should result from combining the quinazolin-4(3H)-ones' natural DHFR inhibitory action with the accessible functional groups in one structure. It is also known that the majority of these functional groups promote lipid solubility.[23-26]

Dihydrofolate reductase (DHFR) inhibition

Following the recommended procedure, all synthesized compounds were tested for their ability to inhibit microbial DHFR, and the results are presented as IC_{50} (M) values (Table 2).[27-29] With IC_{50} values ranging from 4 to 24µM, all the drugs have demonstrated moderate to good DHFR inhibitory action.

Antimicrobial Screening

A panel of standard strains of Gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeuroginosa*), the yeast-like pathogenic fungus *Candida albicans*, and mould *Aspergillus niger* were used to test the synthesized compounds for their *in-vitro* antimicrobial activity. The Muller-Hinton

Table 2: Antimicrobial activity MIC (µg/ml) and DHFR inhibitory activity IC_{50} (µM) of quinazolin-4(3H)-one derivatives.

Sl. No	Compound Code	DHFR Inhibition (IC_{50})	Antimicrobial Activity (MIC)					
			S. A	B. S	E. C	P. A	C. A	A. N
1	QIa	24	18(2.0)	20(2.0)	20(2.0)	---	25(1.0)	28(1.0)
2	QIb	21	24(1.0)	26(1.0)	28(1.0)	18(2.0)	---	---
3	QIc	22	28(1.0)	26(1.0)	25(1.0)	24(1.0)	30(0.2)	32(0.2)
4	QId	18	25(1.0)	24(1.0)	20(2.0)	26(1.0)	21(2.0)	24(1.0)
5	QIe	21	22(2.0)	26(1.0)	18(2.0)	---	25(1.0)	21(2.0)
6	QIf	20	24(1.0)	23(1.5)	20(2.0)	---	---	---
7	QIg	08	32(0.5)	35(0.25)	33(0.5)	34(0.25)	22(1.5)	25(1.0)
8	QIh	10	30(0.5)	28(1.0)	25(1.0)	---	---	---
9	QIi	18	25(1.5)	22(1.5)	26(1.0)	---	---	---
10	QIj	07	35(0.2)	40(0.1)	31(0.5)	---	---	---
11	QIk	12	26(1.0)	22(1.5)	25(1.0)	---	---	---
12	QIl	4	34(0.5)	36(0.5)	35(0.5)	---	---	---
13	QIm	5	32(0.5)	31(0.5)	30(0.5)	---	18(2.0)	20(2.0)
14	QIn	14	20(2.0)	25(1.0)	26(1.0)	---	15(3.0)	---
15	QIIa	20	15(3.0)	18(2.0)	13(2.5)	---	14(3.0)	---
16	QIIb	22	16(2.0)	18(2.0)	19(2.0)	17(2.0)	---	14(3.0)
17	QIIc	24	14(3.0)	---	15(3.0)	14(3.0)	---	---
18	QIId	10	29(0.5)	30(0.5)	32(0.25)	31(0.25)	27(1.0)	24(1.0)
19	QIIe	08	31(0.5)	33(0.25)	32(0.25)	28(0.5)	16(2.0)	22(1.5)
20	QIIf	06	35(0.2)	40(0.1)	32(0.2)	31(0.25)	---	---
21	QIIg	14	18(1.5)	22(1.0)	21(1.5)	24(1.0)	12(3.0)	14(3.0)
22	QIIh	15	12(3.0)	22(1.25)	18(2.0)	---	---	---
23	QIIIa	18	12(3.0)	---	13(3.0)	---	---	---
24	QIIIb	24	16(2.0)	18(2.0)	14(3.0)	15(2.5)	35(1.0)	32(0.25)
25	QIIIc	22	18(2.0)	19(2.0)	16(2.0)	20(2.0)	---	---
26	QIIId	23	30(0.5)	32(0.2)	28(0.5)	25(1.0)	22(1.5)	20(2.0)
27	QIIIe	08	31(0.2)	29(0.5)	30(0.5)	32(0.25)	28(0.5)	29(0.5)
28	QIIIf	18	25(1.0)	28(0.5)	32(0.25)	31(0.25)	35(0.2)	36(0.2)
29	QIIIg	16	18(1.5)	20(1.0)	23(1.0)	16(2.0)	21(1.5)	19(1.0)
30	GEN	---	30(0.25)	32(0.2)	35(0.5)	38(0.1)	Nd	Nd
31	CIP	---	40(0.1)	39(0.1)	38(0.1)	36(0.5)	Nd	Nd
32	CLT	---	Nd	Nd	Nd	Nd	35(0.2)	37(0.2)
33	TMP	10	---	---	---	---	---	---

Inhibition Zone (mm): (---) Not active (5-12 mm), Weak activity (12-18 mm), Moderate activity (18-30 mm), Strong activity (>30 mm). Solvent: DMSO (5-8 mm). MIC (µg/ml) showed in parentheses. Nd, not determined. S.A- *Staphylococcus aureus*, B.S- *Bacillus subtilis*, E.C-*Escherischia coli*, P.A- *Pseudomonas aeruginosa*. GEN; Gentamycin, CIP; Ciprofloxacin, CLT; Clotrimazole, TMP; Trimethoprim.

agar medium was used for the primary screening, which was done using the agar disc diffusion technique. Table 2 displays the findings of preliminary antibacterial tests of the produced compounds.[30]

Molecular modeling study

To comprehend and analyze the peculiar DHFR inhibitory pattern of this class of more recent drugs, a molecular modeling research is mainly required. For modeling and docking, the tertiary complex of dihydrofolate reductase (DHFR, 2W3M), NADPH, and TMP was employed as a reference. The results were particularly fascinating since they showed several types of spatial distance-dependent protein-ligand binding interactions (Figures 4A and 4B, 5A and 5B, 6A and 6B, 7A and 7B, 8A and 8B, and 9A and 9B, respectively).

DISCUSSION

The overall formula for a new series of quinazolin-4(3H)-one derivative is shown in Figure 2. These compounds comprise 2-phenyl, 3-benzylidine amino phenyl, and 3-ethylidine amino

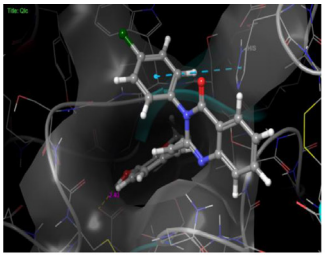

Figure 4A

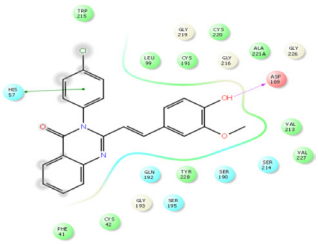

Figure 4B

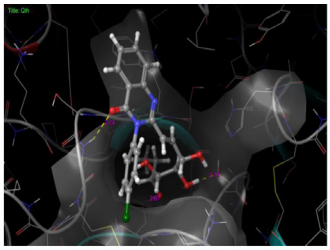

Figure 7A

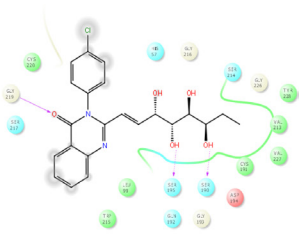

Figure 7B

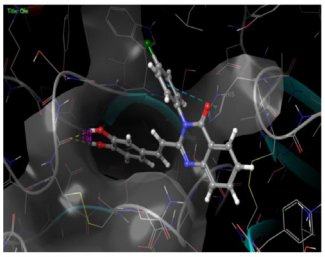

Figure 5A

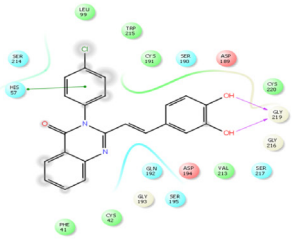

Figure 5B

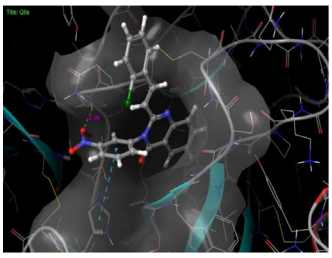

Figure 8A

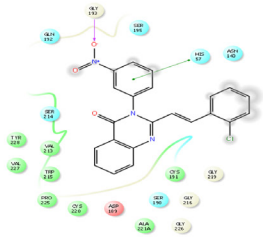

Figure 8B

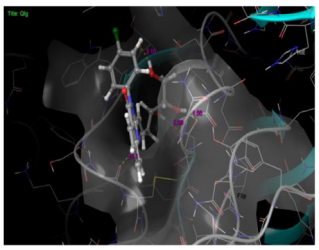

Figure 6A

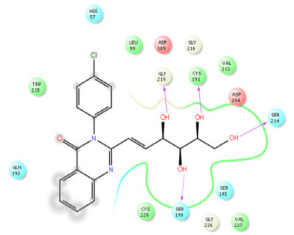

Figure 6B

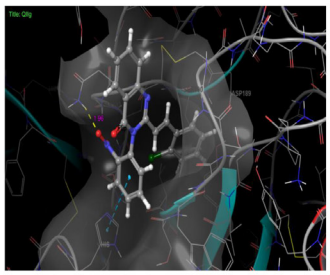

Figure 9A

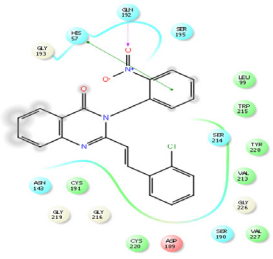

Figure 9B

Figures 4A and 4B, 5A and 5B, 6A and 6B, 7A and 7B, 8A and 8B, and 9A and 9B: Representing different types of binding interactions of quinazoline-4(3H)-ones with microbial DHFR.

phenyl groups as substituents to the quinazoline-4(3H)-one nucleus. Figure 3 illustrates the synthesis route for the chemicals in the title (Schemes 1, 2, and 3). In a straightforward benzoylation reaction followed by ring closure, 2-phenyl-4H-benzo-[1,3]-oxazin-4-one was synthesized using anthranilic acid and benzoyl chloride as starting ingredients. The next stage involves treating various substituted alkyl amines and aryl amines in a way that causes the ring oxygen to be replaced by an amine N-atom, resulting in the creation of a number of compounds listed in Scheme 1 (QIa-n). The intermediate 3-amino-2-phenyl-quinazolin-4(3H)-one is produced *via* the reaction of benzoxazin-4-one with hydrazine hydrate. This intermediate was subsequently developed to produce final products with the Scheme-2 (QIIa-h) benzylidene-amino linkage and Scheme-3 (QIIa-h) ethylidene-amino linkage (QIIIa-g). A series of QII products were created by a straightforward reflux condensation reaction between different substituted aldehydes and a 3-amino intermediate, whereas QIII series compounds, which have an ethylidene amino linkage joining to the N-atom at position-3 of the quinazoline ring, were created by a similar reaction with substituted aromatic ketones.

Compounds like QI-g (IC_{50}, 8μM), QI-j (IC_{50}, 6μM), QI-l (IC_{50}, 4μM), and QI-m (IC, 5μM) have improved inhibitory activity that is 1.2, 2, 2.4, and 3.2 folds larger than positive control TMP (IC_{50}, 10μM) in the inhibition of the DHFR enzyme. However, the compounds QI-h (IC_{50}, 10μM), QI-k (IC_{50}, 12μM), and QI-n (IC_{50}, 14μM) generated a moderate level of DHFR inhibition. With IC_{50} values ranging from 15 to 24μM, compounds including QIa-f, QI-I, QIIa-d, QII-h, and QIIIf-g have exhibited only mild inhibition of the target protein DHFR. Possible electron withdrawing groups, such as -Cl, -OH, -SH, $-SO_2$, $-NO_2$, $-NH_2$, $-SO_2NH_2$, -CS-NH-NH_2, -CONH-NH_2, $-N(CH_3)_2$, or electron releasing groups, such as $-CH_3$, $-OCH_3$, $-COCH_3$, as well as functions made to fit on the quinazoline ring, such as thio-ether, aryl, and hetero-aryl groups are known to contribute in DHFR inhibition activity.

The majority of the compounds displayed varied degrees of inhibition against the tested harmful micro-organisms, according to the results of *in-vitro* antimicrobial screening. Among the investigated micro-organisms, Gram-positive bacteria *B. subtilis* and Gram-negative *E. coli* were shown to be the most sensitive. The compounds having strong antibacterial action are QI-j, QII-f with MIC=0.1-0.2μg/mL. The moderate antibacterial activity

produced by the compounds like QIa-d, QIl-m, and QIII-d, QIIIe-f with MIC ranges from 0.5-2.0µg/mL. However, less active compounds found against tested pathogenic bacteria were Qe-I, QI-k, QI-n, QIIa-c, QIIg-h, and QIIIa-c, QIII-g with observed MIC=2.0-3.0µg/mL. In the screening of antifungal activity, compounds with potent activity were QI-c, QII-b, and QIII-f with MIC=0.1-0.2µg/mL. Moderate antifungal activity was produced by compounds as QIc-e, QI-g, QIm-n, QII-d, QIII-b, and QIII-e with MIC=0.5-2.0µg/mL. QIIa-b and QII-g, with MICs of 2.0–3.0 g/mL, were less effective chemicals against fungus.

Using the micro-dilution susceptibility method in Muller-Hinton Broth, the minimum inhibitory concentration (MIC) for the test drugs against bacteria was determined. Compared to the well-known medications gentamicin, ciprofloxacin, and clotrimazole, some of the compounds discussed in our study have shown exceptional antibacterial and antifungal potency.

In the docking and molecular modeling study, the best fit ligand observed from Scheme-1 was QI-c showing aromatic interaction with His57, H-bond with Asp189 (Figure 4A and 4B), QI-e showing aromatic interaction with His57, and two collective H-bond interactions with Gly219 (Figure 5A and 5B), QI-g showing four different H-bond interactions with Gly219, Cys191, Ser214 and Ser190 (Figure 6A and 6B), however, ligand QI-h showing strong hydrophobic π-charge interaction with Gly219 and two different H-bond interactions with Ser190 and Ser195 (Figure 7A and 7B). One of the ligands from Scheme-2, QII-a shows strong hydrophobic π-charge interaction with Gly193 and strong aromatic interaction with His57 (Figure 8A and 8B). Whereas, ligand QIII-g from Scheme-3 was found for perfect fit showing hydrophobic π-charge interaction with Gly192 and strong aromatic interaction with His57 (Figure 9A and 9B). Overall binding interactions and docking investigations have verified that ligand molecules bind perfectly in the target protein DHFR's active domain. Through DHFR inhibition, the docking interaction results may aid in the further development of novel antimicrobial drugs.

Structure activity correlation (SAR)

Three separate sets of compounds were created, including 2-Phenyl-3-substituted quinazolin-4(3H)-one (Scheme 1), 3-(Benzylidene-amino)-2-phenyl-3H-quinazolin-4-one (Scheme 2), and (1-phenyl-ethylideneamino)-3H-quinazolin-4-one (Scheme 3). The 2-phenyl-quinazoline-4(3H)-one moiety, which appears to be the phenyl moiety at position-2 of the quinazoline nucleus, is the core moiety in the structural skeleton of every molecule that has been synthesized. Other substituents to the nucleus' position-3N-atom can also change the bioactivity. The benzylidene and ethylidene amino- moieties have substituents at the phenyl rings that have an electron-donating and an electron-withdrawing character such as -OH, $-OCH_3$, $-NH_2$,-NO2, -COOH, $-CONH_2$, $-CSNH_2$, $-CONH-NH_2$, $-CS-NH-NH_2$, $-SO_2NH_2$, and $-SO_2NH-COCH_3$. These substituents have brought the compounds towards the DHFR inhibitory activity side with IC_{50} range of 4-24µM.

CONCLUSION

The three sets of compounds, Scheme 1 (QIa-n), Scheme-2 (QIIa-h), and Scheme-3 (QIIIa-g), which are all based on the same scaffold as quinazolin-4(3H)-one, were synthesized. Changing the kind of the substituent at position-3 of the scaffold appeared to change the profile of biological activity, although the phenyl group substituent at position-2 of the nucleus appeared to remain common. The critical amino acids identified by molecular modeling studies are Gly219, Gly192, Gly193, and His57a. These residues are crucial for both actual binding to the target protein's active site and additional DHFR inhibition. A docking research revealed that the ligands QII-f and QIII-g perfectly matched the protein's binding domain's active amino acids.

When compared to the conventional medication trimethoprim, which has an IC_{50} of 10µM, the test compounds' found IC_{50} of 4-24µM indicated a considerable inhibition of the target protein by DHFR. Strongly active chemicals according to an inhibition investigation were QI-g (IC_{50}, 8µM), QI-j (IC_{50}, 6µM), QI-l (IC_{50}, 4µM), and QI-m (IC_{50}, 5µM). The test compounds with a MIC range of 0.1-3.0µg/mL shown considerable antimicrobial activity against pathogenic bacterial and fungal strains when compared to the corresponding standards, gentamycin and ciprofloxacin (antibacterial), and clotrimazole (antifungal). Compounds from the current study that make up the skeleton shown in Figure 2 may serve as helpful models for the future development of effective antimicrobial drugs that cause infectious diseases and produce DHFR inhibition.

ACKNOWLEDGEMENT

All the contributing authors are grateful to the Principal, Bharati Vidyapeeth's College of Pharmacy, Kolhapur for providing a necessary facility for the successful completion of this research work.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

REFERENCES

1. Samira I, Patel S, Hasmin M, Patel S. Biological profile of quinazoline. Int J Pharm Chem Sci. 2012;1:1863-72.
2. Raghavendra NM, Thampi P, Gurubasavarajaswamy PM, Sriram D. Synthesis and antimicrobial activities of some novel substituted 2-imidazolyl-N-(4-oxo-quinazolin-3(4H)-yl)-acetamides. Chem Pharm Bull (Tokyo). 2007;55(11):1615-19. doi: 10.1248/cpb.55.1615, PMID 17978522.
3. Al-Omary FA, Abou-Zeid LA, Nagi MN, Habib el-SE, Abdel-Aziz AA, El-Azab AS, *et al*. Non-classical antifolates. Part 2: Synthesis, biological evaluation, and molecular modeling study of some new 2,6-substituted-quinazolin-4-ones. Bioorg Med Chem. 2010;18(8):2849-63. doi: 10.1016/j.bmc.2010.03.019, PMID 20350811.
4. Khalil AA, Abdel-Hamide SG, Al-Obaid AM, El-Subbagh HI. Substituted quinazolines, Part 2. Synthesis and *in-vitro* anticancer evaluation of new 2-substituted mercapto-3H-quinazoline analogs. Arch Pharm (Weinheim). 2003;336(2):95-103. doi: 10.1002/ardp.200390011, PMID 12761762.

5. Hricovíniová J, Hricovíniova Z, Kozics K. Antioxidant, cytotoxic, genotoxic, and DNA-protective potential of 2,3-substituted quinazolinones: Structure-activity relationship study. Int J Mol Sci. 2021;22(2):610-5. doi: 10.3390/ijms22020610, PMID 33435390.
6. Gangjee A, Jain HD, Kurup S. Recent advances in classical and non-classical antifolates as antitumor and antiopportunistic infection agents: Part II. Anticancer Agents Med Chem. 2008;8(2):205-31. doi: 10.2174/187152008783497064, PMID 18288923.
7. Dinakaran M, Selvam P, DeClercq E, Sridhar SK. Synthesis, antiviral and cytotoxic activity of 6-bromo-2,3-disubstituted-4(3H)-quinazolinones. Biol Pharm Bull. 2003;26(9):1278-82. doi: 10.1248/bpb.26.1278, PMID 12951471.
8. Nerkar AG, Saxena AK, Ghone SA, Thaker AK. *In silico* screening, synthesis and *in-vitro* evaluation of some quinazolinone and pyridine derivatives as dihydrofolate reductase inhibitors for anticancer activity. E-journal of chemistry. 2009;6;Suppl 1:S97-S102.
9. Singla P, Luxami V, Paul K. Quinazolinone-benzimidazole conjugates: Synthesis, characterization, dihydrofolate reductase inhibition, DNA and protein binding properties. J Photochem Photobiol B Biol. 2017;168:156-64. doi: 10.1016/j.jphotobiol.2017.02.009.
10. Dohle W, Jourdan FL, Menchon G, Prota AE, Foster PA, Mannion P, *et al*. Quinazolinone-Based Anticancer Agents: Synthesis, Antiproliferative SAR, Antitubulin Activity, and Tubulin Co-crystal Structure. J Med Chem. 2018;61(3):1031-44. doi: 10.1021/acs.jmedchem.7b01474, PMID 29227648.
11. Farghaly AM, Mohsen A, Omar ME, Khalil MA, Gaber MA. Synthesis of 3-aryl-2-substituted-4(3H)-quinazolines as potential antimicrobial agents. Farmaco. 1990;45(4):431-8. PMID 2119178.
12. Khalil MA, Habib NS. Synthesis of thiadiazol derivatives of 4(3H)-quinazolinone as potential antimicrobial agents. Farmaco Sci. 1987;42(12):973-78. PMID 3449395.
13. Jafari E, Khajouei MR, Hassanzadeh F, Hakimelahi GH, Khodarahmi GA. Quinazolinone and quinazoline derivatives: Recent structures with potent antimicrobial and cytotoxic activities. Res Pharm Sci. 2016;11(1):1-14. PMID 27051427.
14. Khan I, Ibrar A, Abbas N, Saeed A. Recent advances in the structural library of functionalized quinazoline and quinazolinone scaffolds: Synthetic approaches and multifarious applications. Eur J Med Chem. 2014;76:193-244. doi: 10.1016/j.ejmech.2014.02.005, PMID 24583357.
15. Gatadi S, Lakshmi TV, Nanduri S. 4(3H)-Quinazolinone derivatives: Promising antibacterial drug leads. Eur J Med Chem. 2019;170:157-72. doi: 10.1016/j.ejmech.2019.03.018, PMID 30884322.
16. Nanda AK, Ganguli S, Chakraborty R. Antibacterial activity of some 3-(Arylideneamino)-2-phenyl quinazoline-4 (3H)-ones: Synthesis and preliminary QSAR studies. Molecules. 2007;12(10):2413-26. doi: 10.3390/12102413, PMID 17978766.
17. Rakesh KP, Kumara HK, Ullas BJ, Shivakumara J, Channe Gowda D. Amino acids conjugated quinazolinone-Schiff's bases as potential antimicrobial agents: Synthesis, SAR and molecular docking studies. Bioorg Chem. 2019;90:103093. doi: 10.1016/j.bioorg.2019.103093, PMID 31288137.
18. Al-OmaryFatmah AM, Abou-zeid LA, Nagi MN, Habib EE, Abdel-AzizAlaa AM, El-Azab AS, *et al*. Non-classical antifolates. Part 2: Synthesis, biological evaluation, and molecular modeling study of some new 2,6-substituted-quinazolin-4-ones. Bioorganic and Medicinal Chemistry. 2010;18(8):2849-63.
19. Al-Rashood ST, Hassan GS, El-Messery SM, Nagi MN, Habib EE, Al-Omary FAM, *et al*. Synthesis, biological evaluation and molecular modeling study of 2-(1,3,4-thiadiazolyl-thio and 4-methyl-thiazolyl-thio)-quinazolin-4-ones as a new class of DHFR inhibitors. Bioorg Med Chem Lett. 2014;24(18):4557-67. doi: 10.1016/j.bmcl.2014.07.070, PMID 25139568.
20. Sharma PK. A review: Different approach of bioactive pyrimido-benzothiazole synthesis. Drug Invent Today. 2017;9(3):18-22.
21. Grasso S, Micale N, Monforte AM, Monforte P, Polimeni S, Zappalà M. Synthesis and *in vitro* antitumour activity evaluation of 1-aryl-1H,3H-thiazolo[4,3-b]quinazolines. Eur J Med Chem. 2000;35(12):1115-19. doi: 10.1016/s0223-5234(00)01195-8, PMID 11248410.
22. Sharma PK, Fogla A, Rathore BS, Kumar M. Synthesis and antimicrobial activity of structurally flexible heterocycles with the 1,4-thiazine heterosystem. Res Chem Intermed. 2011;37(8):1103-11. doi: 10.1007/s11164-011-0320-0.
23. Kung PP, Casper MD, Cook KL, Wilson-Lingardo L, Risen LM, Vickers TA, *et al*. Structure-activity relationships of novel 2-substituted quinazoline antibacterial agents. J Med Chem. 1999;42(22):4705-13. doi: 10.1021/jm9903500, PMID 10579832.
24. Beena KP, Akelesh T. Synthesis and antibacterial activity of quinazolinone derivatives. Int J Pharm Pharm Sci. 2010;2(3):166-8.
25. Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Wood. In: GL Washington, editor JA: Manual of clinical microbiology. Washington, DC: American Society for Microbiology; 1995.
26. Bahar A, Abdul S, Mohsin H. Molecular modeling studies, synthesis and antimicrobial screening of some novel sulphonamide quinazolin-4(3H)-one fused derivatives. Int J Pharm Pharm Sci. 2014;6(4):312-17.
27. Huang S, Feng K, Ren Y. Molecular modelling studies of quinazolinone derivatives as MMP-13 inhibitors by QSAR, molecular docking and molecular dynamics simulations techniques. Chem Commun. 2019;10(1):101-15. doi: 10.1039/C8MD00375K.
28. Auti PS, George G, Paul AT. Recent advances in the pharmacological diversification of quinazoline/quinazolinone hybrids. RSC Adv. 2020;10(68):41353-92. doi: 10.1039/d0ra06642g, PMID 35516563.
29. Algul O, Paulsen JL, Anderson AC. 2,4-Diamino-5-(2'-arylpropargyl)pyrimidine derivatives as new nonclassical antifolates for human dihydrofolate reductase inhibition. J Mol Graph Model. 2011;29(5):608-13. doi: 10.1016/j.jmgm.2010.11.004, PMID 21146434.
30. Sharma S, Sharma K, Pathak S, Kumar M, Sharma PK. Synthesis of medicinally important quinazolines and their derivatives: A review. Open Med Chem J. 2020;14(1):108-21. doi: 10.2174/1874104502014010108.

Cite this article: Harer S, Bhatia M, Sonar P. Evaluation of DHFR Inhibition and Antimicrobial Activity of Some Newly Synthesized Quinazolin-4(3H)-one Scaffold Coupled with Benzylidene and Ethylidene Amino Motifs. Int. J. Pharm. Investigation. 2023;13(1):62-73.