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Cyclization of Chalcone Derivatives: Design, Synthesis, *In Silico* Docking Study, and Biological Evaluation of New Quinazolin-2,4-diones Incorporating Five-, Six-, and Seven-Membered Ring Moieties as Potent Antibacterial Inhibitors

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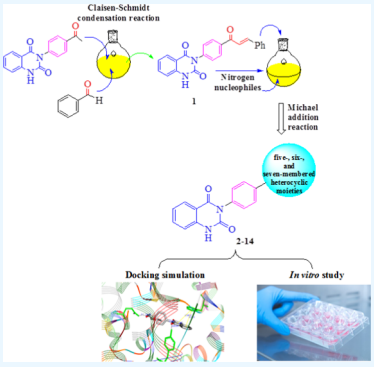
ABSTRACT: Four novel series of quinazolin-2,4-diones bearing five-, six-, and seven-membered heterocyclic moieties **2−14** (such as pyrazole, oxazole, pyrimidine, and azepines) through the 1,4-phenyl linkage were designed, synthesized, and evaluated in terms of their antibacterial activities. Analytical and spectral techniques (FT-IR, ^{1}H NMR, ^{13}C NMR, and Mass) were utilized for the structural elucidation of all of the synthesized compounds **2−14**. Furthermore, the potential antibacterial activity of the thirteen compounds was further evaluated *in vitro* against two different Gram-negative G^{-ve} bacterial strains (named *Escherichia coli* ATCC 25955, *Pseudomonas aeruginosa* ATCC 10145) and two Gram-positive G^{+ve} bacterial strains (named *Bacillus subtilis* ATCC 6633 and *Staphylococcus aureus* NRRL B-767). Investigation of the antibacterial potential indicated that the newly synthesized compounds, especially **13**, exhibited remarkable antibacterial activity against pathogens, comparable to the standard drug ciprofloxacin (a known potent antibacterial agent). Additionally, compounds **2−14** and ciprofloxacin were assessed *in silico* using molecular docking studies against the target thymidine phosphorylase enzyme (PDB ID: 4EAD). Moreover, the structure activity relationship (SAR) for these compounds was also described to give guidance about the effective molecules that could play an important role in identifying potential antibacterial agents. Finally, the drug-likeness and physicochemical parameters of the newly synthesized molecules **2−14** were *in silico* investigated. Among them, we found that the compound 3-[4-(6-phenyl-6,7-dihydro-5-oxa-9-aza-benzocyclohepten-8-yl)-phenyl]-1*H*-quinazolin-2,4-dione **13** with the highest binding affinity showed a strong fit to the active site of the tested enzyme, indicating **13** as a promising drug candidate for designing and developing novel classes of antibiotics.



1. INTRODUCTION

Despite the progress in the development of drugs to treat infectious diseases, antimicrobial resistance (AMR) remains a significant challenge in this century.[1]

Quinazolin-2,4-diones are a significant class of nitrogen heterocyclic cores that exhibit a broad spectrum of biological activities such as anticancer, anti-inflammatory,[2] anticholera,[3] antimalaria,[4] and antibacterial.[5]

Chalcone is a privileged synthon for the preparation of various heterocyclic rings.[6] In addition, chalcone derivatives showed promising biological activities, including antiparasitic, anticancer, and antioxidant.[7−11]

Furthermore, five-, six-, and seven-membered heterocyclic moieties with one or two nitrogen atoms, such as pyrazole, oxazole, pyrimidine, and azepine, have been reported as very important skeletons in medicinal chemistry that have diverse biological and pharmaceutical activities such as antibacterial, anti-inflammatory, antidepressant, and antioxidant.[12−16]

The molecular hybridization strategy is a widely used tool in drug discovery.[17−19] The hybridization of the quinazolinedione scaffold with various bioactive nitrogen moieties has recently been reported to show a different mechanism of action with a broad spectrum of biological and pharmaceutical activities.[20]

On the other hand, four Gram-positive and -negative pathogens, namely *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Staphylococcus aureus*, were found to be responsible for a variety of infectious diseases and to have the ability to resist the exciting antibiotics.

Herein, the structure activity relationship (SAR) study showed that the quinazolinedione moiety is crucial for antimicrobial efficacy, as presented in Figure 1[5] Further, five-, six-, and seven-heterocyclic nitrogen scaffolds such as pyrazole, oxazole, pyrimidine, and azepine enhanced the antibacterial efficacy.[15,21,22] In addition, the hydrophobic domain in an organic molecule may increase its antibacterial

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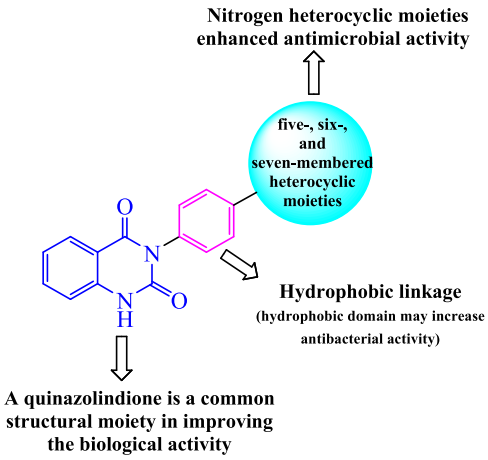


Figure 1. Design of the target compounds **2**−**14**.

activity.[23] Overall, the newly synthesized thirteen quinazolin-2,4-diones linked to various N-heterocyclic rings through the hydrophobic linkage are unique templates for the design of antibacterial inhibitors.

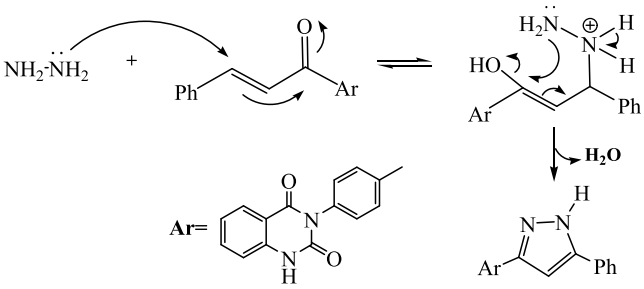
In light of the above information and as a part of the ongoing effort to synthesize highly selective compounds with efficient biological activities, the present study was carried out in an attempt to synthesize a new series of quinazolin-2,4-dione derivatives attached to five-, six-, and seven-membered heterocyclic moieties such as pyrazole, oxazole, pyrimidine, and azepine through the 1,4-phenyl linkage **2**−**14** by cyclization of chalcone **1** with various nitrogen nucleophiles. Furthermore, the 13 ligand molecules were docked to the binding site of thymidine phosphorylase enzyme, and the suitable interactions were represented based on their bond lengths and conformational energies. Finally, *in silico* ADMET predictions for the prepared quinazolin-2,4-diones were investigated with the objective of gaining an insight into their pharmacokinetic, safety, and drug-likeness profile.

2. RESULTS AND DISCUSSION

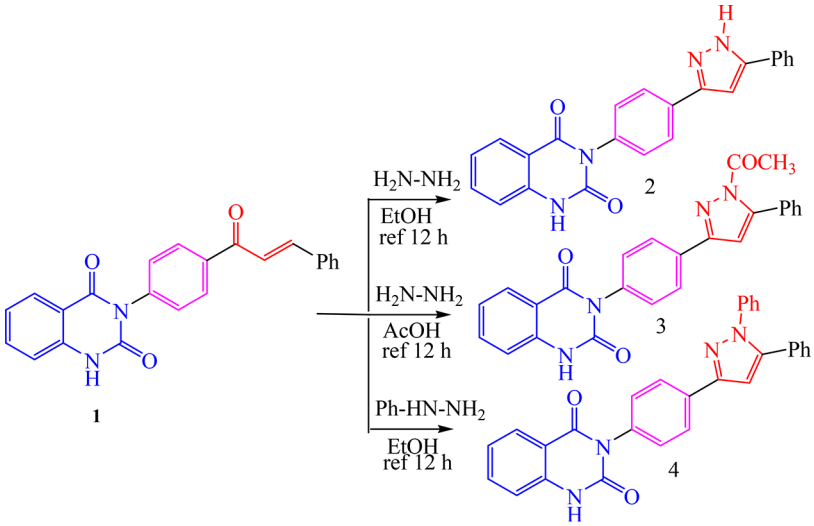
2.1. Chemistry. The objective of our ongoing study was to synthesize a new class of quinazolinedione analogues linked to five-, six-, and seven-membered heterocyclic moieties. The general procedure for the synthesis of key molecule chalcone **1** was previously described by Abdelmonsef et al.,[24] by treatment of 3-(4-acetyl-phenyl)-1*H*-quinazolin-2,4-dione with benzaldehyde and a catalytic amount of base NaOH through Claisen−Schmidt condensation reaction. This compound **1** is used as a good intermediate for the facile synthesis of the target compounds with heterocyclic moieties **2**−**14**. In the present study, four novel classes of quinazolinediones attached to five-, six-, and seven-membered heterocyclic moieties **2**−**14** were synthesized by treatment of 3-[4-(3-phenyl-acryloyl)-phenyl]-1*H*-quinazolin-2,4-dione **1** with different nitrogen nucleophilic reagents including hydrazine, phenyl hydrazine, hydroxylamine, thiosemicarbazide, semicarbazide HCl, amino guanidine HCl, urea, thiourea, guanidine HCl, *o*-phenylenediamine, *o*-aminophenol, and *o*-aminothio-phenol through the ring-closing reaction, as represented in Schemes 1−8.

As shown in Scheme 1, the reaction of the key starting chalcone **1** *via* cyclo-condensation reaction with a series of hydrazine derivatives (hydrazine hydrate and phenyl hydrazine) yielded three new pyrazoline derivatives, **2**−**4**. The hydrazinolysis of compound **1** in ethanol under reflux afforded the compound **2**. On the other hand, the addition of acetic acid furnished the expected pyrazoline **3**. The reaction of chalcone **1** with phenyl hydrazine under reflux yielded the pyrazoline **4**. Scheme 2 represents the proposed mechanism for

Scheme 2. Suggested Mechanism for Preparation of the Pyrazole Moiety Attached to the Quinazolin-2,4-dione Skeleton



Scheme 1. Formation of Pyrazoline Derivatives 2−4



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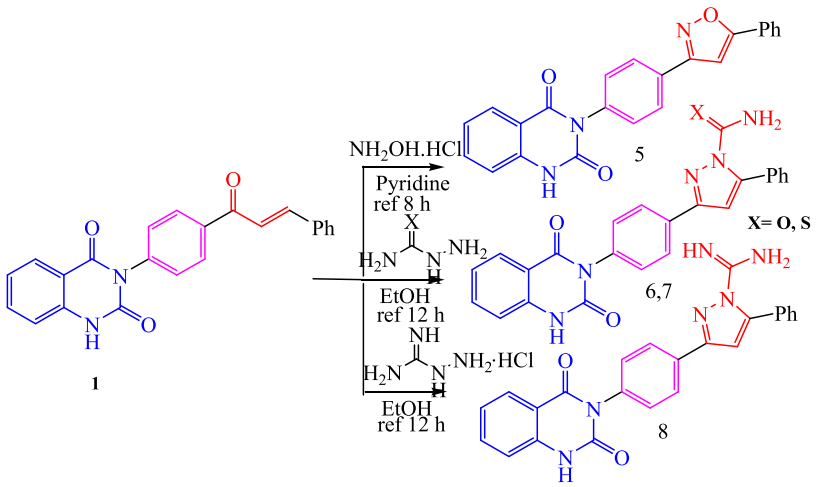
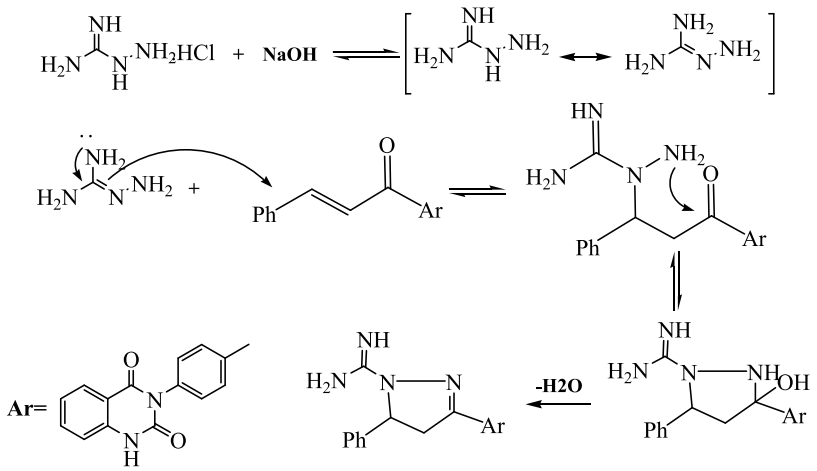
Scheme 3. Formation of Various Five-Membered Rings of Oxazole and Pyrazole Attached to the Quinazolinedione Moiety 5−8



Scheme 4. Formation Mechanism of Pyrazole-1-carboxamidine Attached to the Quinazolin-2,4-dione Skeleton



the synthesis of the pyrazole ring. The spectral data and elemental analysis were utilized for identification of the chemical structures of the compounds. The Fourier transform infrared (FT-IR) spectral data showed the disappearance of the characteristic peak for the conjugated carbonyl group at 1680 cm^{-1}, along with the C═N stretching band at 1604 cm^{-1}, C═C stretching band at 1549 cm^{-1}, and the appearance of an NH stretching band at 3225 cm^{-1}. Considering the ^1H NMR spectra of the new pyrazoline derivatives **2**−**4**, NH protons of quinazolinedione moieties were observed as singlet signals in the δ range 11.2−11.3 ppm. In addition, the aromatic protons were observed at δ 7.21−7.96 ppm. On the other hand, the methyl protons of derivative **2** were found at δ 1.85 ppm as a singlet peak. The mass spectrum of **2** represented a molecular ion peak [M^+] at m/z 380, which is in agreement with its molecular formula $C_{23}H_{16}N_4O_2$.

Treatment of chalcone **1** with hydroxylamine in boiling pyridine gave the corresponding oxazole derivative **5**. In addition, the route to the pyrazole skeleton attached to quinazolin-2,4-dione moiety **6**−**8** is *via* reaction of chalcone **1** with semicarbazide HCl, thiosemicarbazide, and/or amino guanidine HCl under the condition of a basic catalyst NaOH, as represented in Scheme 3. The formation mechanism of the pyrazole-1-carboxamidine attached to quinazolin-2,4-dione skeleton **8**, is represented in Scheme 4.

The newly synthesized compounds **6**−**8** were structurally elucidated by their spectral analyses. The FT-IR spectra of these compounds exhibited characteristic absorption bands at 3433−3400 and 3278−3200 for NH_2 and NH, and 1717−1667 cm^{-1} for C═O groups.

^1H NMR of **7**, for example, showed the presence of a singlet signal for the $-NH_2$ group at δ 10.3 ppm, in addition to a singlet signal at 8.2 ppm related to the olefinic ═CH proton, aromatic protons as multiplet signals at δ 7.06−7.95 ppm, and a singlet signal at δ 11.4 ppm assigned to the NH of quinazolinedione moiety. The mass spectrum of **7** represented [M^+] at m/z 439, which is in agreement with its molecular formula $C_{24}H_{17}N_5O_2S$.

On the other hand, the formation of pyrimidine moieties bound to the quinazolinedione skeleton **9**−**11** was carried out by cyclization of chalcone **1** with urea and its analogues thiourea and guanidine in the presence of ethanol and a catalytic amount of NaOH, as depicted in Scheme 5. The structures of the newly synthesized molecules **9**−**11** were elucidated based on spectral analyses. The stretching frequency of compound **9**, for example, was observed at 3500, 3282, 1668, and 1585 cm^{-1} for O−H, N−H, C═O, and C═C,

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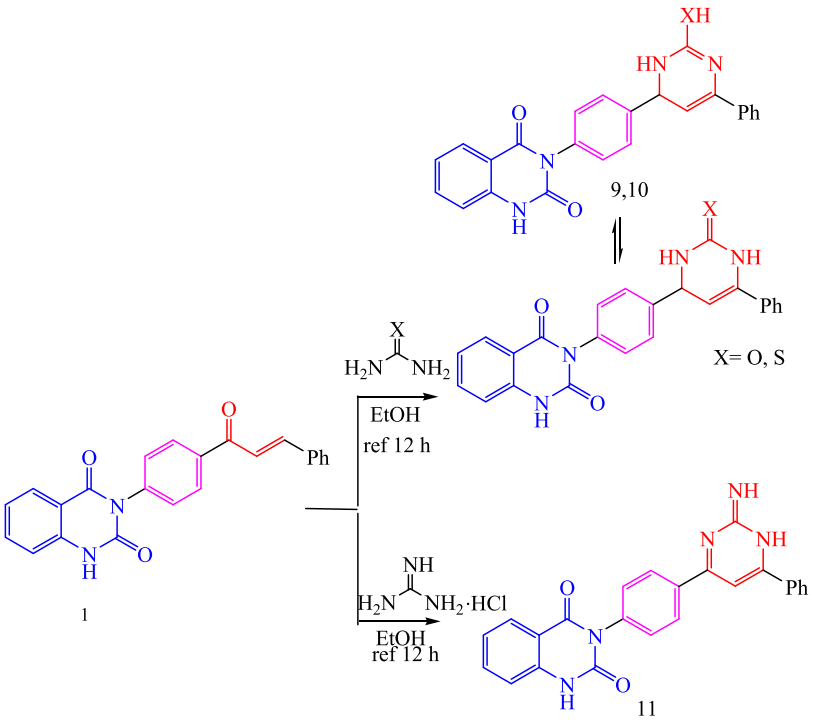
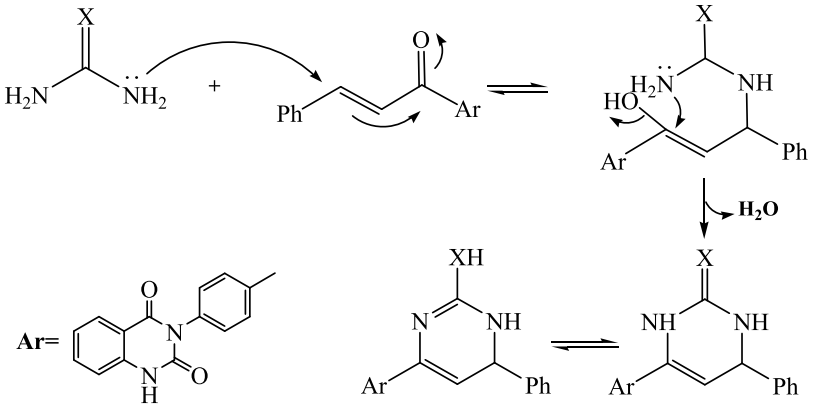
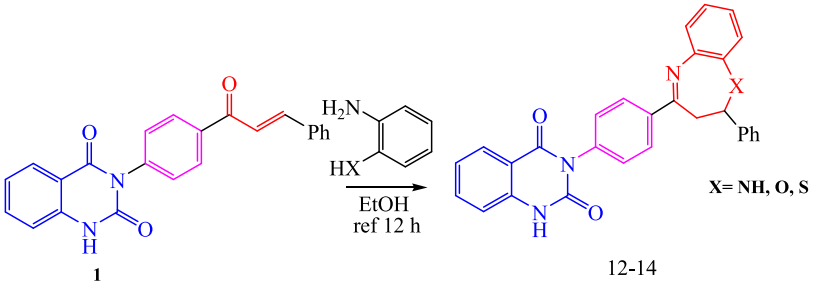
Scheme 5. Formation of Six-Membered Rings Attached to the Quinazolinedione Moiety 9−11



Scheme 6. Proposed Mechanism for the Synthesis of Compounds 9−11



Scheme 7. Formation of Seven-Membered Rings Attached to the Quinazolinedione Moiety 12−14



respectively. Its ^{1}H NMR spectrum showed chemical shifts as a singlet peak at δ 6.57 ppm for −CH−Ph of the pyrimidine ring, multiplet peaks at δ 7.08−7.97 ppm assigned to the aromatic protons, and a singlet peak for ═CH of the pyrimidine ring at δ 8.38 ppm, in addition to three singlet peaks at δ 10.13, 10.39, and 11.53 ppm assigned to the OH and 2 NH groups of pyrimidine and quinazolinedione moieties. The mass spectrum of **9** exhibited [M$^+$] at m/z 410, which is consistent with its chemical formula $C_{24}H_{18}N_4O_3$. The reactions proceeded according to the plausible mechanism represented in Scheme 6.

Finally, Michael addition reaction of chalcone **1** with *o*-phenylenediamine, *o*-aminophenol, and/or *o*-aminothiophenol in ethanol catalyzed by NaOH gave benzodiazepine,

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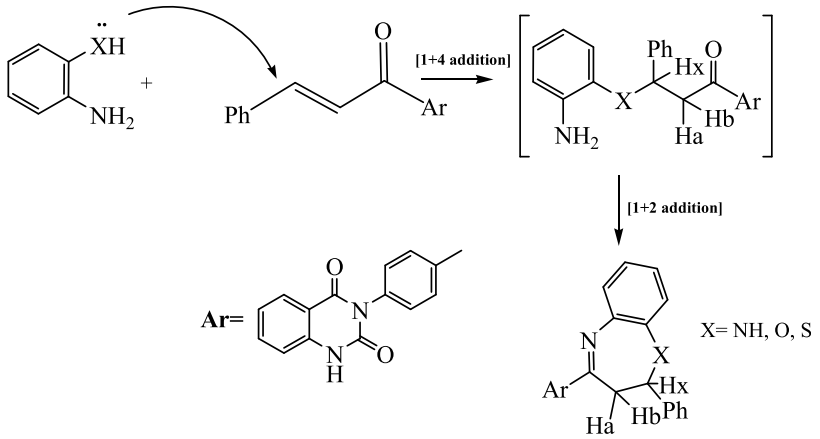
Scheme 8. Suggested Mechanism for the Synthesis of Compounds 12−14



benzoxazepine, and/or benzothiazepine, respectively, as presented in Scheme 7. The addition was carried out using an XH group (NH, O, and/or S) to the enone functional group [1, 4 addition] of chalcone to form intermediates, followed by cyclization [1, 2 addition] to yield azepines **12−14**, as shown in Scheme 8.

The chemical structures of the prepared azepines **12−14** were mainly confirmed on the basis of spectroscopic methods, where their IR spectra revealed the presence of a band characteristic of a CH_2-aliphatic compound at 3002 cm^{-1}, and a C═N stretching peak at 1604 cm^{-1}. Their ^1H NMR spectra showed the appearance of two doublet of doublet signals characteristic of CHa and CHb at δ 3.83 and δ 4.34 ppm, in addition to the triplet signal at δ 4.86 ppm related to CHx. The mass spectra of compounds **12−14** revealed [M^+] at m/z 458, 459, and 475, which are in agreement with the suggested molecular formulas $C_{29}H_{22}N_4O_2$, $C_{29}H_{21}N_3O_3$, and $C_{29}H_{21}N_3O_2S$, respectively. The spectral analyses of all compounds are included in the Supplementary Data File, as Figures S1−S38.

2.2. *In Vitro* Biological Screening. In our investigation, the antibacterial efficacy of a new class of N-heterocyclic molecules **2−14** against various pathogenic microbes was investigated. The minimum inhibitory concentration (MIC) of the tested newly synthesized compounds **2−14** was also calculated, as shown in Table 1.

MIC is the average of the lowest concentrations that can inhibit pathogenic microbial growth. The obtained results revealed that most of the tested ligand molecules (at low concentrations) can inhibit the microbial growth of the selected microbes. For instance, compounds **2** (1*H*-pyrazole derivative), **6** (1*H*-pyrazole-1-carboxamide derivative), and **8** (1*H*-pyrazole-1-carboximidamide) exhibited a strong inhibition potency against the tested microbes at low concentrations ranging from 10 to 20 μg/mL. Meanwhile, compounds **4**, **5**, and **9** showed significant antimicrobial effect at low concentrations against the two tested G^{-ve} bacterial strains. The results also demonstrated that compound **13** with benzoxazepine moiety showed excellent performance against all of the tested microbes at low concentrations ranging from 2.5 to 10 μg/mL. In addition, compound **13** exhibited a strong effect against the G^{-ve} bacteria (*E. coli*) at a very low concentration, 2.5 μg/mL lower than that of the standard drug ciprofloxacin. The initial observations in the structure activity relationship (SAR) study showed that the antibacterial activity

Table 1. Antimicrobial Efficacy and MIC of Compounds 2−14 and Ciprofloxacin[a]

	minimum inhibitory concentration (MIC, μg/mL)			
sample no.	*E. coli*	*P. aeruginosa*	*B. subtilis*	*S. aureus*
2	20	20	10	15
3	80	40	160	40
4	20	40	16	ND
5	40	60	120	40
6	10	20	20	ND
7	40	ND	ND	120
8	20	12	20	20
9	20	40	ND	ND
10	ND	160	ND	ND
11	120	40	80	80
12	ND	40	ND	ND
13	2.5	10	5	7
14	10	40	80	120
ciprofloxacin	5	7	2.5	1.25

[a]ND: not determined.

exhibited an essential effect on changing the moieties on the quinazolin-2,4-dione ring. In the SAR study of introducing the benzoxazepine moiety attached to the quinazolin-2,4-dione skeleton through hydrophobic linkage in the title compound **13**, it was found that the antibacterial activity increased against both G^{+ve} and G^{-ve} bacterial strains at low concentrations. The observed antibacterial activity of compound **13** is corroborated by the strong binding nature that was observed by *in silico* molecular docking studies against the target.

2.3. Molecular Docking Study. To investigate the possible mode of actions and druggability of the newly molecules, molecular docking and drug-likeness properties were studied. Thymidine phosphorylase (TP) is found in a wide range of organisms, including bacteria. Literature has revealed that abnormal levels of TP cause various bacterial diseases.[25] Herein, thymidine phosphorylase is chosen as a promising target for performing the docking study. The crystal structure of the enzyme was downloaded from the RCSB Protein Data Bank web server (PDB ID: 4EAD). All of the prepared compounds and standard drug were docked into the binding site of the enzyme using PyRx software. In the present study, enzyme-docking examination was performed on a potential target enzyme for bacterial diseases both to support the *in vitro* findings of the compounds and to explore their

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Table 2. Binding Energies (in kcal/mol) of Ligand Molecules 2−14 and the Standard Drug against the Target Enzyme

entry	binding energy (kcal/mol)	docked complex (amino acid−ligand) interactions	distance (Å)
2	−10.3	H-bonds	
		THR87:O–compound **2**	2.24
		arene−cation	
		PHE24–compound **2**	4.67
3	−10.2	H-bonds	
		TYR168:OH–compound **3**	2.43
		arene−cation	
		LYS190:NZ–compound **3**	4.26
		ARG171:NH1–compound **3**	5.44
		ARG171:NH1–compound **3**	5.28
		arene−cation	
		PHE210–compound **3**	3.60
4	−10.3	arene−cation	
		LYS190:NZ–compound **4**	4.28
		ARG171:NH1–compound **4**	5.46
		ARG171:NH1–compound **4**	5.70
		ARG171:NH2–compound **4**	5.19
		LYS165:NZ–compound **4**	5.76
		arene−cation	
		PHE210–compound **4**	3.83
5	−10.1	H-bonds	
		GLN156:NE2–compound **5**	2.40
		SER186:OG–compound **5**	2.25
		arene−arene	
		TYR168–compound **5**	3.92
		TYR168–compound **5**	4.32
		arene−cation	
		ARG115:NH2–compound **5**	5.91
		ARG171:NH1–compound **5**	5.26
6	−10.0	H-bonds	
		TYR168:OH–compound **6**	2.17
		arene−cation	
		LYS190:NZ–compound **6**	4.24
		ARG171:NH1–compound **6**	5.44
		ARG171:NH1–compound **6**	5.06
		arene−sigma	
		PHE210–compound **6**	3.60
7	−9.4	H-bonds	
		TYR168:OH–compound **7**	2.47
		arene−cation	
		TYR170:OH–compound **7**	4.33
		GLY193:N–compound **7**	4.44
		GLN196:NE2–compound **7**	5.94
		arene−sigma	
		PHE210–compound **7**	3.66
8	−10.4	H-bonds	
		GLN156:NE2–compound **8**	2.80
		THR87:O–compound **8**	2.43
		arene−arene	
		TYR168–compound **8**	4.11
		arene−cation	
		LYS165:NZ–compound **8**	5.72
		LYS190:NZ–compound **8**	5.13
		LYS165:NZ–compound **8**	5.80
		ARG171:NH1–compound **8**	5.39
9	−9.9	H-bonds	
		GLN156:NE2–compound **9**	2.39
		HIS85:NE2–compound **9**	2.16
		arene−arene	
		TYR168–compound **9**	4.16
		arene−cation	
		LYS165:NZ–compound **9**	5.75
		LYS165:NZ–compound **9**	5.11
		ARG171:NH1–compound **9**	5.79
		ARG171:NH1–compound **9**	5.23
		ARG171:NH1–compound **9**	5.26
		ARG171:NH2–compound **9**	5.30
		arene−sigma	
		PHE210–compound **9**	3.41
		LYS165:CG–compound **9**	3.88
10	−10.0	arene−cation	
		LYS190:NZ–compound **10**	4.05
		ARG171:NH1–compound **10**	5.35
		ARG171:NH1–compound **10**	5.05
		ARG171:NH2–compound **10**	5.65
		LYS165:NZ–compound **10**	5.81
		LYS165:NZ–compound **10**	5.70
11	−10.3	H-bonds	
		TYR168:OH–compound **11**	2.36
		arene−cation	
		LYS190:NZ–compound **11**	4.00
		ARG171:NH1–compound **11**	5.38
		ARG171:NH1–compound **11**	5.62
		ARG171:NH2–compound **11**	6.00
		LYS165:NZ–compound **11**	5.73
		arene−sigma	
		PHE210–compound **11**	3.67
12	−11.1	H-bonds	
		TYR168:OH–compound **12**	2.33
		arene−cation	
		LYS190:NZ–compound **12**	4.22
		ARG171:NH1–compound **12**	5.45
		ARG171:NH1–compound **12**	5.10
		ARG171:NH2–compound **12**	5.26
		LYS165:NZ–compound **12**	5.84
		arene−sigma	
		PHE210–compound **12**	3.67
13	−11.5	H-bonds	
		TYR168:OH–compound **13**	2.28
		arene−cation	
		LYS190:NZ–compound **13**	4.22
		ARG171:NH1–compound **13**	5.42
		ARG171:NH1–compound **13**	5.04
		LYS165:NZ –compound **13**	5.96
		LYS165:NZ–compound **13**	5.02
		arene−sigma	
		PHE210–compound **13**	3.63
14	−10.8	arene−cation	
		LYS190:NZ–compound **14**	4.42

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Table 2. continued

entry	binding energy (kcal/mol)	docked complex (amino acid−ligand) interactions	distance (Å)
		ARG171:NH1–compound **14**	5.63
		ARG171:NH1–compound **14**	5.32
		PHE210–compound **14**	5.96
		arene−sigma	
		PHE210–compound **14**	3.91
ciprofloxacin	−8.3	H-bonds	
		ARG171:NH1–**ciprofloxacin**	2.27
		ARG171:NH2–**ciprofloxacin**	2.34
		LYS190:NZ–**ciprofloxacin**	2.21
		GLY121:O–**ciprofloxacin**	1.82

binding affinities to the target enzyme. The docking studies exhibited that all of the prepared ligand molecules energetically favored the enzyme. They revealed good to excellent binding energies against the target. The binding affinities of the docked molecules to the target protein are represented in Table 2. The *in silico* docking study disclosed that the molecules nicely docked with the active site pockets of the target enzyme through various interactions as shown in Figure 2. Most of the ligand molecules and the standard drug docked to the binding site of the enzyme represented in the residues Lys165, Tyr168, Arg171, Lys190, and Phe210 through H-bonds, arene−arene, arene−cation, and arene−sigma interactions. The docking study showed that the oxo of quinazolinedione moiety and NH groups are responsible for HB interactions, while the phenyl and pyrimidinone moieties are responsible for arene−arene, arene−cation, and arene−sigma interactions. The compound **13**, the potent *in vitro* antibacterial derivative, exhibited the highest binding affinity (−11.5 kcal/mol). The mode of binding represented that it binds to the enzyme *via* seven noncovalent interactions such as H-bond and π-stacking with the residues Tyr168, Arg171, Lys165, and Phe210 at distances of 2.28, 4.22, 5.42, 5.04, 5.96, and 3.63 Å, respectively.

In conclusion, the molecular docking approach exhibited that the quinazolinedione moiety, hydrophobic fragment, and N-heterocyclic rings can act as hydrogen bond donors/acceptors to generate hydrogen bond interactions as represented above. On the other hand, the drug-likeness and ADMET analyses of the target molecules were investigated by ADMETlab and SwissADME web tools, and it was found that all of the synthesized molecules comply well with Lipinski's rule with zero violation. All molecules showed a drug-like nature, nontoxicity, and >80% absorption, indicating that they are appropriate for oral administration. All compounds have high gastrointestinal absorption GI. In addition, the compounds have good bioavailability scores as all have TPSA ≤ 140 Å, indicating that they can easily penetrate the cell membranes. The target molecules cannot pass BBB, which indicates their good CNS safety profile, as shown in Table 3. The compounds have average solubility in water; therefore they can be absorbed in the body. Finally, the physicochemical parameters and bioavailability of compound **13** were calculated using ADMETlab tool (Figure 3). In summary, the synthesized molecules have appreciable drug-likeness properties and should be considered for further investigation in the search for antibacterial agents.

3. EXPERIMENTAL SECTION

3.1. Chemistry. All chemicals and solvents were obtained from Aldrich Chemical Co. and Merck and used without purification. The melting points of all molecules were determined in capillary tubes using MEL-TEMP II and were uncorrected. The completion of the reactions was monitored by thin-layer chromatography (TLC) using benzene/EtOH (3:1) as the mobile phase, and the spots were visualized by irradiation with UV light (254 nm). FT-IR, NMR, and MS spectra were recorded using Shimadzu 408, Bruker Vect. 22, JEOL, and Mass 5988 Mass spectrometer, respectively. Elemental analyses were performed at Cairo University, Egypt.

3.1.1. 3-[4-(5-Phenyl-1H-pyrazol-3-yl)-phenyl]-1H-quinazolin-2,4-dione ***2***. A mixture of chalcone **1** (0.36 gm, 0.001 mol) and hydrazine hydrate (0.1 mL, 0.002 mol) in ethanol (15 ml) was refluxed for 10 h. The formed precipitate was collected by filtration, dried, and recrystallized from benzene/ethanol to yield **2**, as white crystals. Yield: 88%; mp: 274−276 °C; FT-IR (KBr, υ, cm^{-1}) = 3323 (N−H), 1648 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 5.46 (s, 1H, NH), 7.21−7.96 (m, 14H, Ar−H + CH), 11.41 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) = 114.57, 115.12, 115.62, 122.01, 127.91, 137.76, 138.28, 140.37, 151.03, 168.59, 169.17, 169.65; MS (EI): m/z (%) = 392 [M^+]; anal. calcd for $C_{23}H_{16}N_4O_2$: C, 72.62%; H, 4.24%; N, 14.73%. Found: C, 72.71%; H, 4.30%; N, 14.65%.

3.1.2. 3-[4-(1-Acetyl-5-phenyl-1H-pyrazol-3-yl)-phenyl]-1H-quinazolin-2,4-dione ***3***. To a solution of chalcone **1** (0.36 gm, 0.001 mol) in glacial acetic acid (15 mL), hydrazine hydrate (0.1 mL, 0.002 mol) was added portionwise and the reaction mixture was refluxed for 12 h. The formed precipitate was collected by filtration, dried, and recrystallized from ethanol to give compound **3**, as white crystals. Yield: 85%; mp: 256−258 °C; FT-IR = 3225 (N−H), 1669, 1620 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 1.85 (s, 3H, CH_3), 7.23−7.99 (m, 13H, Ar−H), 8.77 (s, 1H, CH), 9.59 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) = 19.63, 114.81, 115.73, 115.82, 123.05, 128.03, 137.76, 138.28, 140.37, 151.03, 168.59, 169.17, 169.65; MS (EI): m/z (%) = 422 [M^+]; anal. calcd for $C_{25}H_{18}N_4O_3$: C, 71.08%; H, 4.29%; N, 13.26%. Found: C, 71.16%; H, 4.45%; N, 13.12%.

3.1.3. 3-[4-(1,5-Diphenyl-1H-pyrazol-3-yl)-phenyl]-1H-quinazolin-2,4-dione ***4***. A mixture of chalcone **1** (0.36 gm, 0.001 mol) and phenyl hydrazine (0.11 gm, 0.001 mol) in absolute ethanol (15 mL) was heated under reflux for 10 h. The formed precipitate was collected by filtration, dried, and recrystallized from benzene/ethanol to obtain compound **4**, as yellowish white crystals. Yield: 84%; mp: 144−146 °C; FT-IR = 3464 (N−H), 1668 (C═O); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm) = 7.24−8.10 (m, 19H, Ar−H + CH−pyrazole), 11.44 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) = 114.85, 115.44, 115.76, 123.07, 128.12, 128.36, 128.79, 128.17, 129.44, 135.74, 140.36, 150.67, 157.81; MS (EI): m/z (%) = 426 [M^+]; anal. calcd for $C_{29}H_{20}N_4O_2$: C, 76.30%; H, 4.42%; N, 12.27%. Found: C, 76.51%; H, 4.45%; N, 12.21%.

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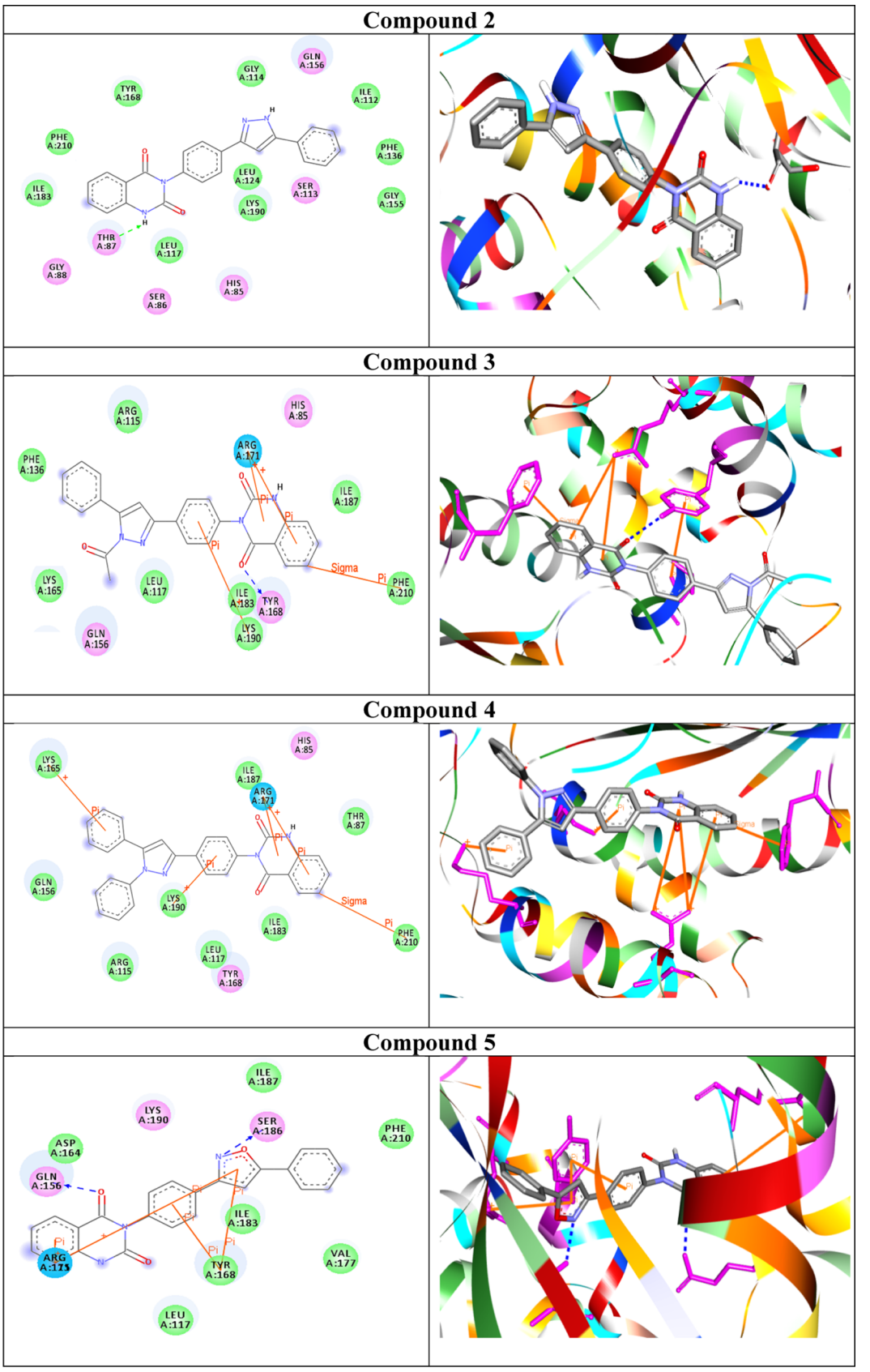

Figure 2. continued

https://doi.org/10.1021/acsomega.3c02478
ACS Omega 2023, 8, 27216−27230

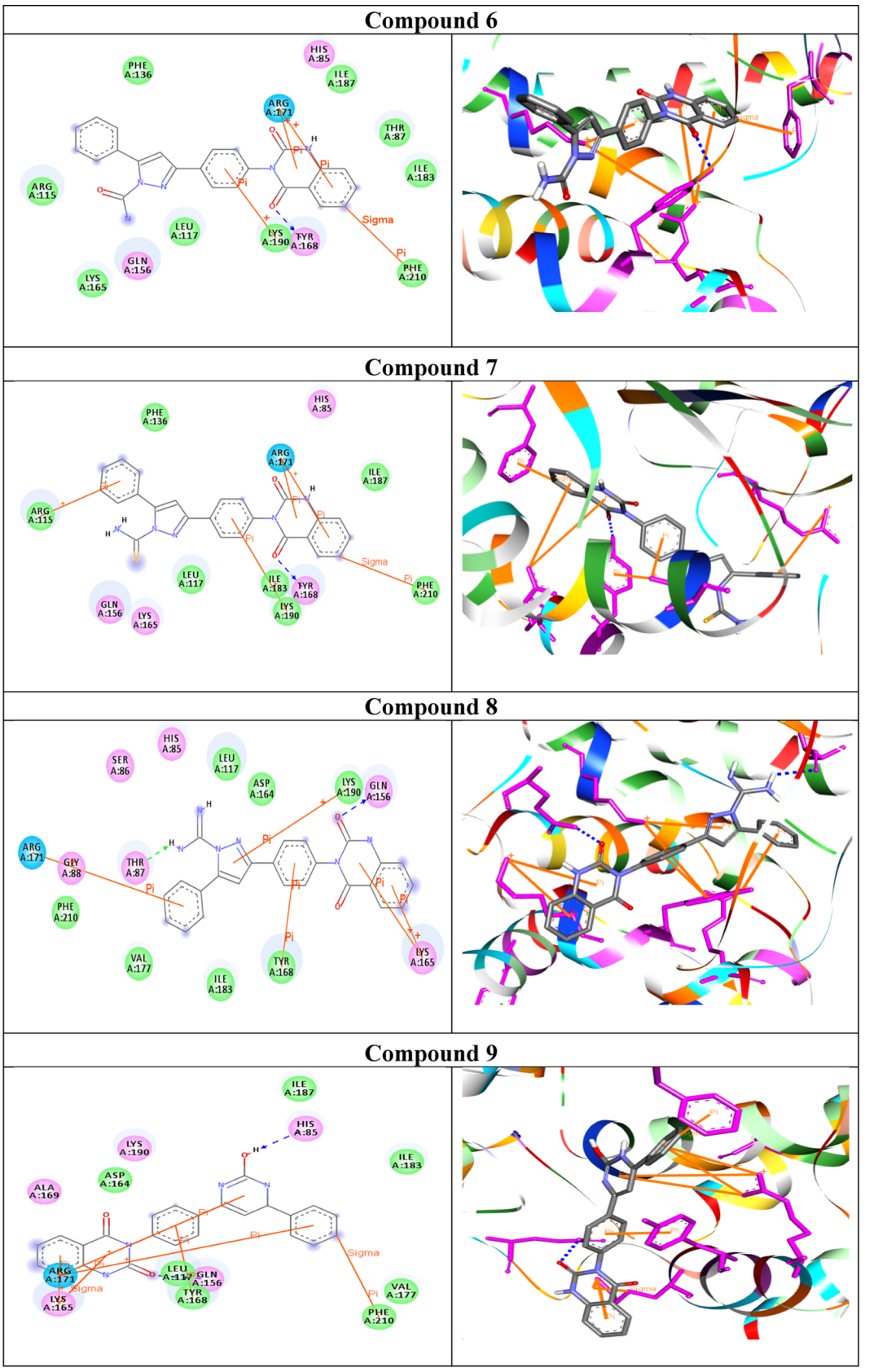

Figure 2. continued

https://doi.org/10.1021/acsomega.3c02478
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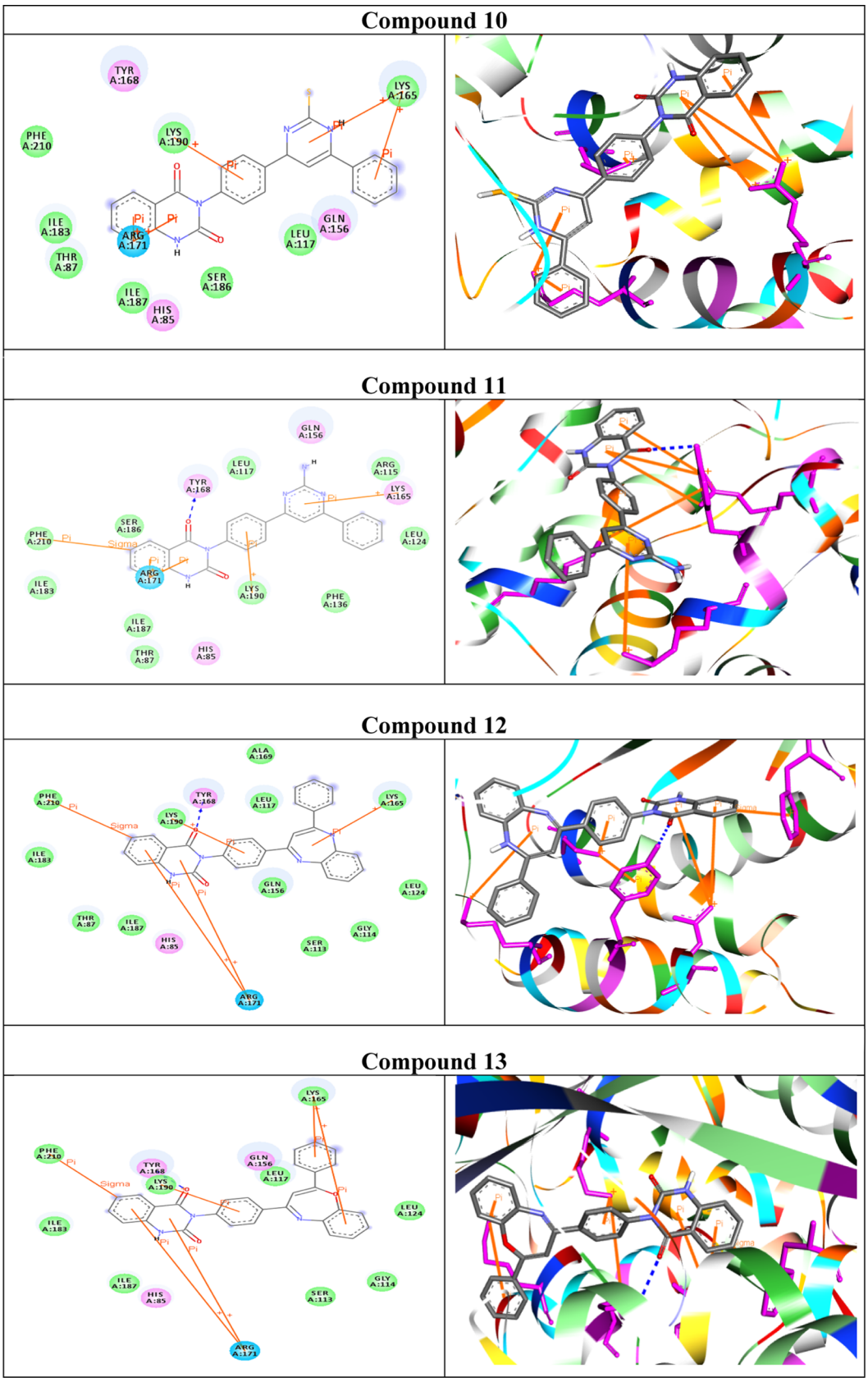

Figure 2. continued

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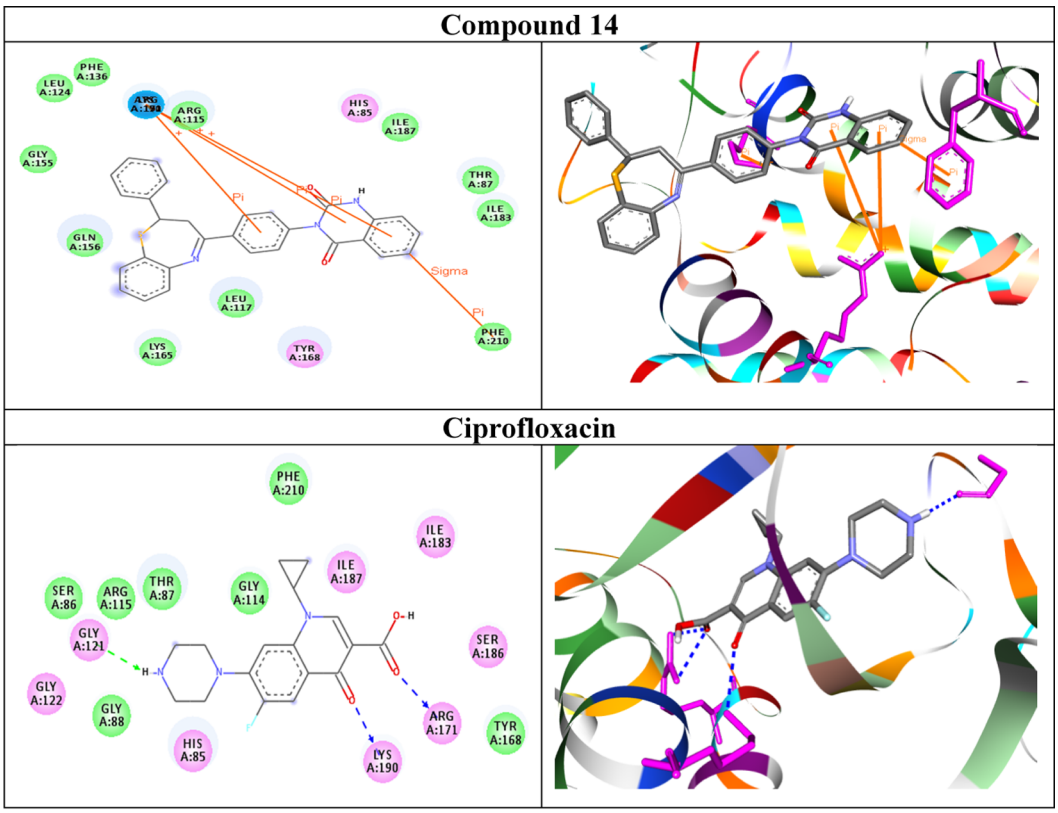

Figure 2. Left side: two-dimensional (2D), and right side: three-dimensional (3D) orientations of docked complexes. H-bonds are shown in blue dotted lines. Pi-stacked interactions are shown in orange lines.

Table 3. Physicochemical and ADMET Parameters of Molecules 2−14, and Ciprofloxacin[a]

entry	molecular weight (g/mol)	% (HIA$^+$)	log p	TPSA (A^2)	HBA	HBD	N rotatable	N violations	volume (A^3)	GI absorption	bioavailability score	BBB permeant
reference range	130−500	<25 poor; >80 high	<5	≤140	2−20	0−6	≤10	≤1	500−2000			
2	380.41	99.39	4.02	83.54	6	2	3	0	390.87	high	0.55	no
3	442.14	98.56	4.25	89.75	7	1	4	0	431.62	high	0.55	no
4	456.16	100.00	4.24	72.68	6	1	4	0	478.18	high	0.55	no
5	381.11	100.00	3.67	80.89	6	1	3	0	388.66	high	0.55	no
6	423.13	100.00	3.92	115.77	8	3	4	0	425.32	high	0.55	no
7	439.11	97.88	4.31	98.70	7	3	4	0	435.04	high	0.55	no
8	422.15	95.88	3.27	122.55	8	4	4	0	427.52	high	0.55	no
9	410.14	98.86	2.97	96.32	7	2	3	0	416.96	high	0.55	no
10	426.12	90.86	3.36	79.25	6	2	3	0	426.68	high	0.55	no
11	407.14	99.72	3.02	107.39	7	3	3	0	416.53	high	0.55	no
12	458.17	98.23	4.49	79.25	6	2	3	0	480.82	high	0.55	no
13	459.16	99.70	4.44	76.45	6	1	3	0	478.61	high	0.55	no
14	475.14	99.21	4.88	67.22	5	1	3	0	488.33	high	0.55	no
ciprofloxacin	331.34	97.95	1.28	74.57	5	2	3	0	285.46	high	0.55	no

[a]log p, logarithm ratio of the partition coefficient between n-octanol and water; TPSA, topological polar surface area; HBA, number of hydrogen bond acceptors; HBD, number of hydrogen bond donors; N rotatable, number of rotatable bonds.

3.1.4. 3-[4-(5-Phenyl-isoxazol-3-yl)-phenyl]-1H-quinazolin-2,4-dione ***5***. A mixture of chalcone **1** (0.36 gm, 0.001 mol) and hydroxylamine (0.11 gm, 0.001 mol) in pyridine (15 mL) was refluxed for 8 h. The mixture was allowed to cool and then poured onto H_2O/HCl. The formed precipitate was collected by filtration, dried, and recrystallized from benzene/ethanol to furnish **5**, as yellowish white crystals. Yield: 84%; mp: 210−212 °C; FT-IR = 3238 (N−H), 1667, and 1619 (C═O); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm) = 7.10−7.99 (m, 14H, Ar−H + CH−oxazole), 11.40 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) = 114.51, 115.33, 115.60, 122.32, 128.91, 137.76, 138.52, 140.37, 151.03, 168.59, 169.17, 169.65; MS (EI): m/z (%) = 383 [M$^+$]; anal. calcd for $C_{23}H_{15}N_3O_3$: C, 72.43%; H, 3.96%; N, 11.02%. Found: C, 72.51%; H, 4.05%; N, 10.93%.

3.1.5. 3-[4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-phenyl]-5-phenyl-pyrazole-1-carboxylic Acid Amide ***6***. A mixture of chalcone **1** (0.36 gm, 0.001 mol) and semicarbazide

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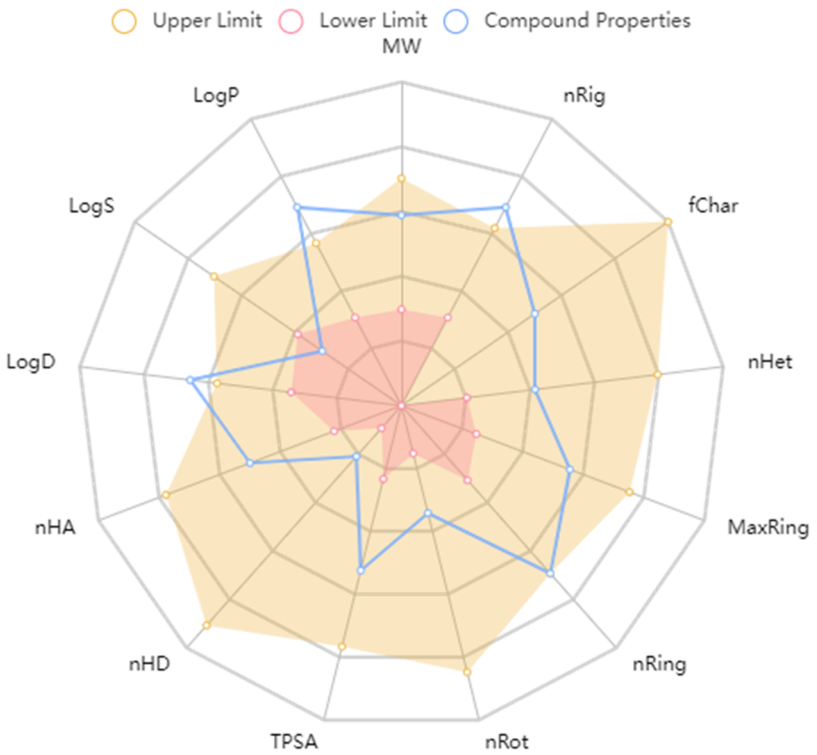

Figure 3. ADMET parameters of **13** calculated using ADMETlab tool.

hydrochloride (0.11 gm, 0.001 mol) in absolute ethanol (15 mL) was refluxed for 10 h. The formed precipitate was collected by filtration, dried, and recrystallized from benzene/ethanol to furnish **6**, as white crystals. Yield: 83%; mp: 180−182 °C; FT-IR = 3335 (N−H), 1669 (C═O); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm) = 7.19−7.99 (m, 13H, Ar−H), 8.31 (s, 1H, CH−pyrazole), 10.50 (s, 2H, NH_2), 11.54 (s, 1H, NH); ^{13}C NMR (125 MHz, DMSO-d_6, δ, ppm) = 108.79, 112.00, 112.17, 114.90, 117.32, 122.84, 126.58, 128.47, 130.89, 133.56, 135.65, 139.66, 140.85, 141.93, 151.32, 168.53; MS (EI): m/z (%) = 423 [M$^+$]; anal. calcd for $C_{24}H_{17}N_5O_3$: C, 68.08%; H, 4.05%; N, 16.54%. Found: C, 68.16%; H, 4.18%; N, 16.21%.

*3.1.6. 3-[4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-phenyl]-5-phenyl-pyrazole-1-carbothioic Acid Amide **7**.* Thiosemicarbazide (0.11 gm, 0.001 mol) was mixed with chalcone **1** (0.36 gm, 0.001 mol) in absolute ethanol (15 mL) and the mixture was refluxed for 10 h. The formed precipitate was collected by filtration, dried, and recrystallized from benzene/ethanol to obtain **7**, as white crystals. Yield: 84%; mp: 192−194 °C; FT-IR = 3433 (NH_2), 3278 (N−H), 1667 (C═O), 1178 (C═S); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 7.06−7.95 (m, 13H, Ar−H), 8.32 (s, 1H, CH−pyrazole), 10.40 (s, 2H, NH_2), 11.32 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 108.79, 112.00, 114.90, 117.32, 122.84, 126.58, 128.21, 128.47, 128.64, 130.89, 133.56, 135.65, 139.66, 140.79, 140.85, 141.93, 148.40, 150.32, 161.20, 181.11; MS (EI): m/z (%) = 439 [M$^+$]; anal. calcd for $C_{24}H_{17}N_5O_2S$: C, 65.59%; H, 3.90%; N, 15.93%, S, 7.30%. Found: C, 66.63%; H, 3.93%; N, 15.67%; S, 7.32%.

*3.1.7. 3-[4-(2,4-Dioxo-1,4-dihydro-2H-quinazolin-3-yl)-phenyl]-5-phenyl-pyrazole-1-carboxamidine **8**.* A mixture of chalcone **1** (0.36 gm, 0.001 mol) and amino guanidine hydrochloride (0.15 gm, 0.001 mol) in absolute ethanol (20 mL) and sodium hydroxide (0.001 mol) was heated under reflux for 8 h. The formed precipitate was filtrated off, and recrystallized from benzene/ethanol to yield **8**, as white crystals. Yield: 78%; mp: 144−146 °C; FT-IR = 3400 (NH_2), 3279 (N−H), 1667 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 7.03−8.25 (m, 14H, Ar−H + CH−pyrazole), 9.53 (s, 2H, NH_2), 11.21 (s, 1H, NH), 11.52 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 108.79, 112.00, 114.90, 117.32, 122.84, 126.58, 128.21, 128.47, 128.64, 130.89, 133.56, 135.65, 139.66, 140.79, 140.85, 141.93, 148.40, 150.32, 158.60, 161.20; MS (EI): m/z (%) = 408 [M$^+$]; anal. calcd for $C_{24}H_{18}N_6O_2$: C, 68.24%; H, 4.29%; N, 19.89%. Found: C, 68.33%; H, 5.02%; N, 19.83%.

*3.1.8. 3-[4-(2-Hydroxy-6-phenyl-1,6-dihydro-pyrimidin-4-yl)-phenyl]-1H-quinazolin-2,4-dione **9**.* To a stirred solution of chalcone **1** (0.36 gm, 0.001 mol) and urea (0.1 gm, 0.001 mol) in 20 mL of absolute ethanol, a sodium hydroxide (0.001 mol) was added. The mixture was refluxed for 8 h. After leaving to cool, the mixture was poured onto H_2O/HCl. The formed solid product was filtered off, dried, and then recrystallized from benzene/ethanol to give **9**, as yellow crystals. Yield: 85%; mp: 150−152 °C; FT-IR = 3500 (O−H), 3282 (N−H), 1668 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 6.61 (d, 1H, CH-sp^3), 7.08−7.97 (m, 14H, Ar−H + CH−pyrimidine), 10.20 (s, 1H, NH), 10.44 (s, 1H, OH), 10.50 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 108.79, 112.00, 112.17, 114.90, 117.32, 122.84, 126.58, 128.47, 130.89, 133.56, 135.65, 139.66, 140.85, 141.93, 148.41, 151.32, 168.53; MS (EI): m/z (%) = 410 [M$^+$]; anal. calcd for $C_{24}H_{18}N_4O_3$: C, 70.23%; H, 4.42%; N, 13.65%. Found: C, 70.39%; H, 4.47%; N, 13.60%.

*3.1.9. 3-[4-(2-Mercapto-6-phenyl-1,6-dihydro-pyrimidin-4-yl)-phenyl]-1H-quinazolin-2,4-dione **10**.* A mixture of chalcone **1** (0.36 gm, 0.001 mol) and thiourea (0.12 gm, 0.001 mol) in absolute ethanol (20 mL) and sodium hydroxide (0.001 mol) was refluxed for 10 h. The formed precipitate was

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collected by filtration, dried, and recrystallized from benzene/ethanol to yield **10**, as yellowish white crystals. Yield: 82%; mp: 144−146 °C; FT-IR = 3346 (N−H), 2606 (S−H), 1671 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 2.34 (s, 1H, SH), 6.49 (d, 1H, CH-sp^3), 7.12-8.33 (m, 14H, Ar−H + CH−pyrimidine), 10.42 (s, 1H, NH), 11.70 (s, 1H, NH); MS (EI): m/z (%) = 426 [M$^+$]; anal. calcd for $C_{24}H_{18}N_4O_2S$: C, 67.59%; H, 4.25%; N, 13.14; S,7.52%. Found: C, 68.11%; H, 4.27%; N, 12.98; S, 7.54%.

*3.1.10. 3-[4-(2-Amino-6-phenyl-pyrimidin-4-yl)-phenyl]-1H-quinazolin-2,4-dione **11**.* A mixture of chalcone **1** (0.36 gm, 0.001 mol) and guanidine hydrochloride (0.15 gm, 0.001 mol) in absolute ethanol (20 mL) and sodium hydroxide (0.001 mol) was refluxed for 10 h. The formed precipitate was collected by filtration, and recrystallized from benzene/ethanol to give **11**, as yellowish white crystals. Yield: 84%; mp: 152−154 °C; ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm) = 7.08-8.02 (m, 14H, Ar−H + CH−pyrimidine), 10.45 (s, 1H, NH); 11.57 (s, 1H, NH); 13.38 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 97.82, 112.82, 115.17, 115.32, 123.18, 127.57, 127.96, 128.79, 130.20, 131.20, 133.31, 133.64, 135.12, 135.81, 139.33, 150.00, 151.16, 156.17, 157.99, 161.20; anal. calcd for $C_{24}H_{17}N_5O_2$: C, 70.75%; H, 4.21%; N, 17.19%. Found: C, 70.78%; H, 4.27%; N, 17.15%.

*3.1.11. General Procedure for the Synthesis of Azepines **12**−**14**.* A mixture of *o*-phenylenediamine, *o*-aminophenol, and/or *o*-aminothiophenol (1.0 mmol) in ethanol (5.0 mL) was added dropwise with stirring to a solution of chalcone **1** (1.0 mmol) in glacial acetic acid (5.0 mL); then, the reaction mixture was refluxed for 12−14 h. The progress of the reaction was followed by TLC. The solid precipitate of the desired product from each reaction was filtered off and recrystallized from ethanol.

*3.1.11.1. 3-[4-(4-Phenyl-4,5-dihydro-3H-benzo[b][1,4]-diazepin-2-yl)-phenyl]-1H-quinazolin-2,4-dione **12**.* Yield: 83%; mp: 216−218 °C; FT-IR = 3242 (N−H), 1669 (C═O); ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 3.83 (dd, 1H (Ha), CH$_2$), 4.34 (dd, 1H (Hb), CH$_2$), 4.86 (t, 1H (Hx), CH), 5.45 (s, 1H, NH), 7.12−7.40 (m, 17H, Ar−H), 11.41 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 42.27, 57.20, 114.83, 115.28, 115.83, 123.08, 123.36, 128.09, 128.51, 129.02, 129.18, 130.12, 135.77, 139.97, 140.38, 140.67, 150.22, 151.84, 153.06, 163.51, 173.35; MS (EI): m/z (%) = 458 [M$^+$]; anal. calcd for $C_{29}H_{22}N_4O_2$: C, 75.97%; H, 4.84%; N, 12.22%. Found: C, 76.16%; H, 4.95%; N, 12.07%.

*3.1.11.2. 3-[4-(6-Phenyl-6,7-dihydro-5-oxa-9-aza-benzo-cyclohepten-8-yl)-phenyl]-1H-quinazolin-2,4-dione **13**.* Yield: 81%; mp: 210−212 °C; ^{1}H NMR (400 MHz, DMSO-d_6, δ, ppm) = 3.83 (dd, 1H (Ha), CH$_2$), 4.34 (dd, 1H (Hb), CH$_2$), 4.86 (t, 1H (Hx), CH), 7.12−7.40 (m, 17H, Ar−H), 11.41 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 40.27, 77.00, 114.83, 115.28, 115.83, 123.08, 123.36, 128.09, 128.51, 129.02, 129.18, 130.12, 135.77, 139.97, 140.38, 140.67, 150.22, 151.84, 153.06, 163.51; MS (EI): m/z (%) = 460 [M$^+$]; anal. calcd for $C_{29}H_{21}N_3O_3$: C, 75.80%; H, 4.61%; N, 9.14%. Found: C, 76.02%; H, 4.85%; N, 9.11%.

*3.1.11.3. 3-[4-(2-Phenyl-2,3-dihydro-benzo[b][1,4]-thiazepin-4-yl)-phenyl]-1H-quinazolin-2,4-dione **14**.* Yield: 79%; mp: 152−154 °C; FT-IR = 3362 (N−H), 1669, and 1723 (C═O); ^{1}H NMR (500 MHz, DMSO-d_6, δ, ppm) = 3.80 (dd, 1H (Ha), CH$_2$), 4.09 (dd, 1H (Hb), CH$_2$), 5.41 (t, 1H (Hx), CH), 6.39−7.36 (m, 17H, Ar−H), 11.49 (s, 1H, NH); ^{13}C NMR (100 MHz, DMSO-d_6, δ, ppm) = 39.2, 47.3, 112.0, 114.9, 117.3, 122.5, 125.3, 127.5, 127.8, 127.9, 128.2, 128.4, 135.4, 135.6, 135.8, 139.7, 148.9, 150.0, 161.2, 173.5; MS (EI): m/z (%) = 476 [M$^+$]; anal. calcd for $C_{29}H_{21}N_3O_2S$: C, 73.24%; H, 4.45%; N, 8.84%. Found: C, 73.32%; H, 4.61%; N, 8.79%.

3.2. Biological Evaluation. The spread of the microbial chemotherapeutic resistance is considered a significant clinical problem globally.[26] Therefore, searching for new heterocyclic molecules to serve as wide-spectrum antimicrobials could contribute to combating such challenges. In the present work, the antimicrobial activity and MIC of the newly prepared molecules were determined against two G^{-ve} and two G^{+ve} bacteria. The pathogens under study were provided by Al-Azhar University, Egypt. They were cultivated in Mueller Hinton broth at 35 ± 2 °C for 24 h. The antimicrobial susceptibility and MIC testing was carried out as described by Qadir et al.[27]

3.3. *In Silico* Docking Study. The X-ray crystal structure of thymidine phosphorylase enzyme was downloaded from the RCSB database (PDB ID: 4EAD). The discovery studio visualizer was used to remove water, heteroatoms, and co-crystallized ligand, and to prepare the protein further. All synthesized molecules **2**−**14** were used as ligand molecules in the docking process. The 2D chemical structures of the ligands and ciprofloxacin were sketched using ChemDraw software 16.0, and then converted to the 3D structures by using Open Babel GUI tool.[28] The energy minimization for the protein file and ligand molecules was performed using CHARMm[29] and AMBER[30] Force Fields, respectively. PyRx tool[31] is utilized for the docking study of the ligand molecules and the enzyme. The 2D and 3D views of the docked compounds and ciprofloxacin with the target were visualized using Discovery Studio visualizer software. Finally, the pharmacokinetics properties of the compounds were detected using AdmetSAR, SwissADME, and molinspiration web-based tools.

4. CONCLUSIONS

Thirteen new compounds containing in their structures the quinazolinedione skeleton and having five-, six-, and seven-membered heterocyclic moieties **2**−**14** such as pyrazole, oxazole, pyrimidine, and azepines were prepared by cyclization of chalcone **1** with various nitrogen nucleophiles. The compounds were structurally confirmed by their spectral and elemental analyses. Further, the observed antibacterial activity of compound **13** against the tested microbes was confirmed by its strong binding nature, which was observed by *in silico* molecular docking studies against the target. Based on *in vitro* and *in silico* findings, the ligand molecule **13** can be used as a better and potent drug candidate for treatment of infectious diseases. The SAR study suggested the significance of introducing benzoxazepine moiety on quinazolin-2,4-dione. Further synthesis and *in silico* and *in vitro* investigation of this molecule originated a path for development of potential drugs for treatment of bacterial diseases.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c02478.

Additional figures illustrating the characterization of all synthesized compounds by H NMR, C NMR, mass, and IR spectra (PDF)

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Notes

The authors declare no competing financial interest.

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