Article

Synthesis, Antimicrobial Evaluation, and Docking Studies of Novel 4-Substituted Quinazoline Derivatives as DNA-Gyrase Inhibitors

Shireesha Boyapati, Umasankar Kulandaivelu, Srinivas Sangu and Malla Reddy Vanga

Medicinal Chemistry Research Division, Vaagdevi College of Pharmacy, Warangal, A.P, India

Quinazoline derivatives are reported to have anti-inflammatory, analgesic, antibacterial, and anticancer activities. The incorporation of ''OCH_2CONH_2'' (oxymethylcarbamide) at 4^{th} position of the quinazoline ring was found to influence the biological activities of the molecules. With this rationale, some new oxadiazolyl methyloxy quinazolines, pyrazolyl acetoxy methyl quinazolines, triazolylmethyloxy quinazolines were synthesized from anthranilic acid through quinazolyl oxyacetylhydrazide intermediates. All the compounds were characterized by IR, ^{1}H-NMR, EI-MS, and C, H, N analyses and evaluated for their antimicrobial activity. Docking studies on the DNA-gyrase enzyme (1KZN) show their role in the antimicrobial activity of the molecules and explain the higher potency of compounds **6a**, **6b**, **8a**, **8b** based on ReRanking scores and binding poses of the molecules.

Keywords: DNA-Gyrase inhibitors / Antimicrobial activity / 4-Substituted quinazolines

Received: March 2, 2010; Accepted: April 16, 2010

DOI 10.1002/ardp.201000065

Introduction

Quinazolines and condensed quinazolines are found to possess potent anticancer [1], antihistaminic [2], and CNS activities like analgesic and anti-inflammatory [3–6], sedative-hypnotic and anticonvulsant [7] activities. Quinazolinones with substitution of ''OCH_2CONH_2'' (oxymethylcarbamide) group at the 4^{th} position have been reported to enhance the biological activities [8]. Quinazolines were also found to have antibacterial activity [9–15]. Antibiotic resistance in the community is a growing public health concern due to the continued emergence of multi drug resistant bacterial strains. In view of this fact, the design and synthesis of newer antibacterials are of immense significance and continue to attract the attention of numerous medicinal chemists.

The 4-quinolones such as ciprofloxacin, ofloxacin, lomefloxacin, gatifloxacin, are established synthetic antibacterial agents as DNA gyrase inhibitors [16]. DNA gyrase is a prokaryotic type II topoisomerase and a major target of quinolone antibacterials.

Bacterial DNA gyrase belongs to a superfamily of ATPases, known as GHKL (gyrase, Hsp90, histidine, kinase, MutL), which consists of several important targets for infection and cancer treatments. It is involved in the vital processes of DNA replication, transcription, and recombination [17]. It has the ability to catalyze the ATP-dependent introduction of negative supercoils into bacterial DNA and to unknot DNA. This enzyme consists of two subunits, A and B, of molecular masses 97 and 90 kDa, respectively, with the active enzyme being an A2B2 complex. The A-subunit of DNA gyrase is involved in DNA breakage and reunion, whereas the B-subunit catalyzes the hydrolysis of ATP [18]. A majority of mutations conferring resistance to quinolones arise within the quinolone resistance-determining region of GyrA close to the active site (Tyr122) where DNA is bound and cleaved. However, some quinolone resistance mutations are known to exist in GyrB and these residues lie at a considerable distance from the quinolone resistance-determining region. The mutations exert their effect by decreasing the amount of

Correspondence: Dr. Shireesha Boyapati, M. Pharm. PhD, Associate Professor Medicinal Chemistry Research Division, Vaagdevi College of Pharmacy, Hanamkonda, Warangal-506001, India.
E-mail: sirimedchem@yahoo.co.in; sirimedchem@gmail.com
Fax: +91 870 254-4949

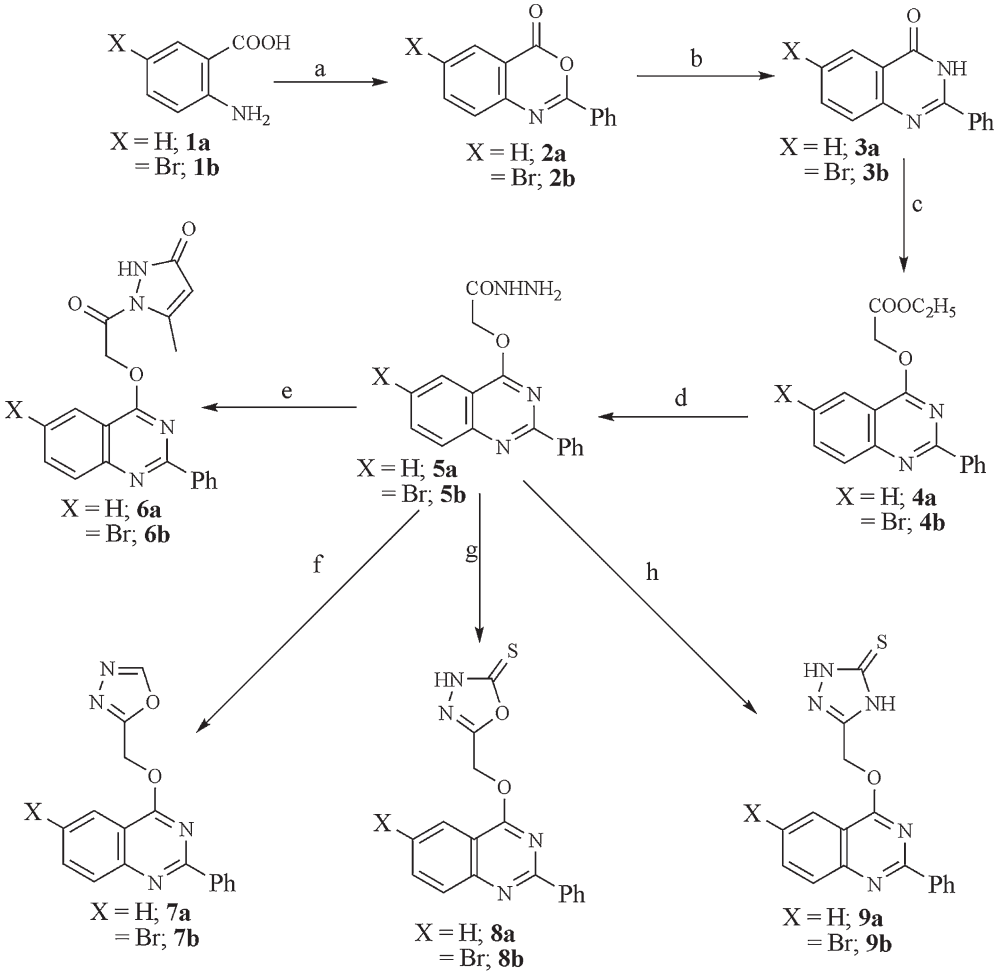
quinolone bound to a gyrase-DNA complex and GyrB residues form part of a quinolone binding pocket that includes DNA and the quinolone resistance-determining region in GyrA. Large conformational changes during the catalytic cycle of the enzyme allow these regions to come into close proximity [19, 20].

Substituted quinazolines were reported as isosters of quinolones to show a variety of antibacterial activities with DNA gyrase inhibition [21, 22]. In continuation of our efforts to synthesize quinazoline derivatives [23–25], we report the synthesis of novel 2,6-substituted quinazoline derivatives with "OCH_2CONH_2" substitution at the 4th position along with their antimicrobial activities. The possibility of an involvement of the DNA-gyrase enzyme in the activity of quinazolines was confirmed from docking studies.

Results and Discussion

Chemistry

Quinazolines with substitutions on the 2nd and 4th positions and either halogens or electron-rich substituents on the 6th position were known to promote activity against bacterial cultures. The synthesis of designed 4-substituted quinazoline derivatives was achieved following the steps outlined in Scheme 1. Anthranilic acid **1** underwent cyclization with benzoyl chloride in pyridine at 0–5°C to give 2-phenyl-(4*H*)-3,1-benzoxazin-4-one **2** by benzoylation followed by dehydration. Compound **2**, on reaction with formamide, results in the more stable 2-phenyl-4(3*H*) quinazolinone **3**. Compounds **2** and **3** were identified by comparing them with earlier reports [26–28] and the physical data of the compounds are given in Table 1.



Reagents and conditions: a) Benzoyl chloride, pyridine; b) $HCONH_2$; c) $ClCH_2COOC_2H_5$; d) $NH_2NH_2 \cdot H_2O$; e) ethylacetoacetate; f) triethyl ortho formate; g) CS_2/KOH; h) KCNS/HCl, NaOH.

Scheme 1. Synthesis of 4-substituted quinazolines.

Table 1. Physical data of benzoxazin-4(3*H*)-ones **2** and quinazolin-4(3H)-ones **3**.

Compound	X	R	m. p. (°C)	Yield (%)
2a	H	Ph	122	82
2b	Br	Ph	182	80
3a	H	Ph	226	84
3b	Br	Ph	230	80

Compound **3** on alkylation with ethyl chloroacetate in dry acetone over anhydrous potassium carbonate yielded exclusively its *O*-alkylated product: ethyl-[(2-phenyl-4-quinazolinyl)-oxy]-acetate **4**. This is attributed not only to the basic medium but also steric factors as the bulky phenyl group is in the 2nd position [29]. Compound **4** was reacted further with hydrazine hydrate (80%) to obtain the corresponding 2-phenyl-4-quinazolinyloxyacetyl hydrazides **5** [30]. The hydrazide **5** on reaction with ethylacetoacetate/triethyl orthoformate/carbon disulfide and potassium hydroxide/potassium thiocyanide in hydrochloric acid led to the formation of pyrazoles **6a**, **6b**, oxadiazoles **7a**, **7b**, oxadiazol-2-thiones **8a**, **8b**, and triazol-2-thiones **9a**, **9b**, respectively. All the compounds synthesized were characterized based on their physical and spectral data.

Antimicrobial evaluation

Antibacterial activity

Compounds **6a**, **6b**, **7a**, **7b**, **8a**, **8b**, and **9a**, **9b** were screened for their *in-vitro* antibacterial activity against two Gram-negative strains, *i. e*., *Escherichia coli* (NCIM 2068) and *Klebsiella pneumonia* (NCIM 2957) and two Gram-positive strains, *i. e*., *Bacillus subtilis* (NCIM 2921) and *Staphylococcus aureus* (NCIM 2079), and their MIC values are presented in Table 2. The antibacterial activity of the molecules was compared with a standard drug, ciprofloxacin. Minimum inhibitory concentrations (MIC values) of ciprofloxacin in various strains were comparable with the reported values [31]. Perusal of the activity data shows that compound **8b** was more potent and compounds **7a**, **7b** were inactive against all the strains. The presence of a bromine at the 6th position of the quinazoline system was found to influence the antibacterial activity in relation to the unsubstituted derivatives: **6a**, **6b** and **8a**, **8b**. Compounds **6b** and **8b** with diazolyl, oxadiazolyl substitutions on the 4th position, respectively, were found to be relatively potent against Gram-negative bacteria with a potency comparable to that of the standard ciprofloxacin. The orders of activity was found to be **8b** > **6b** > **8a** > **9b** > **6a**, **9a** and **8b**, **6b** > **9a** > **6a**, **8a** against Gram-negative and -positive strains, respectively.

Antifungal activity

All the synthesized quinazoline derivatives were evaluated for antifungal activity against two fungal strains: *Candida albicans* (NCIM 3471) and *Aspargillus flavus* (NCIM 555). The activity of all molecules was compared with an antifungal drug, amphoterecin-B as presented in Table 2. The minimum inhibitory concentrations of amphoterecin-B against both strains were compared with literature reports [32, 33]. The antifungal activity of the test compounds was not significant. Only compound **8a** was found to inhibit *C. albicans* with MIC, 24 μg/mL and *A. flavus* with MIC, 22 μg/mL.

Docking studies

In the present investigation, an attempt was made to understand the ligand-receptor interactions of quinazoline derivatives **6–9** against bacterial DNA gyrase as a target enzyme, by performing docking studies using molegro Virtual Docker (MVD) [34] version 2008.3.2.1, installed on an Intel Pentium 4 Machine (Intel Corporation, Santa Clara, CA, USA), probably the most accurate predictive tool of binding geometry, today. All the ligand structures were constructed using Chem 3D ultra 8.0 software (Molecular Modeling and Analysis;

Table 2. Antibacterial activity of 4-substituted quinazolines **6–9**.

Test compound	Antibacterial activity MIC (μg/mL)				Antifungal activity MIC (μg/mL)	
	E. coli NCIM 2068	***K. pneumonia*** NCIM 2957	***S. aureus*** NCIM 2079	***B. subtilis*** NCIM 2921	***C. albicans*** NCIM 3471	***A. flavus*** NCIM 555
6a	45	43	35	50	30	50
6b	4	12	5	4	100	215
7a	–	–	270	275	300	–
7b	–	–	330	375	–	–
8a	20	25	40	34	24	22
8b	2	8	4	5	31	34
9a	45	50	27	27	244	150
9b	25	30	26	24	278	231
Ciprofloxacin	0.05	0.06	0.5	1	–	–
Amphoterecin-B	–	–	–	–	5	4

Table 3. MolDock score and ReRank scores of all quinazoline derivatives.

Ligand	MolDock Score	ReRank Score	HBond
6a	−83.80	−65.09	−0.1017
6b	−109.99	−90.12	−5.6533
7a	−67.58	−20.59	−0.5073
7b	−88.84	−32.54	−0.9796
8a	−94.59	−67.09	−1.0123
8b	−129.09	−90.99	−0.9502
9a	−96.09	−9.88	0.0794
9b	−89.80	−51.78	−0.6498

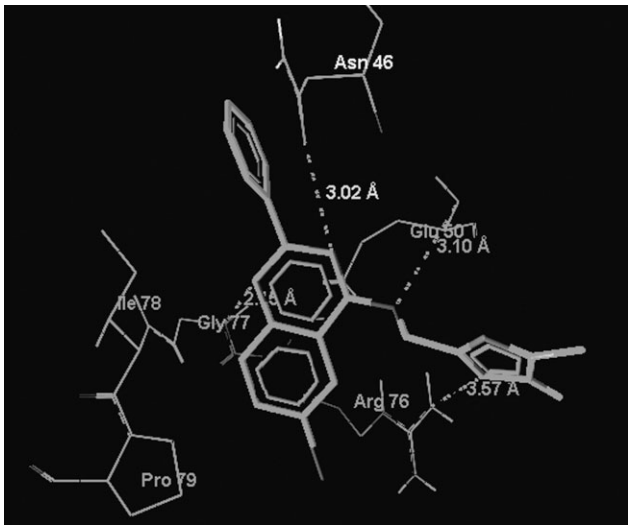


Figure 1. Snapshot of compound **8b** and its hydrogen-bond interactions with 1KZN.

Cambridge Soft Corporation, USA, 2004), and then these structures were energetically minimized by using MOPAC (semi-empirical quantum mechanics), Jop Type with 100 iterations and minimum RMS gradient of 0.01, and saved as MDL molFile (*.mol). The crystal structure of the target protein isomerase (1KZN) is the crystal structure of *E. coli* 24-kda domain in complex with clorobiocin [35] being retrieved from the Protein Data Bank (www.rcsb.org/pdb/welcome.-do).

Docking studies were performed to identify the nature and amount of interactions of the synthesized quinazoline derivatives with DNA-gyrase enzyme (1KZN). The MolDock scores and ReRank scores of molecules are presented in Table 3. Perusal of Table 3 shows ReRank scores of −90.12, −90.99 for compounds **6b** and **8b**, respectively, which are in correlation with the experimental values. The obtained pose showed four hydrogen-bond interactions. Atoms of protein (1KZN) and ligand **8b** involved in hydrogen-bond interactions are presented along with their bond distance in Table 4, indicating that the docking method was most appropriate for clarifying the binding mode of 4-substituted quinazolines as DNA-gyrase inhibitors, as illustrated in Figs. 1 and 2.

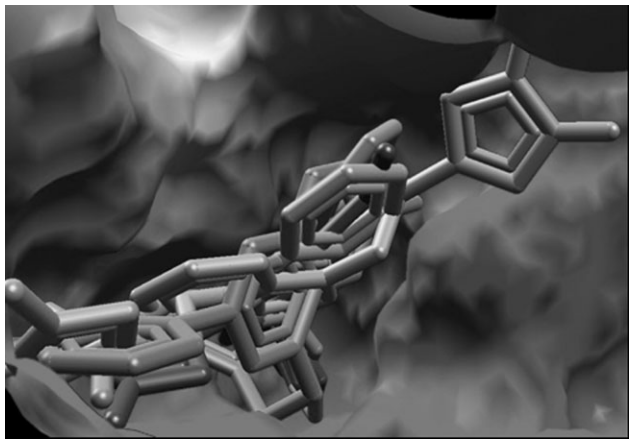


Figure 2. Snap shot of superimposed quinazolines in the binding cavity of 1KZN.

Experimental

Chemistry

Melting points were recorded on Casia-siamia (VMP-AM) melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer FT-IR 240-C spectro photometer using KBr optics (Perkin-Elmer. USA). ^{1}H-NMR spectra were recorded on Gemini Varian (Varian, USA) 200 MHz, Bruker (Bruker Bioscience, USA) AV 300 MHz, and Unity (Varian) 400 MHz spectrometer in DMSO-d_6 or $CDCl_3$ using TMS as an internal standard. Electron impact (EI) and chemical ionization mass spectra were recorded on a VG 7070 H instrument (Micromass, UK) at 70 eV. All reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck silica gel (100–200 mesh; Merck, Germany) was used for chromatography. CHN analyses were recorded on a Vario EL analyzer.

Table 4. Amino acid residues and atoms of ligand **8b** involved in hydrogen bonding and their bond lengths.

Residues	Atom ID	Pdb Atom Name	Ligand atom	Distance (Å)
Asp 76	464	N (Donor)	N (Acceptor) oxadiazolyl	3.57
Glu 50	266	N (Donor)	O (Acceptor) etherial oxygen	3.10
Asn 46	243	O (Donor)	N-2 (Acceptor) quinazolyl	3.02
Gly 77	466	N (Acceptor)	N-1 (Donor) quinazolyl	2.15

*Synthesis of 1,3-benzoxazin-4-ones **2a**, **2b** [26–28]*

The physical data of the compounds are presented in Table 1 and compared with the reported values.

*Synthesis of 4-quinazolinones **3a**, **3b** [26–28]*

The physical data of the compounds are presented in Table 1 and compared with the reported values.

*Synthesis of quinazolinyl-4-oxy-ethylacetate **4a**, **4b** [30]*

Compound **3a** or **3b** (0.03 mol), ethyl chloroacetate (0.03 mol), potassium carbonate (0.03 mol) were taken up in 10 mL of dry acetone and the mixture was heated at reflux for a period of 24 h. Completion of the reaction was monitored by TLC. After the reaction, the mixture was added to about 100 mL of ice-cold water and the solution was kept in a refrigerator overnight. The resultant compound was extracted with ether; the removal of ether *in vacuo* yielded the product. The product ester thus obtained was used in the next step of synthesis without further purification.

*Synthesis of quinazolinyl-4-oxy-acetyl hydrazine **5a**, **5b***

Compound **4a** or **4b** (0.001 mol) and hydrazine hydrate (0.001 mol) were taken up in ethanol (10 mL) and heated on a water bath at reflux for 6 h. The solid that precipitated on cooling was filtered, dried, and purified by column chromatography.

*[(2-Phenyl-4-quinazolinyl)oxy]-acetyl hydrazine **5a***

Yield: 68% (395 mg); m. p.: 210°C; IR (KBr, cm^{-1}): 1290 (C–O–C), 1616 (C=N), 1762 (C=O), 3296 (N–H), 3056, 2918 (C–H); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.2 (s, 2H, OCH_2), 4.4 (s, 2H, NH_2), 7.4–7.6 (m, 4H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.4 (s,1H, Ar-H), 9.3 (s, 1H, N–H); EI-MS: 294 [M^+] (19%). Anal. calcd. for $C_{16}H_{14}N_4O_2$: C, 65.30; H, 4.79; N, 19.04. Found: C, 65.19; H, 4.72; N, 19.01.

*[(6-Bromo-2-phenyl-4-quinazolinyl)oxy]-acetyl hydrazine **5b***

Yield: 55% (205 mg); m. p.: 195°C; IR (KBr, cm^{-1}): 1768 (C=O), 1620 (C=N), 3285 (N–H), 3080, 2945 (C–H); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.3 (s, 2H, OCH_2), 4.7 (s, 2H, NH_2), 7.5–7.7 (m, 3H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.5 (s,1H, Ar-H), 9.4 (s,1H, N-H); EI-MS: 373 [M^+] (19%). Anal. calcd. for $C_{16}H_{13}BrN_4O_2$: C, 51.49; H, 3.51; N, 15.01. Found: C, 51.35; H, 3.42; N, 14.92.

*General procedure for synthesis of [(2-phenyl-4-quinazolinyl)-oxy]acetyl-3-methyl pyrazol-5-ones **6a**, **6b***

Ethyl acetoacetate (0.001 mol) and compound **5a** or **5b** (0.01 mol) were taken up in ethanol (15 mL) along with 4 to 5 drops of HCl and heated on a water bath at reflux for 5 h. Completion of the reaction was monitored by TLC. Ethanol was removed *in vacuo* and the residue was triturated with cold water. The solid obtained was filtered under pressure, dried, and purified by using column chromatography.

*[(2-Phenyl-4-quinazolinyl)-oxy]acetyl-3-methyl pyrazol-5-one **6a***

Yield: 57% (205 mg); m. p.: 196°C; IR (KBr, cm^{-1}): 1707 (C=O), 1625 (C=N), 3320 (N–H); ^{1}H-NMR ($CDCl_3$, ppm) δ: 2.2 (s, 3H, CH_3), 4.4 (s, 2H, OCH_2), 7.4–7.6 (m, 4H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.5 (s,1H, Ar-H), 8.6 (s, 1H, pyrazole-H), 9.1–9.3 (s, 1H, N–H); EI-MS: 360 [M^+] (20%). Anal. calcd. for $C_{20}H_{16}N_4O_3$: C, 66.66; H, 4.48; N, 15.55. Found: C, 66.45; H, 4.31; N, 15.40.

*[(2-Phenyl-6-bromo-4-quinazolinyl)-oxy]acetyl-3-methyl pyrazol-5-one **6b***

Yield: 50% (219.5 mg); m. p.: 185°C; IR (KBr, cm^{-1}): 1720 (C=O), 1628 (C=N), 3345 (N–H); ^{1}H-NMR ($CDCl_3$, ppm) δ: 2.2 (s, 3H, CH_3), 4.6 (s, 2H, OCH_2), 7.6–7.7 (m, 3H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.3–8.4 (t, 2H, Ar-H), 8.5 (s, 1H, Ar-H), 8.6 (s, 1H, pyrazole-H), 9.1–9.3 (s, 1H, NH); EI-MS: 439 [M^+] (65%). Anal. calcd. for $C_{20}H_{15}BrN_4O_3$: C, 54.69; H, 3.44; N, 12.75. Found: C, 54.52; H, 3.19; N, 12.57.

*General procedure for synthesis of 4-oxadiazolylmethoxy-2-phenylquinazolines **7a**, **7b***

Compound **5a** or **5b** (0.01 mol) and triethyl orthoformate (0.01 mol) were taken in 10 mL of ethanol and heated at reflux on a water bath for 18 h. Completion of the reaction was monitored by TLC. The solvent was evaporated *in vacuo* and the residue was washed with a small amount of ethanol and filtered. The product was dried and purified by column chromatography.

*4-(1,3,4-Oxadiazol-2-ylmethoxy)-2-phenylquinazoline **7a***

Yield: 52% (158 mg); m. p.: 176°C; IR (KBr, cm^{-1}): 1326 (C–O–C); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.4 (s, 2H, OCH_2), 7.4–7.6 (m, 4H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.4 (s, 1H, Ar-H), 8.5 (s, 1H, oxadiazole-H); EI-MS: 305 [M + 1] (95%). Anal. calcd. for $C_{17}H_{12}N_4O_2$: C, 67.10; H, 3.97; N, 18.41. Found: C, 66.95; H, 3.82; N, 18.24.

*6-Bromo-4-(1,3,4-oxadiazol-2-ylmethoxy)-2-phenyl-quinazoline **7b***

Yield: 51% (158 mg); m. p.: 184°C; ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.5 (s, 2H, OCH_2), 7.5–7.6 (m, 3H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.5 (s, 1H, Ar-H), 8.6 (s, 1H, oxadiazole-H); EI-MS: 384 [M + 1] (65%). Anal. calcd. for $C_{17}H_{11}BrN_4O_2$: C, 53.28; H, 2.89; N, 14.62. Found: C, 53.13; H, 2.76; N, 14.47.

*Synthesis of 5-{[(2-phenylquinazolin-4-yl)oxy]methyl}-1,3,4-oxadiazol-2(3H)-thione derivatives **8a**, **8b***

Compounds **5a** & **5b** (0.001 mol) and carbon disulphide (0.003 mol) were taken in ethanolic solution of KOH (0.1 mol, 15 mL) and heated at reflux on a water bath for 20 h. Completion of the reaction is monitored by TLC. The solvent was evaporated under reduced pressure and the residue was triturated with cold water. Neutralization of the solution with acetic acid yielded a precipitate which was filtered, dried, and purified using column chromatography.

*5-{[(2-Phenylquinazolin-4-yl)oxy]methyl}-1,3,4-oxadiazol-2(3H)-thione **8a***

Yield: 61% (205 mg); m. p.: 198°C; IR (KBr, cm^{-1}): 3448 (N-H), 1204 (C=S), 1632 (C=N), 3051, 2851 (C–H); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.3 (s, 2H, OCH_2), 7.3 (s, 1H, N-H), 7.4–7.6 (m, 4H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.5 (s, 1H, Ar-H); EI-MS: 336 [M^+] (100%). Anal. calcd. for $C_{17}H_{12}N_4O_2S$: C, 60.70; H, 3.60; N, 16.66. Found: C, 60.65; H, 3.72; N, 16.75.

5-{[(6-Bromo-2-phenylquinazolin-4-yl)oxy]methyl}-1,3,4-oxadiazol-2(3H)-thione ***8b***

Yield: 52% (215 mg); m. p.: 204°C; IR (KBr, cm^{-1}): 3450 (N–H), 1220 (C=S), 1630 (C=N), 2865 (C-H); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.4 (s, 2H, OCH_2), 7.3 (s, 1H, N–H), 7.6–7.8 (m, 4H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.3–8.5 (s, 1H, Ar-H); EI-MS: 415 [M + 1] (60%). Anal. calcd. for $C_{17}H_{11}BrN_4O_2S$: C, 49.17; H, 2.67; N, 13.49. Found: C, 49.15; H, 2.43; N, 13.56.

Synthesis of 5-{[(2-phenylquinazolin-4-yl)oxy]methyl}-2,4-dihydro-3H-1,2,4-triazole-3-thiones ***9a, 9b***

A mixture of compound **5a** or **5b** (0.01 mol) and potassium thiocyanate (0.01 mol) was taken up in ethanolic solution of sodium hydroxide (0.1 mol) and the resulting solution was heated at reflux on a water bath for 24 h. Completion of the reaction was monitored by TLC. The solvent was removed *in vacuo* and the residue was triturated with ice-cold water. Neutralization of the solution using 5% hydrochloric acid solution yielded a product which was filtered and dried. The product was purified by column chromatography.

5-{[(2-Phenylquinazolin-4-yl)oxy]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-thione ***9a***

Yield: 60% (200 mg); m. p.: 84°C; IR (KBr, cm^{-1}): 3450 (NH), 1320 (C-O), 1230 (C=S); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.3 (s, 2H, OCH_2), 7.4–7.6 (m, 4H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.3 (s, 1H, Ar-H), 11.1 (s, 2H, N-H); EI-MS: 336 [M + 1] (80%). Anal. calcd. for $C_{17}H_{13}N_5OS$: C, 60.88; H, 3.91; N, 20.88. Found: C, 60.84; H, 3.87; N, 20.73.

5-{[(6-Bromo-2-phenylquinazolin-4-yl)oxy]methyl}-2,4-dihydro-3H-1,2,4-triazol-3-thione ***9b***

Yield: 53% (219 mg); m. p. 93°C; IR (KBr, cm^{-1}): 3426 (NH), 1315 (C-O), 1224 (C=S); ^{1}H-NMR ($CDCl_3$, ppm) δ: 4.6 (s, 2H, OCH_2), 7.6–7.7 (m, 3H, Ar-H), 7.8–7.9 (t, 2H, Ar-H), 8.2–8.3 (t, 2H, Ar-H), 8.4 (s, 1H, Ar-H), 11.2 (s, 2H, N-H); EI-MS: 415 [M + 1] (60%). Anal. calcd. for $C_{17}H_{12}BrN_5OS$: C, 49.29; H, 2.92; N, 16.90. Found: C, 49.22; H, 2.95; N, 16.76.

Antimicrobial activity

All the compounds were screened for their *in-vitro* antibacterial activity against two Gram-negative strains, *i. e.*, *Escherichia coli* (NCIM 2068) and *Klebsiella pneumonia* (NCIM 2957) and two Gram-positive strains, *i. e.*, *Bacillus subtilis* (NCIM 2921) and *Staphylococcus aureus* (NCIM 2079), and for their antifungal activity against two fungal strains *Candida albicans* (NCIM 3471) and *Aspergillus flavus* (NCIM 555). The specified stains of organisms were procured from the National Chemical Laboratory, Pune, India, and were used for the evaluation of the test compounds by broth dilution method [36]. Cultures of test organisms were maintained on nutrient agar slants and were subcultured in nutrient broth prior to testing. The inoculum size was approximately 10 colony forming units (CFU/mL). The media used was nutrient agar for bacterial strains and sabouraud dextrose agar medium for *Candida albicans* and czapexs dox agar medium for *Aspergillus flavus* procured from Himedia Laboratories, Mumbai, India. All the compounds were dissolved in dimethyl sulfoxide to give a concentration of 1 mg/mL. The test compounds were prepared in different concentrations from 5 μg/mL to 500 μg/mL in DMSO. Ciprofloxacin was used as the standard for antibacterial activity and amphoterecin-B for antifungal activity, while keeping DMSO as control.

In-silico studies

The protein structure of 1KZN was imported in MVD, and missing bond orders, hybridization states, and angles were then assigned. To obtain better potential binding sites in the protein, a maximum of five cavities were detected using parameters such as molecular surface (expanded van der Waals), maximum number of cavities ($n = 5$), minimum cavity volume (10), probe size (1.20), maximum number of ray checks ($n = 16$), minimum number of ray hits ($n = 12$), and grid resolution (0.80). The chosen cavity was further refined using side-chain minimization by selecting the add-visible option at a maximum of steps per residue (10 000) and a maximum of global steps (10 000). The setup for side-chain flexibility by selection of the add-visible option, the setting for the selected flexible side chain during the docking option, and other parameters, all were kept in default.

All docking calculations were carried out using the grid-based MolDock score (GRID) function with a grid resolution of 0.30 A. The binding site on the receptor was defined as extending in *X*, *Y*, and *Z* directions around the Dock molecule with a radius of approximately 10 to 17 Å. The MolDock optimization search algorithm with a maximum of ten runs was used through the calculations, with all other parameters kept as defaults. One pose per run was retained based on root mean square division clustering using a heavy atom threshold set at 2.0 Å and an energy penalty of 100. All the poses were examined manually and the best poses were retained.

Optimization of the parameter for suitable docking

To obtain better potential binding sites in the protein (1KZN), a maximum of three cavities was detected using default parameters. Out of the detected cavities, cavity number 1 (cavity volume, 58 894 Å) was selected for further studies. The chosen cavity was further refined using side-chain minimization by selection of an add-visible option set at a maximum of 10 000 steps per residue and at a maximum of 10 000 global steps. The side-chain flexibility was set by selecting the add-visible option. The same was selected during docking, and the remaining parameters were kept as fixed variables. Furthermore, the docking simulation was run and the best pose for the set derivatives was selected on the basis of the MolDock score and ReRank score.

Authors are thankful to the Secretary, Viswambhara Educational Society for providing the facilities.

The authors have declared no conflict of interest.

References

[1] P. M. Chandrika, T. Yakaiah, A. R. Rao, B. Narsaiah, *et al.*, *Eur. J. Med. Chem.* **2008**, *43*, 846–852.

[2] V. Alagarsamy, M. Rupeshkumar, K. Kavitha, S. Meena, *et al.*, *Eur. J. Med. Chem.* **2008**, *43*, 2331–2337.

[3] V. Alagarsamy, V. Muthukumar, N. Pavalarani, P. V. Asanthanathan, R. Revathi, *Biol. Pharm. Bull.* **2003**, *26*, 557–559.

[4] R. Tyagi, B. Goel, V. K. Srivastava, A. Kumar, *Indian J. Pharm. Sci.* **1998**, *60*, 283–286.

[5] A. Kumar, S. Sharma, B. Archana Kiran, S. Shipra, *et al*., *Bioorg. Med. Chem.* **2003**, *11*, 5293–5299.

[6] M. R. Yadav, S. T. Shirude, A. Parmar, R. Balaraman, R. Giridhar, *Chem. Heterocycl. Comp.* **2006**, *42*, 1038–1045.

[7] J. Varsha, M. Pradeep, K. Sushil, J. P. Stables, *Eur. J. Med. Chem.* **2008**, *43*, 135–141.

[8] D. Chakravarti, R. N. Chakravarti, L. A. Cohen, B. Dasgupta, *et al*., *Tetrahedron* **1961**, *16*, 224–226.

[9] P. M. S. Bedi, V. Kumar, M. P. Mahajan, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 5211–5213.

[10] S. Jantova, S. Stankovsky, K. Spirkova, *Biologia* **2004**, *59*, 741–752.

[11] V. Alagarsamy, G. Murugananthan, R. V. Perumal, *Biol. Pharm. Bull.* **2003**, *26*, 1711–1714.

[12] T. P. Tran, E. L. Ellsworth, M. A. Stier, J. M. Domagala, *et al*., *Bioorg. Med. Chem. Lett.* **2004**, *14*, 4405–4409.

[13] S. S. Ibrahim, A. M. A. Halim, Y. Gabr, S. Ei, *et al*., *Indian J. Chem.* **1998**, *37B*, 62–67.

[14] R. Rohinia, P. M. Reddy, K. Shankera, A. Hu, V. Ravindera, *Eur. J. Med. Chem.* **2010**, *45*, 1200–1205.

[15] V. Gupta, S. K. Kashaw, V. Jatav, P. Mishra, *Med. Chem. Res.* **2008**, *17*, 2–7.

[16] H. J. Boehm, M. Boehringer, D. Bur, H. Gmuender, *et al*., *J. Med. Chem.* **2000**, *43*, 2664–2674.

[17] J. Heddle, A. Maxwell, *Antimicrob. Agents Chemother.* **2002**, *46*, 1805–1815.

[18] S. C. Jain, M. K. Pandey, R. K. Upadhyay, R. Kumar, *et al*., *Phytochemistry* **2006**, *67*, 1005–1010.

[19] R. Thomsen, M. H. Christensen, *J. Med. Chem.* **2006**, *49*, 3315–3321.

[20] D. Lafitte, V. Lamour, P. O. Tsvetkov, A. A. Makarov, *et al*., *Biochemistry* **2002**, *41*, 7217–7223.

[21] M. D. Huband, M. A. Cohen, M. Zurack, D. L. Hanna, *et al*., *Antimicrob. Agents Chemother.* **2007**, *51*, 1191–1201.

[22] T. P. Tran, E. L. Ellsworth, J. P. Sanchez, B. M. Watson, *et al*., *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1312–1320.

[23] S. Ravikanth, G. V. Reddy, K. H. Kishore, P. S. Rao, *et al*., *Eur. J. Med. Chem.* **2006**, *41*, 1011–1016.

[24] T. Yakaiah, B. P. V. Lingaiah, B. Narsaiah, B. Shireesha, *et al*., *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3445–3453.

[25] T. Yakaiah, B. P. V. Lingaiah, B. Narsaiah, K. Pranay Kumar, U. S. N. Murthy, *Eur. J. Med. Chem.* **2008**, *43*, 341–347.

[26] P. Manichandrika, T. Yakaiah, B. Narsaiah, V. Sridhar, *et al*., *Indian J. Chem.* **2009**, *48B*, 840–847.

[27] D. J. Connolly, D. Cusack, T. P. O'Sullivan, P. J. Guiry, *Tetrahedron* **2005**, *61*, 10153–10202.

[28] M. E. Azab, E. A. Kassab, M. A. El-Hashash, R. S. Ali, *Phosphorus Sulfur Silicon Relat. Elem.* **2009**, *184*, 610–625.

[29] B. Sirisha, B. Narsaiah, T. Yakaiah, G. Gayatri, *et al*., *Eur. J. Med. Chem.* **2010**, *45*, 1739–1745.

[30] S. Bahadur, N. Srivastava, S. Mukherji, *Curr. Sci.* **1983**, *52*, 910–912.

[31] H.-J. Yun, Y.-H. Min, M.-J. Kim, J.-A. Lim, *et al*., *Antimicrob. Agents Chemother.* **2002**, *46*, 3071–3074.

[32] P. D. Rogers, R. E. Kramer, J. K. Crews, R. E. Lewis, *J. Antimicrob. Chemother.* **2003**, *51*, 305–312.

[33] M. Zarrin, M. R. Najafi, *Pak. J. Med. Sci.* **2007**, *23*, 323–325.

[34] R. Thomsen, M. H. Christensen, *J. Med. Chem.* **2006**, *49*, 3315–3321.

[35] D. Lafitte, V. Lamour, P. O. Tsvetkov, A. A. Makarov, *et al*., *Biochemistry* **2002**, *41*, 7217–7223.

[36] NCCLS, *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria, which Grows Aerobically*, 5thEd., NCCLS, Villanova, PA, USA, **2000**, Approved Standard M 7-A 5.

