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RESEARCH ARTICLE

New Derivatives of (E)-3-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one: Searching for New Antifungal and Antibacterial Agents

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ABSTRACT

Objective: The objective of the paper was to evaluate the antifungal and antibacterial potential of new derivatives of (*(E)*-3-(5-((substitutedphenylamino)methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one. **Materials and Methods:** Various syntheses of *(E)*-3-(5-(substitutedaminomethyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one derivatives have been synthesized by reacting 2-substituted benzoxazin-4-one with *(E)*-2-(4-Substituedstyryl)-4H-benzo[d] [1,3]oxazin-4-one. All synthesized compounds have been characterized by the infrared, 1HNMR, and mass spectral analysis. Proposed compounds have been evaluated for antifungal and antibacterial activity. The antimicrobial activity of synthesized compounds (QNM-1 to QNM-15) has been carried through the serial dilution method. For bacterial screening, bacterial species were taken includes *Staphylococcus aureus* (MTCC-96), *Bacillus subtilis* (MTCC-441), *Pseudomonas aeruginosa* (MTCC-424), and *Escherichia coli* (MTCC-40). Norfloxacin (1-Ethyl-6-fluoro-1,4,dihydro-4-oxo-7-(1-piperazinyl)-3-quinoline carboxylic acid) was used as the standard drug for antibacterial study. For antifungal screening, the following fungal species were used includes *Aspergillus niger* (MTCC-281), *Candida albicans* (MTCC-227), and *Fusarium oxysporum* (MTCC-284). Clotrimazole was selected as a standard drug for antifungal study. **Results and Discussion:** QNM-1, QNM-2, QNM-3, QNM-5, QNM-7, QNM-9, QNM-12, QNM-14, and QNM-15 were the most active compounds [Table 1] in the synthesized series which were active against both Gram-positive and Gram-negative organisms by the antibacterial screening. In the case of antibacterial activity, the presence of electronegative group (Cl, Br, F, and NO_2) at both R may enhance the activity when they are p-substituted, but the compounds QNM-6 (R1=-C_6H_5Br (o); Ar=-C_6H_5), QNM-10 (R_1 = -C_6H_5F (o); Ar= -C_6H_5F), QNM-11 (R1 =-$C_6H_5NO_2$ (p); Ar=-C_6H_5F), and QNM-4 (R1 =-C_6H_5F (m); Ar=-C_6H_5) with given substitution may result in diminishing the activity. In case of antifungal activity, compounds QNM-1, QNM-5, QNM-7, QNM-9, QNM-11, QNM-12, QNM-14, and QNM-15 were the most active compounds in the synthesized series which were active against both Gram-positive and Gram-negative organisms. In that series, compounds QNM-14, QNM-11, QNM-5, and QNM-7 have shown the highest activity. Compounds QNM-3, QNM-6, QNM-10, and QNM-13 have the least active. This result has also concluded that o-substituted compounds, i.e., -C_6H_5Cl(o), -C_6H_5Cl (m), -C_6H_5Br(o), -C_6H_5F (o), -C_6H_5F (p) at R_1 position my resulted in diminishing or lower the activity.

Keywords: Antibacterial, antifungal, clotrimazole, norfloxacin, quinazoline, serial dilution method

INTRODUCTION

The searching and finding of a new compound with therapeutic potentials numerous approaches explained and explored by the scientist, and this research resulted in the form of dosages that help to cure diseases as well as to maintain health. The development of new drugs has been responsible for decreasing human morbidity and mortality more than any other scientific endeavor. These products have dramatically improved the quality of life

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Table 1: Antibacterial activity of the synthesized compounds

Codes	Ar	R_1	R_2	Antibacterial activity MIC in µg/ml			
				Staphylococcus aureus **(MTCC-96)**	***Bacillus subtilis*** **(MTCC-441)**	***Pseudomonas aeruginosa*** **(MTCC-424)**	***Escherichia coli*** **(MTCC-40)**
QNM-1	$-C_6H_5$	$-C_6H_5$	H	6.14±0.24	12.34±0.14	9.20±1.08	9.14±0.26
QNM-2	$-C_6H_5$	$-C_6H_5Cl$ (o)	H	6.16 ±0.63	16.46±0.66	11.88±0.56	10.68±0.66
QNM-3	$-C_6H_5$	$-C_6H_5Cl$ (m)	H	5.44±0.36	14.66±0.46	10.28±1.6	11.04±0.36
QNM-4	$-C_6H_5$	$-C_6H_5F$ (m)	H	8.18 ±0.64	14.64±0.88	9.22±0.66	9.44±0.66
QNM-5	$-C_6H_5$	$-C_6H_5NO_2$ (p)	H	4.42±0.40	7.32±0.16	8.23 ±1.02	9.13 ±0.20
QNM-6	$-C_6H_5$	$-C_6H_5Br$(o)	H	7.22±0.75	15.20±0.80	12.36±0.44	10.22±0.88
QNM-7	$-C_6H_5Br$	$-C_6H_5Br$ (p)	H	4.40±0.22	8.62±1.80	8.44 ±0.66	8.44 ±0.25
QNM-8	$-C_6H_5Br$	$-C_6H_5F$ (p)	H	5.83±0.26	10.43±0.22	9.42 ±0.25	11.6 ±0.90
QNM-9	$-C_6H_5Br$	$-C_6H_5NO_2$ (p)	H	4.46±0.12	6.40±0.80	8.73 ±1.86	8.58±0.98
QNM-10	$-C_6H_5F$	$-C_6H_5F$ (o)	H	9.12±0.22	15.22±0.26	9.34 ±0.82	12.28±0.36
QNM-11	$-C_6H_5F$	$-C_6H_5NO_2$ (p)	H	8.16±0.70	13.45±0.20	8.56±0.29	8.58±0.86
QNM-12	$-C_6H_5CH_3$	$-C_6H_5Cl$ (p)	H	4.40±0.28	8.28±0.60	9.28±0.76	8.54±0.86
QNM-13	$-C_6H_5CH_3$	$-C_6H_5F$	H	6.45±1.60	15.45±0.80	9.62 ±0.22	8.67±0.80
QNM-14	$-C_6H_5CH_3$	$-C_6H_5NO_2$	H	4.58±0.34	8.68±0.38	8.65±1.64	9.36±0.26
QNM-15	$-C_6H_5CH_3$	$-C_6H_5Br$	H	4.62±0.24	9.60±0.48	9.26±1.66	8.24±0.40
Norfloxacin				4.36±0.20	14.48±0.72	8.48±0.96	8.49±0.28

across all ages.[1] They can prevent illness or when illness occurs, speed recovery, reduces hospital stays, and decreases the need for surgery. To explore new drugs, many hurdles and difficulties were arise and to solve this many rational approach has been developed, i.e., many chemical mediators or enzymes or specific receptor have been identified that play a crucial role in the cure, treatment and to defining the pathological condition. This helps to identify the diseases and their treatment. Candidate drugs designed and synthesized partly on the basis of such known mediators, hormones, metabolites, or substrates.[2]

Quinazoline is an aromatic heterocyclic with a bi-cyclic structure consisting of two fused six-member aromatic rings, a benzene ring, and pyrimidine ring. Quinazoline is a compound made up of two fused six-member aromatic rings, a benzene ring, and a pyrimidine ring. Quinazoline is a fused bicyclic compound earlier known as benzo-1,3-diazine was first prepared in the laboratory by Gabriell. Depending on the position of the keto or oxo group, these compounds may be classified into three types including 4(3H)-quinazolinone, 2(1H) quinazolinone, and 2,4 (1H,3H)-quinazolinedione, of the three quinazolinone structures 4(3H)-quinazolinone are most prevalent, either as intermediates or as natural products in many proposed biosynthetic pathways.

Quinazolinone is a potent hypnotic agent and has been reported to exhibit analgesic, anesthetic, antifungal,[3,4] antibacterial,[5] anticancer, anticonvulsant, antihypertensive, anti-inflammatory, antioxidant, diuretic, muscle relaxant, sedative, anti-hepatitis-A virus, and tranquilizer properties. The 4(3H)-quinazoline and its derivatives have been reported to exhibit anticonvulsant, antimicrobial,[6,7] sedative, tranquilizer, antiviral,[8] analgesic,[9] antibacterial,[10] anesthetic, anticancer, antimalarial,[11] diuretic, antihypertensive, anti-inflammatory, and muscle relaxant properties. 2-Methyl-3-o-tolyl-4(3H)-quinazolinone (Methaqualone) is the most frequently prescribed quinazolinone derivative as a safe sedative-hypnotic and anticonvulsant drug.

A literature survey revealed that the presence of substituted aromatic ring at 3rd position and methyl/phenyl group at 2nd position of 4(3H)-quinazolinone are necessary requirements for the antibacterial activity. This hypothesis encourages us to build the modification of quinazolinone at 2nd and 3rd position. In this paper, 15 compounds have been synthesized using different substitution of benzaldehyde at R_1 position and -o,-m,-p substitution at Ar position

in the 4(3H)-quinazolinone has design to improve the antifungal and antibacterial activity. The objective of the papers was to design, synthesize, and evaluation of synthesized compounds for antifungal and antibacterial activity.

MATERIALS AND METHODS

2-chloroacetyl chloride, thiosemicarbazide, and formaldehyde were purchased from Sigma-Aldrich, New Delhi. Substituted anilines (Aniline, o-fluoro aniline, m-fluoro aniline, p-chloro aniline, o-chloro aniline, m-chloro aniline, o-bromo aniline, m-bromo aniline, p-bromo aniline, and p-nitro aniline) were purchased from HiMedia. Acetic anhydride, di-methyl formamide, glacial acetic acid substituted, and benzaldehyde (Benzaldehyde, p- fluorobenzaldehyde, p-Bromobenzaldehyde, and p-Tolualdehyde) were purchased from CDH (Chemical Drug House), New Delhi, India. The chemical used for experimental work was synthetic grade. The melting points of the synthesized compounds were determined in open glass capillaries. Infrared (IR) spectra were recorded on ALPHA (Bruker) Fourier transform IR spectrometer. Elemental analysis was performed, and found values were within 0.4% of theoretical values. 13C NMR spectra were recorded on Bruker Avance 400 spectrophotometer at 400 MHz, 5 mm multi-nuclear inverse probe head, low, and high-temperature facility, and HRMAS accessory. Mass spectra were recorded using Mass Spectrometers Jeol SX-102 (Fast atom bombardment [FAB]) by ESI.

Chemistry

The synthesis of (E)-3-(5-(substitutedaminomethyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one is accompanied in Figure 1.

Present synthesis comprises

1. Synthesis of 1,3,4-thiadiazole
2. Synthesis of (E)-3-(5-(((4-Substitutedphenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one.

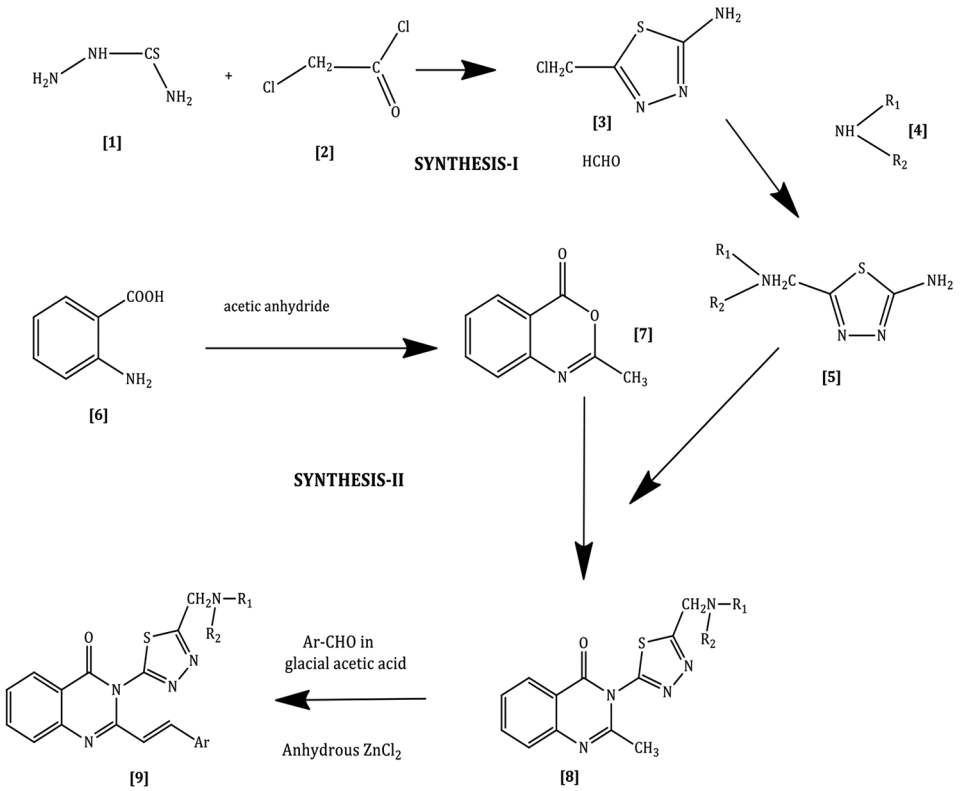

Figure 1: Schematic representation of synthesis-I and scheme-II

SYNTHESIS-I

Synthesis of 1,3,4-thiadiazole

Step 1: Synthesis of 5-(chloromethyl)-1,3,4-thiadiazol-2-amine

In that reaction, substituted amino thiadiazole **[3]** was prepared by the conventional method by the following procedure: In this reaction, 2-chloroacetyl chloride **[2]** (0.1M) and thiosemicarbazide **[1]** (0.1M) were mixed and refluxed with Conc. sulfuric acid for 2½ h. When the reaction is completed, the reaction mixture was cooled in ice bath and neutralized with the ammonia solution (2.5%). The reaction was monitored by the thin-layer chromatography (TLC) method.[12] The solid product thus obtained was filtration and re-crystallize using 75% ethanol. The product is characterized by 1HNMR (6.99 ppm N-H; 4.62 ppm CH_2), and ultraviolet (UV)-spectral analysis. The compounds were shown peak at 280 nm by UV spectroscopic analysis.

Step 2: Synthesis of 5-(substituted-amino methyl)-1,3,4-thiadiazol-2-amine

In that reaction, 5-(chloromethyl)-1,3,4-thiadiazol-2-amine **[3]** (0.1M) was taken in round bottom flask, and formaldehyde was dissolved in methanol (3.0 ml) and was added dropwise with continuous stirring.[13] The resulting mixture was stirred for half an hour to complete the mixing. To this reaction mixture, methanol solution of Aniline, o-fluoro aniline, m-fluoro aniline, p-chloro aniline, o-chloro aniline, m-chloro aniline, o-bromo aniline, m-bromo aniline, p-bromo aniline, and p-nitro aniline (0.1M) **[4]** was mixed and reflux for 2 h at 65–70°C. Then, after the reaction mixture was cool at room temperature and solution poured in cold water. The solidification of compounds arise, and obtained solid was filtered and washed with hot distilled water.[14] The obtained solid product was air-dried for further synthesis. The obtained compound **[5]** was characterized by IR, 1HNMR and was found consistent with an expected structure. The IR data of 3270.5 (N-H str.); 3082.5 (Ar. C-H); 1515.3 (C=N str.); 642.5 (C-S str.); and 1466.9 (N=O asym. str.) confirm the compound N-((5-amino-1,3,4-thiadiazol-2-yl)methyl) nitramide. This compounds further confirmed by the 1HNMR (167 C_2-1,3,4-thiadiazole, 56 ppm CH_2-NH). TLC has been performed each and every step to confirm the completion of the reaction.

SYNTHESIS-II

Synthesis of (E)-3-(5-(((4-Substitutedphenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

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

In this reaction, anthranilic acid [6] (0.01 M) was refluxed under the anhydrous condition for 4 h using acetic anhydride as a solvent the remaining un-reacted acetic anhydride was distilled off to get product N-acetyl anthranillic acid. Then, N-acetyl anthranillic acid was further refluxed with acetic anhydride, under anhydrous condition for 4 h to obtain the solid mass of 2-methyl benzoxazin-4-one [7]. The products were dried and recrystallized from petroleum ether.[15] Reaction was monitored by the TLC for the completion of the reaction. The compounds 7 (2-methyl benzoxazine-4-one) was characterized by 1H-NMR spectra (7.09-8.128 (δ ppm) = m, 4H (Ar); 2.511 (δ ppm) = s, 3H, CH_3). The 2-methyl benzoxazine-4-one was also confirmed by the IR analysis, IR peak shows at N-H str. (primary amine 3580 cm^{-1}), Ar-CH (3200 cm^{-1}).

Step 2: Synthesis of 3-(5-((Substitutedamino)methyl)-1,3,4-thiadiazol-2-yl)-2 methyl quina zolin-4(3H)-one

In that reaction, 2-methyl-4H-benzo[d][1,3]oxazin-4-one **[7]** (0.1 M) and obtained compounds [5] (0.1 M) was suspended in glacial acetic acid[7] and refluxed for 4 h. After completion of reaction, the reaction mixture was cooled at room temperature, and then it was poured into crushed ice and kept overnight in the refrigerator.[16] The obtained solid product **[8]** was filtered, washed with cold water and recrystallized from hot ethanol (75%). The synthesis was monitored by the TLC for the completion of the reaction.

Step 3: Synthesis of (E)-3-(5-(((4-Substitutedphenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

In that reaction, equimolar quantity of compound **[8]** (0.2 M) was taken in round bottom

flask, benzaldehyde and substituted benzaldehyde (p- fluorobenzaldehyde; p-Bromobenzaldehyde/p-Tolualdehyde) were dissolved in glacial acetic acid (0.2 M)and refluxed at 130–140°C for 2 h by the addition of anhydrous zinc chloride (0.1 g). After, reaction completion, the mixture was washed with cold water to dissolve un-reacted zinc chloride.[17] The obtained solid residue after filtration was washed with cold ethanol. Purification of the synthesized compounds **[9]** was done by dissolving the compounds in the minimum quantity of dimethylformamide (DMF) and then added this solution to distilled water. This synthesis was monitored by the TLC to confirm the completion of the reaction.

Antimicrobial activity

Evaluation of antimicrobial activity

Evaluation of the antimicrobial activity has been carried through the serial dilution method. Minimum inhibitory concentration (MIC) was determined by the serial dilution method.[18]

Antibacterial screening of the synthesized compounds (QNM-1 to QNM-15)

For the bacterial screening of the synthesized compounds **(QNM-1 to QNM-15),** the following bacterial species were taken includes *Staphylococcus aureus* (MTCC-96);[19] *Bacillus subtilis* (MTCC-441);[20] *Pseudomonas aeruginosa* (MTCC-424); and *Escherichia coli (MTCC-40)*. Norfloxacin(1-Ethyl-6-fluoro-1,4,dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid) was used as the standard drug for antibacterial study. It is active against Gram-positive and Gram-negative bacteria both. It acts by inhibiting the subunit of DNA *gyrase*, which is essential for the reproduction of bacterial DNA.[21]

Preparation of Solution of Standard Drug

A stock solution of norfloxacin (1 mg/ml) was prepared in DMF (N,N-DMF). Further dilutions were made accordingly using the same solvent as per the requirements.

Preparation of solution of the synthesized compounds

A stock solution of each synthesized compound (1 mg/ml) was made in DMF. Further dilutions were made as above according to requirements.

Measurement of activity

Determination of MIC

The serial dilution method has been used for the determination of MIC. A set of "8" sterilized test tubes were taken, and different solutions were transferred aseptically to each test tube as per the quantities given below:

Test tube no. 6, 7, and 8 was controls. Test tube 6 contained no inhibitor that confirmed the culture was viable and no solvent effect. Test tube 7 contained neither inhibitor nor organism, which confirmed the sterility of the culture, test tube 8 contained a high concentration of inhibitor but no organism to detect the precipitation caused by the interaction of broth constituents and test compounds. In the case of the standard, a set of "8" sterilized test tube was taken, to each of the test tubes, different solutions were transferred aseptically as per the quantities given below:

All the test tubes were kept for incubation for 48 h at 37°C, examined for growth of the test organism.

Test tube No.	Test comp. (100 µg/ml)	Inoculum	Nutrient broth	Final Conc. of test comp. (µg/ml)	Solvent blank (dimethylformamide)
1.	0.4 ml	0.1 ml	9.5 ml	4.0	-
2	0.6 ml	0.1 ml	9.3 ml	6.0	-
3	0.8 ml	0.1 ml	9.1 ml	8.0	-
4	1.0 ml	0.1 ml	8.9 ml	10.0	-
5	1.2 ml	0.1 ml	8.7 ml	12.0	-
6	-	0.1 ml	9.4 ml	-	0.5 ml
7	-	-	10 ml	-	-
8	1.1 ml	-	8.9 ml	11.0	-

Test tube No.	Norfloxacin (100 µg/ml)	Inoculum	NB	Final concentration of norfloxacin (µg/ml)
1.	0.05 ml	0.1 ml	9.85 ml	0.5
2.	0.1 ml	0.1 ml	9.8 ml	1.0
3.	0.2 ml	0.1 ml	9.7 ml	2.0
4.	0.4 ml	0.1 ml	9.5 ml	4.0
5.	0.6 ml	0.1 ml	9.3 ml	6.0
6.	0.8 ml	0.1 ml	9.1 ml	8.0
7.	1.0 ml	0.1 ml	8.9 ml	10.0
8.	1.2 ml	0.1 ml	8.7 ml	12.0

The MIC of the test compound was between the lowest concentration inhibiting growth and the highest concentration allowing growth. These two concentrations for each synthesized were noted. The exact MIC of each synthesized compound was determined by repeating the experiment, using a range of concentration between these two concentrations. For example, the lowest concentration inhibiting growth and highest concentration allowing growth were 10 µg/ml and 20 µg/ml, respectively, this means the MIC of the test compound was between 10 µg/ml and 20 µg/ml. The determination was repeated with test compound concentration of 10 µg/ml, 11 µg/ml, 12 µg/ml, 20µg/ml to get the exact MIC of the test compound. The final result of the MIC determination of the synthesized compounds is given in Table 1.

Antifungal screening of the synthesized compounds

For antifungal screening, the following fungal species were used includes

- *Aspergillus niger* (MTCC-281): It belongs to the class deuteromycota. It reproduces by means of a sexual spore formation known as conidiospore, which is a unicellular or multi-cellular spore that is not enclosed in a sac.[22] It is used in the fermentation industry for the production of glucuronic acid as well as citric acid. Many species of *Aspergillus* are responsible for the human disease called aspergillosis, and *A. niger* is a mold that is rarely reported as a cause of pneumonia.[23] The less thermotolerant, ideal temperature for growth is 30–34 °C, making germination difficult in human body temperature of at least 37°C[24]
- *Candida albicans* (MTCC-227): It is pathogenic yeast, which belongs to the same class as *A. niger*. It is responsible for the disease known as candidiasis that can affect skin, mucous membranes, and nails.[25] It produces chlamydospore, a thick-walled spore formed by rounding and enlargement within the hyphae segment. Morphologically, it is yeast like with pseudohyphae
- *Fusarium oxysporum* (MTCC-284): It is a frequent agent for a mycotic eye infection, most commonly affecting the cornea. It is also occasionally involved in a variety of infections, including mycetoma, sinusitis, septic arthritis, and nail infection[25]
- Clotrimazole (1-(o-chloro-α, α-diphenyl) benzyl imidazole) was selected as standard drug for antifungal study. It is a broad-spectrum antifungal agent.[26]

Preparation of solution of standard drug

A stock solution of clotrimazole (1 mg/ml) was prepared in DMF and further diluted as reported for antibacterial studies.

Preparation of solution of the synthesized compounds

The solutions were prepared in the same way as mentioned under antibacterial screening.

Measurement of activity

MIC for standard drug, i.e., clotrimazole and for synthesized compounds was determined using the same procedure as described under antibacterial screening. The result is shown in Table 2. Initial

antimicrobial activity data for the quinazolinone analogs are reported in Tables 1 and 2, along with the literature data on clotrimazole.

RESULTS AND DISCUSSION

Spectral analysis

The structures of the synthesized compounds (QNM-1 to QNM-15) were characterized by IR, 13C NMR spectra, and mass spectroscopy. The IR spectra of the synthesized compounds showed characteristic absorption band between 1680 and 1700 cm^{-1} due to C=O str (quinazolinone ring); between 1600 and 1650 cm^{-1} due to C=C str. (vinyl group); between 1520 and 1560 cm^{-1} due to C=N str. (1,3,4-thiadiazole and quinazolinone ring); between 1210 and 1250 due to C-N str of quinazolinone ring; between 550 and 780 cm^{-1} due to C-S str. (1,3,4-thiadiazole ring); 1090 cm^{-1} due to Ar-Cl str. and between 400 and 500 cm^{-1} due to aryl C-Cl in chloro containing compounds and 3163.3 C-H str. (Aromatic ring).

In 13C-NMR spectra of the synthesized compounds C-2 and C-4 of quinazolinone were observed between 160–165 and 167–168 (δ, ppm), respectively, C-11 and (C-5, C-6, C-7, C-8, C-9, C-10, C-12, C-13, C-14 and C18, C16, C15 and C17, and C16) of quinazolinone were observed between 112–115 and 122.1–147.8 (δ, ppm) respectively. Methyl carbons were observed at 21.3 ppm. In addition, peaks at δ 77.0 ppm for CDCl3 (solvent) and at δ 39.0 ppm for dimethyl sulfoxide -d6 (solvent) were also observed in respective cases. Elemental analysis of all synthesized compounds was within the ±0.4% of the theoretical values. Generation of dense sooty flame and formation of oily layer after nitration of the compounds confirmed the presence of aromatic ring in all the synthesized compounds. In the FAB mass spectra, two prominent peaks were observed. TLC has been executed for the monitored of reaction and purity of the synthesized compounds using silica gel G in various solvent systems such as hexane/ethanol (95%)/chloroform/ benzene, and iodine chamber has been used for the visualization and in some cases UV chamber used. All these characterization parameters showed that the structure of the synthesized compounds was near to expected.

QNM-1: (E)-3-(5-((phenylamino)methyl)-1,3,4-thiadiazol-2-yl)-2-styrylquinazolin-4(3H)-one

Molecular formula: $C_{25}H_{19}N_5OS$; Molecular weight: 437.52; TLC (Rf value):0.45; element analysis

Table 2: Antifungal activity of the synthesized compounds

Codes	Ar	R_1	R_2	Antifungal activity minimum inhibitory concentration in µg/ml		
				Aspergillus niger (MTCC-281)	C. albicans (MTCC-227)	*Fusarium oxysporum* (MTCC-284)
QNM-1	$-C_6H_5$	$-C_6H_5$	H	12.30±0.24	10.28±1.08	10.58±0.18
QNM-2	$-C_6H_5$	$-C_6H_5Cl$ (o)	H	14.48±0.64	13.88±0.34	13.20±0.44
QNM-3	$-C_6H_5$	$-C_6H_5Cl$ (m)	H	15.28±1.64	14.84±1.88	14.48±1.46
QNM-4	$-C_6H_5$	$-C_6H_5F$ (m)	H	12.66±0.88	10.44±0.85	12.64±0.32
QNM-5	$-C_6H_5$	$-C_6H_5NO_2$ (p)	H	11.42±0.22	7.23±1.02	10.13±0.20
QNM-6	$-C_6H_5$	$-C_6H_5Br$(o)	H	16.24±0.26	12.38±0.66	13.46±0.60
QNM-7	$-C_6H_5Br$	$-C_6H_5Br$ (p)	H	11.46±0.58	7.04 ±0.82	10.41±0.40
QNM-8	$-C_6H_5Br$	$-C_6H_5F$ (p)	H	13.56±0.66	7.58±0.20	12.34±0.57
QNM-9	$-C_6H_5Br$	$-C_6H_5NO_2$ (p)	H	11.90±0.76	6.88±1.20	10.68±0.54
QNM-10	$-C_6H_5F$	$-C_6H_5F$ (o)	H	14.84±0.70	13.68 ±0.66	14.64 ±0.22
QNM-11	$-C_6H_5F$	$-C_6H_5NO_2$ (p)	H	11.40±0.21	7.85±0.52	10.60±0.98
QNM-12	$-C_6H_5CH_3$	$-C_6H_5Cl$ (p)	H	11.64±0.30	6.75±0.50	9.95±0.82
QNM-13	$-C_6H_5CH_3$	$-C_6H_5F$ (p)	H	13.44±0.36	6.60±1.10	11.26±0.46
QNM-14	$-C_6H_5CH_3$	$-C_6H_5NO_2$(p)	H	11.40±0.26	7.30±0.62	10.26±0.76
QNM-15	$-C_6H_5CH_3$	$-C_6H_5Br$ (p)	H	11.68±0.36	6.44 ±0.58	10.20±0.24
Clotrimazole				11.56±0.32	6.40±0.26	9.88±0.71

found (Calculated): Nitrogen (%) 16.01 (15.98); sulfur (%) 7.33 (7.31); oxygen (%) 3.66 (3.64). IR (cm^{-1}): 3020 (C-H str.); 760 (C-H def.); 1700 (C=O str.); 1174 (-C_6H_5); 1516 (C=C str.) 2856 (C-H str.); 3120 (C-H str.); 1461 (C-H str.); 1580 (C-C str.); 1614 (C=C str.); 1326 (C-N str.) 1555 (C=N str.); 760 (C-S str.) 13C NMR (ppm): 113.3 (C11 due to styryl group attached to 4-quinazolinone ring); 126.7 (C8 due to 4-quinazolinone ring); 128.5 (C_{14} and C^{18} due to phenyl substituted styryl group attached to 4-quinazolinone ring); 145.5 (C9, due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring); 127.9 (C_{16} due to phenyl substituted styryl group attached to 4-quinazolinone ring); 127.3 (C6 due to 4-quinazolinone ring); 128.6 (C15 and C17, due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring); 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.6, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 135.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.1, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, C_{14} due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.9, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; and 51.3a, due to CH2–NH attached to 1,3,4 thiadiazole ring. FAB Mass (m/z): 437.11.

QNM-2: (E)-3-(5-(((4-chlorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one

Molecular formula:$C_{25}H_{18}ClN_5OS$; Molecular weight: 471.96; TLC (Rf value): 0.65; elemental analysis found (Calculated): Nitrogen (%) 14.82 (14.84); sulfur (%) 6.72 (6.79); oxygen (%) 3.37 (3.39); IR (cm^{-1}): 3020 C-H str.; 760 C-H def.; 1700 C=O str.; 1174 -C6H5; 1516 C=C str.; 2856 C-H str.; 3120 C-H str.; 1461 C-H str.; 1580 C-C str.; 1614 C=C str.; 1326 C-N str.; 1555 C=N str.; 760 C-S str.; 1538 C-Cl str.; 13C NMR (ppm):113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.7, C8 due to 4-quinazolinone ring; 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 145.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring 128.6, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.6, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 135.2 C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.1, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.9, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; and 51.3 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 472.00.

QNM-3: (E)-3-(5-(((4-chlorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}ClN_5OS$; molecular weight: 471.96; TLC (Rf value): 0.65; elemental analysis: Found (Calculated): Nitrogen (%) 14.82 (14.84); sulfur (%) 6.72 (6.79); oxygen (%) 3.37 (3.39); IR (cm^{-1}): 3020 C-H str.; 760 C-H def; 1700 C=O str.; 1174 -C6H5; 1516 C=C str.; 2856 C-H str.; 3120 C-H str.; 1461 C-H str.; 1580 C-C str.; 1614 C=C str.; 1326 C-N str.; 1555 C=N str.; 760 C-S str.; 1542 C-Cl str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.7, C8 due to 4-quinazolinone ring; 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 145.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 128.6, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.6, C5 due to 4-quinazolinone ring; 133.4,

C7 due to 4-quinazolinone ring; 135.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.1, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.9, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; and 51.3a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 472.

QNM-4: (E)-3-(5-(((2-fluorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}FN_5OS$; molecular weight: 455.51; TLC (Rf value): 0.68; elemental analysis: Found (Calculated): Nitrogen (%) 15.35 (15.37); sulfur (%) 7.02 (7.04); Oxygen (%) 3.45 (3.51); IR (cm^{-1}): 3261 C-H str.; 886 C-H def (oop);1700 C=O str.; 1174 -C6H5; 1540 N=0 str.; 1320 N-O str.; 1593 C=C str.; 2856 C-H str.; 3057 C-H str.; 1382 C-H def; 1442 C-C str.; 1620 C=C str.; 1274 C-N str.; 740 C-S str.; 650 C-F str.; 13C NMR (ppm): 113.5, C11 due to styryl group attached to 4-quinazolinone ring; 126.9, C8 due to 4-quinazolinone ring; 128.1, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.4, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 136.3, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 127.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.3, C5 due to 4-quinazolinone ring; 133.6, C7 due to 4-quinazolinone ring; 135.1, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.6, C12 due to styryl group attached to 4-quinazolinone ring; 155.4, phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.7, C2 due to 4-quinazolinone ring; 160.9, C4 due to 4-quinazolinone ring; and 51.1 a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB mass (m/z): 455.31.

QNM-5: (E)-3-(5-(((4-nitrophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}N_6O_3S$; molecular weight: 482.51; TLC (Rf value): 0.67; elemental analysis found (Calculated): Nitrogen (%) 17.38 (17.42); sulfur (%) 6.60 (6.65); oxygen (%) 9.90 (9.95); IR (cm^{-1}): 3261 C-H str.; 831 C-H def (oop); 1700 C=O str.;1174 -C6H5; 1540 N=0 str.; 1320 N-O str.; 1593 C=C str.; 856 C-H str.; 3057 C-H str.; 1382 C-H def; 1442 C-C str.; 1620 C=C str.; 1274 C-N str.; 740 C-S str.; 13C NMR (ppm): 113.5, C11 due to styryl group attached to 4-quinazolinone ring; 126.9, C8 due to 4-quinazolinone ring; 128.1, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.4, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.9, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 136.3, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 127.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.3, C5 due to 4-quinazolinone ring; 133.6, C7 due to 4-quinazolinone ring; 135.1, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.6, C12 due to styryl group attached to 4-quinazolinone ring; 155.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.7, C2 due to 4-quinazolinone ring; 160.9, C4 due to 4-quinazolinone ring; and 51.1a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 482.

QNM-6: (E)-3-(5-(((4-bromophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}BrN_5OS$; molecular weight: 516.41; TLC (Rf value): 0.65; elemental analysis found (Calculated): Nitrogen (%) 13.46 (13.56); sulfur (%) 6.18 (6.21); oxygen (%) 3.09 (3.10); IR (cm^{-1}): 3211 C-H str.; 774 C-H def (oop); 1701 C=O str.; 1596 C=C str.; 2896 C-H str.; 3060 C-H str.; 1447 C-H def; 1470 C-C str.; 1637 C=C

str.; 1316 C-N str.; 1530 C=N str.; 719 C-S str.; 570 C-Br str.; 13C NMR (ppm): 113.2, C11 due to styryl group attached to 4-quinazolinone ring; 126.4, C8 due to 4-quinazolinone ring; 129.0, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 133.5, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.7, C6 due to 4-quinazolinone ring; 115.1, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 132.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.4, C5 due to 4-quinazolinone ring; 133.8, C7 due to 4-quinazolinone ring; 135.3, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.5, C12 due to styryl group attached to 4-quinazolinone ring; 148.3, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.1, C10 due to 4-quinazolinone ring; 158.4, C2 due to 4-quinazolinone ring; 160.6, C4 due to 4-quinazolinone ring; and 51.1a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB mass (m/z): 516.13.

QNM-7: (E)-3-(5-(((4-nitrophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin -4(3H)-one

Molecular formula: $C_{25}H_{18}N_6O_3S$; molecular weight: 482.51; TLC (Rf value): 0.67; elemental analysis: Found (Calculated): Nitrogen (%) 17.38 (17.42); sulfur (%) 6.60 (6.65); oxygen (%): 9.90 (9.95); IR (cm-1): 3125 (C-H str.); 808 (C-H def (oop); 1700 (C=O str.; 1590 C=C str.; 2945 C-H str.; 3050 C-H str.; 1450 C-H def.; 1570 C-C str.; 1630 C=C str.; 1348 C-N str.; 1560 C=N str.; 575 C-S str.; 520 C-Br str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 126.2 C8 due to 4-quinazolinone ring: 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 118.9, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.8, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.9, C6 due to 4-quinazolinone ring; 155.2, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 116.3, phenyl ring attached to 1,3,4 thiadiazole ring; 126.3, C5 due to 4-quinazolinonering;133.7,C7dueto4-quinazolinone ring; 135.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.5, C12 due to styryl group attached to 4-quinazolinone ring; 144.9, phenyl ring attached to 1,3,4 thiadiazole ring; 120.5, C10 due to 4-quinazolinone ring 158.2, C2 due to 4-quinazolinone ring; 160.1, C4 due to 4-quinazolinone ring; and 51.5a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 482.51.

QNM-8: (E)-3-(5-(((4-nitrophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-styryl quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{18}N_6O_3S$; molecular weight: 482.51; TLC (Rf value): 0.67; elemental analysis found (Calculated): Nitrogen (%) 17.38 (17.42); sulfur (%) 6.60 (6.65); oxygen (%) 9.90 (9.95); IR (cm^{-1}): 3074 C-H str.; 718 C-H def (oop); 1734 C=O str.; 1580 N=O str.; 1350 N-O str.; 1597.1 C=C str.; 2944 C-H str.; 3020 C-H str.; 1380 C-H def.; 1456 C-C str.; 1634 C=C str.; 1239 C-N str.; 658 C-S str.; 520 C-Br str.; 13C NMR (ppm): 113.2, C11 due to styryl group attached to 4-quinazolinone ring; 126.4 C8 due to 4-quinazolinone ring; 128.4 C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.9, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 133.5, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 129.1, C6 due to 4-quinazolinone ring; 136.3, C15 and C17, C due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2, C5 due to 4-quinazolinonering;133.4,C7dueto4-quinazolinone ring; 135.1, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring;138.2, C12 due to styryl group attached to 4-quinazolinone ring; 155.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.2, C10 due to 4-quinazolinone ring; 158.6, C2 due to 4-quinazolinone ring; 160.3, C4 due

to 4-quinazolinone ring; and 51.1a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 482.51.

QNM-9: (E)-2-(4-bromostyryl)-3-(5-(((4-nitrophenyl)amino)methyl)-1,3,4-thiadiazol-2-yl) quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}BrN_6O_3S$; molecular weight: 561.41; TLC (Rf value): 0.65; elemental analysis found (Calculated): Nitrogen (%) 14.92 (14.97); sulfur (%) 5.68 (5.71); oxygen (%) 8.45 (8.55); IR (KBr, cm-1): 3159 C-H str.; 761 C-H def (oop); 1701 C=O str.; 1562 C=C str.; 2907 C-H str.; 3010 C-H str.; 1375 C-H def; 1439 C-C str.; 1693 C=C str.; 1274 C-N str.; 754 C-S str.; 520 C-Br str.; 1540 N=0 str.; 1320 N-O str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4 quinazolinone ring; 126.2, C8 due to 4-quinazolinone ring; 128.4, C14 and C18 due to phenyl substituted styryl group attached to 4 quinazolinone ring; 113.4, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.1, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.4, C6 due to 4-quinazolinone ring; 136.7, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 128.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2, C5 due to 4-quinazolinone ring; 133.5, C7 due to 4-quinazolinone ring; 135.5, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.7, C12 due to styryl group attached to 4-quinazolinone ring; 146.5, due to phenyl ring attached to 1,3,4 thiadiazole ring;120.2, C10 due to 4-quinazolinone ring;158.3, C2 due to 4-quinazolinone ring; 160.6 C4 due to 4-quinazolinone ring; and 51.5 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 561.41.

QNM-10: (E)-3-(5-(((2-fluorophenyl)amino) methyl)-1,3,4-thiadiazol-2-yl)-2-(4-fluoro styryl)quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}F_2N_5OS$; molecular weight: 473.50; TLC (Rf value):0.62; elemental analysis found (Calculated): Nitrogen (%) 14.75 (14.79); sulfur (%) 6.74 (6.77); oxygen (%) 3.32 (3.38); IR (KBr, cm-1): 3157 C-H str.; 819 C-H def (oop); 1703 C=O str.; 1080 C-O-C str.; 1559 C=C str.; 2909 C-H str.; 3050 C-H str.; 1417 C-H def.; 1450 C-C str.; 1609 C=C str.; 1252 C-N str.; 1519 C=N str.; 615 C-S str.; 650 C F str.; 13C NMR (ppm): 113.1, C11 due to styryl group attached to 4-quinazolinone ring; 126.2, C8 due to 4-quinazolinone ring; 128.3, C14 and C18 due to phenyl substituted styryl group attached to 4 quinazolinone ring; 113.3, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 127.6, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 151.7, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 126.5, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 135.7, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.8, C12 due to styryl group attached to 4-quinazolinone ring; 120.3, C10 due to 4-quinazolinone ring; 158.6, C2 due to 4-quinazolinone ring; and 160.8, C4 due to 4-quinazolinone ring; 51.5 a, due to CH_2–NH attached to 1,3,4 thiadiazole ring: FAB mass (m/z): 473.16.

QNM-11: (E)-2-(4-fluorostyryl)-3-(5-(((4-nitrophenyl)amino)methyl)-1,3,4-thiadiazol-2-yl)quinazolin-4(3H)-one

Molecular formula: $C_{25}H_{17}FN_6O_3S$; molecular weight: 500.50; TLC (Rf value): 0.75; elemental analysis: Found (Calculated): Nitrogen (%) 16.78 (16.79); sulfur (%) 6.39 (6.41); oxygen (%) 9.57 (9.59); IR (KBr, cm^{-1}): 3160 C-H str; 760 C-H def (oop); 1693 C=O str.; 1590 C=C str.; 2902 C-H str.; 3020 C-H str.; 1373 C-H def.; 1437 C-C str.; 1580 N=0 str.; 1370 N-O str.; 1610 C=C str.; 1316 C-N str.; 1568 C=N str.; 667 C-S str.; 650 C-F str.; 13C NMR (ppm): 113.1, C11 due to styryl group attached to 4-quinazolinone ring; 126.3, C8 due to 4-quinazolinone ring; 129.0, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.9, C9 due to

4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 147.1, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 123.8, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.1, C5 due to 4-quinazolinone ring; 133.7 C7 due to 4-quinazolinone ring; 141.3, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.2, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.8, C10 due to 4-quinazolinone ring; 158.2 C2, due to 4-quinazolinone ring; 160.3, C4 due to 4-quinazolinone ring; and 51.3 a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; FAB Mass (m/z): 500.50.

QNM-12: (E)-3-(5-(((4-chlorophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-(4-methylstyryl) quinazolin-4(3H)-one

Molecular formula: $C_{26}H_{20}ClN_5OS$; molecular weight: 485.99; TLC (Rf value): 0.62; elemental analysis found (Calculated): Nitrogen (%) 14.35 (14.41); sulfur (%) 6.56 (6.60);Oxygen (%) 3.25 (3.29); IR (KBr, cm^{-1}): 3117.3 C-H str.; 752.8 C-H def (oop); 1689.5 C=O str.; 1594 C=C str.; 2917 C-H str.; 3020 C-H str.; 1448.3 C-H def.; 1240 C-C str.; 1610 C=C str.; 1346 C-N str.; 1519 C=N str.; 608 C-S str.; 464.4 C-Cl str.; 13C NMR (ppm): 113.4, C11 due to styryl group attached to 4-quinazolinone ring; 127.3, C8 due to 4-quinazolinone ring; 128.5, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.4, C9, due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 137.6, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.1, C6 due to 4-quinazolinone ring; 128.9, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.4due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.5, C5 due to 4-quinazolinone ring; 133.4, C7 due to 4-quinazolinone ring; 132.2, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.4, C12 due to styryl group attached to 4-quinazolinone ring; 147.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.1, C2 due to 4-quinazolinone ring; 160.7, C4 due to 4-quinazolinone ring; 51.1a, due to CH_2–NH attached to 1,3,4 thiadiazole ring; 15.4 CH3; and phenyl substituted styryl group attached to 4-quinazolinone ring; FAB Mass (m/z): 486.13.

QNM-13: (E)-3-(5-(((4-fluorophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-(4-methylstyryl) quinazolin-4(3H)-one

Molecular formula: $C_{26}H_{20}FN_5OS$; molecular weight: 469.53; TLC (Rf value): 0.80; elemental analysis: Found (Calculated): Nitrogen (%) 14.95 (14.92); sulfur (%) 6.82 (6.83); oxygen (%) 3.38 (3.41); IR (KBr, cm^{-1}): 3163.3 C-H str.; 822 C-H def (oop); 1691.4 C=O str.; 1568.1 C=C str.; 2911 C-H str.; 3032.5 C-H str.; 1374.6 C-H def. 1438.2 C-C str.; 1600 C=C str.; 1313.7 C-N str.; 1520 C=N str.; 614 C-S str.; 650 C-F str.; 13C NMR (ppm): 113.3, C11 due to styryl group attached to 4-quinazolinone ring; 127.5, C8 due to 4-quinazolinone ring; 128.3, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 137.5, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.6, C6 due to 4-quinazolinone ring; 118.7, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 132.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2, C5 due to 4-quinazolinone ring; 133.1, C7 due to 4-quinazolinone ring; 132.1, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.2, C12 due to styryl group attached to 4-quinazolinone ring; 148.5, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.1, C10 due to 4-quinazolinone ring; 158.7, C2 due to 4-quinazolinone ring; 160.2, C4 due to 4-quinazolinone ring; 51.3 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; and 15.4, CH_3 phenyl substituted styryl group attached to 4-quinazolinone ring; FAB mass (m/z): 469.23

QNM-14: (E)-2-(4-methylstyryl)-3-(5-(((4-nitrophenyl)amino)methyl)-1,3,4-thia diazol-2 yl)quinzolin-4(3H)-one

Molecular formula: $C_{26}H_{20}N_6O_3S$; molecular weight: 496.54; TLC (Rf value): 0.72; elemental analysis found (Calculated): Nitrogen (%)16.90 (16.93); sulfur (%) 6.45 (6.46); oxygen (%) 9.65 (9.67); IR (KBr, cm^{-1}): 3166.3 C-H str.; 826.4 C-H def (oop); 1690.1 C=O str.; 1579.6 C=C str.; 1540 N=0 str.; 1320 N-O str.; 2791 C-H str.; 3020 C-H str.; 1369.7 C-H def; 1439.1 C-C str.; 1610 C=C str.; 1325 C-N str.; 1540 C=N str.; 666 C-S str.; 13C NMR (ppm): 113.3 C11 due to styryl group attached to 4-quinazolinone ring; 127.1 C8 due to 4-quinazolinone ring; 130.2 C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.9 C9, C and C due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 159.8 C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.4 C6 due to 4-quinazolinone ring; 114.2 C15 and C17, C due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 129.6 C and C due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.2 C5 due to 4-quinazolinone ring; 133.5 C7 due to 4-quinazolinone ring; 127.5 C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.3 C12 due to styryl group attached to 4-quinazolinone ring; 147.4 C due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.1 C10 due to 4-quinazolinone ring; 158.3 C2 due to 4-quinazolinone ring; 160.4 C4 due to 4-quinazolinone ring; 51.3 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; 15.4 CH3; and phenyl substituted styryl group attached to 4-quinazolinone ring; FAB Mass (m/z): 496.50.

QNM-15: (E)-3-(5-(((4-bromophenyl) amino)methyl)-1,3,4-thiadiazol-2-yl)-2-(4-methylstyryl) quinazolin-4(3H)-one

Molecular formula: $C_{26}H_{20}BrN_5OS$ molecular weight: 530.44; TLC (Rf value): 0.63; elemental analysis: Found (Calculated): Nitrogen (%) 13.19 (13.20); sulfur (%) 6.02 (6.04); oxygen (%): 2.97 (3.02); IR (KBr, cm^{-1}): 3163 C-H str.; 824 C-H def (oop); 1695 C=O str.; 1565 C=C str.; 2909 C-H str.; 3050 C-H str.; 1368 C-H def; 1440 C-C str.; 1600 C=C str.; 1322 C-N str.; 1520 C=N str.; 619 C-S str.; 720 C-Br str.; 13C NMR (ppm): 113.3 C11, due to styryl group attached to 4-quinazolinone ring; 127.5, C8 due to 4-quinazolinone ring; 130.3, C14 and C18 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 114.5, C9 due to 4-quinazolinone ring and phenyl ring attached to 1,3,4 thiadiazole ring; 159.6, C16 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 127.3, C6 due to 4-quinazolinone ring; 115.1, C15 and C17 due to phenyl substituted styryl group attached to 4-quinazolinone ring and phenyl ring attached to 1,3,4-thiadiazole ring; 132.4, due to phenyl ring attached to 1,3,4 thiadiazole ring; 126.6, C5 due to 4-quinazolinone ring; 133.1, C7 due to 4-quinazolinone ring; 127.6, C13 due to phenyl substituted styryl group attached to 4-quinazolinone ring; 138.7, C12 due to styryl group attached to 4-quinazolinone ring; 148.3, due to phenyl ring attached to 1,3,4 thiadiazole ring; 120.3, C10 due to 4-quinazolinone ring; 158.1 C2 due to 4-quinazolinone ring; 160.7, C4 due to 4-quinazolinone ring; 51.3 a, due to CH2–NH attached to 1,3,4 thiadiazole ring; 15.4, CH_3; and phenyl substituted styryl group attached to 4-quinazolinone ring; FAB Mass (m/z): 530.42.

Antimicrobial activity

Antibacterial activity

The MIC values as determined for the most potent compounds were found to be to 4.40 μg/ml–9.12 μg/ml for *S. aureus*, 6.40 μg/ml–16.46 μg/ml for *B. subtilis*, 8.44 μg/ml–12.36 μg/ml for *P. aeruginosa,* and 8.24 μg/ml–12.28 μg/ml for *E. coli*. QNM-1, QNM-2, QNM-3, QNM-5, QNM-7, QNM-9, QNM-12, QNM-14, and QNM-15 were the most active compounds [Table 1] in the synthesized series which were active against both Gram-positive and Gram-negative organisms.

Antifungal activity

Compounds QNM-1, QNM-5, QNM-7, QNM-9, QNM-11, QNM-12, QNM-14, and QNM-15 were

the most active compounds in the synthesized series which were active against both Gram-positive and Gram-negative organisms. In that series, compounds QNM-14, QNM-11, QNM-5, and QNM-7 have shown highest activity. Compounds QNM-3, QNM-6, QNM-10, and QNM-13 have least active. The majority of the compounds showed activity against Gram-positive strains as compared to Gram-negative strains. Range of MIC was 11.40 μg/ml–15.28 μg/ml for *A. niger*, 6.44 μg/ml–14.84 μg/ml for *C. albicans,* and 9.95 μg/ml–14.48 μg/ml for *F. oxysporum* [Table 2]. Critical observation suggests that biological activity involves correlation with the physicochemical properties of the synthesized compound. As the log p (lipophilicity) values of the synthesized compounds increases, the antimicrobial activity also increases.

CONCLUSION

The presence of electronegative group (Cl, Br, and NO_2) either at AR (substituted aromatic ring at 5th position of 1,3,4 thiadiazole) is required for the potent antimicrobial activity. In the case of antibacterial activity, the presence of electronegative group (Cl, Br, F, and NO_2) at both R may enhance the activity when they are p-substituted but the compounds QNM-6 (R_1=-C_6H_5Br (o); Ar=-C_6H_5), QNM-10 (R_1=-C_6H_5F (o); Ar=-C_6H_5F), QNM-11 (R_1=-$C_6H_5NO_2$ (p); Ar=-C_6H_5F), QNM-4 (R_1=-C_6H_5F (m); (Ar=-C_6H_5) with given substitution may result in diminish the activity.

In the case of antifungal activity, the presence of electronegative group (Cl, Br, F, and NO_2) at both R may enhance the activity when they are p-substituted but the addition of F may diminish the activity and Ar position lead to the generation of potent compounds which contains C_6H_5, C_6H_5Br, $C_6H_5CH_3$, but the substitution of C_6H_5F at Ar position may diminish the activity. This result has also concluded that o-substituted compounds, i.e., -C_6H_5Cl(o), -C_6H_5Cl (m), -C_6H_5Br(o), -C_6H_5F (o), and -C_6H_5F (p) at R_1 position my resulted in diminishing or lower the activity.

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CONFLICTS OF INTEREST

The author declares that they have no conflicts of interest

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