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Synthesis and Antiviral Bioactivities of 2-Aryl- or 2-Methyl-3-(substituted- Benzalamino)-4(3*H*)-quinazolinone Derivatives

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Abstract: A simple and general method has been developed for the synthesis of various 4(3H)-quinazolinone derivatives by the treatment of the appropriate 3-amino-2-aryl-4(3H)-quinazolinone with a substituted benzaldehyde in ethanol. The structures of the compounds were characterized by elemental analysis, IR, ^{1}H-NMR and ^{13}C-NMR spectra. The title 2-aryl- or 2-methyl-3-(substituted-benzalamino)-4(3H)-quinazolinone compounds **III-1~III-31** were found to possess moderate to good antiviral activity. Semi-quantitative PCR and Real Time PCR assays were used to ascertain the target of action of compound **III-31** against TMV. The studies suggest that **III-31** possesses antiviral activity due to induction of up-regulation of PR-1a and PR-5, thereby inhibiting virus proliferation and movement by enhancement of the activity of some defensive enzyme.

Keywords: 4(3H)-Quinazolinone, Schiff base, synthesis, antiviral activity

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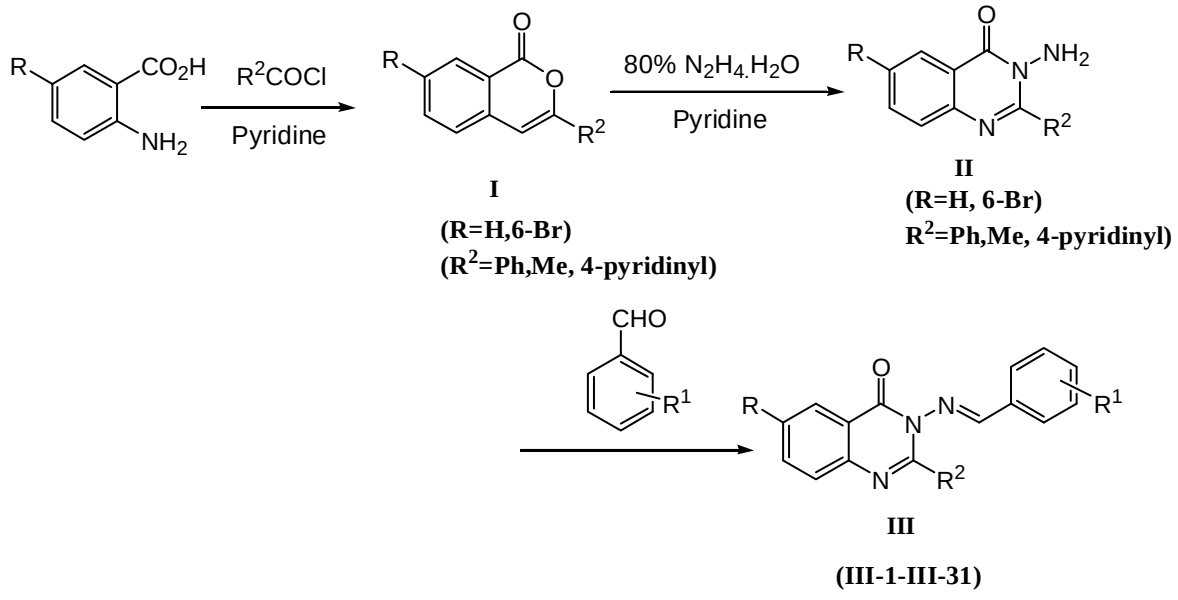
Introduction

4(3H)-Quinazolinones and their derivatives constitute an important class of heterocyclic compounds. They occupy an important position in medicinal and pesticide chemistry, presenting a wide range of bioactivities. As medicines, many of them display antifungal [1], antimicrobial [2], anti-HIV [3], antitubercular [4], anticancer [5], antiinflammatory [6], anticonvulsant [7], antidepressant [8], hypolipidemic [9], antiulcer [10], analgesic [11] or immunotropic activities [12] and are also known to act as thymidyalate synthase [13], poly(ADP-ribose) polymerase (PARP) [14], and protein tyrosine kinase [15] inhibitors. As pesticides, they are used as insecticides [16], fungicides [17] and antiviral agents [18] such as TMV , CMV inhibitors. In light of the growing number of applications in recent years there has been an enormous increase in the interest among biologists and chemists in their synthesis and bioactivity of quinazoline derivatives. In our previous work in this area we reported that some of these compounds showed antifungal activities [17]. Nanda and his co-workers synthesized ten 3-(arylideneamino)-2-phenylquinazoline-4(3H)-ones, which were investigated for their antimicrobial activity against both Gram-positive (*Staphylococcus aureus* 6571 and *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli* K12 and *Shigella dysenteriae* 6) using a turbidometric assay method. It was found that the incorporation of the 3-arylideneamino substituent enhanced the anti-bacterial activity of the quinazolone system [19]. However, no attention has been paid to the antiviral activities for 3-(arylideneamino)-2-phenylquinazoline-4(3H)-ones against TMV (tobacco mosaic virus).

For this report we have designed and synthesized a series of 2-aryl- or 2-methyl-3-(substituted-benzalamino)-4(3H)-quinazolinone derivatives **III-1~III-31** and investigated their antiviral bioactivities. The synthetic route used is shown in Scheme 1. Substitution patterns and optimized yields are given in Table 3. The structures of these compounds were firmly established by well defined IR, ^{1}H-NMR, ^{13}C-NMR data and elemental analysis. Preliminary bioassay tests showed that some compounds displayed *in vivo* antiviral activity against TMV at 500 μg/mL.

Scheme 1. Synthetic route to the title compounds.



Results and Discussion

Different homologues of 3-amino-2-aryl-4(3H)-quinazolinone **II** were prepared following a literature procedure [20]. Reaction conditions were non-homogeneous and the use of an increased amount of hydrazine hydrate did not afford successful results. The reaction conditions for the synthesis of **II-1** (R=H, R^1=H, R^2=Ph) were optimized in various solvents at different temperatures for different times and the results are shown in Table 1. An 87 % yield could be obtained when the reaction mixture was heated in pyridine at 116 °C for 0.5 h.

Table 1. The effect of reaction conditions on yield of intermediate **II-1** (R=H, R^1=H, R^2=Ph).

Entry	Solvent	Reaction temperature (°C)	Reaction time (h)	Yield (%)
1	Anhydrous ethanol	78	3	24
2	Isopropanol	85	3	31
3	*n*-Butanol	117	3	35
4	-	118	3	22
5	Pyridine	116	0.5	87
6	Pyridine	116	3	89

In order to optimize the reaction conditions for the title compounds, the synthesis of **III-1** (R=H, R^1=3, 5-di-chloro，R^2=Ph) was examined under different conditions. First, the role of the catalyst (HOAc) in accelerating the reaction rate was ascertained. While in the presence of catalyst, a 72% yield of **III-1** was achieved in only 10 h (Table 2, entry 1), a yield of only 52% was obtained with a prolonged reaction period of 30 h in the absence of the catalyst (Table 2, entry 2). In addition, we also examined the effects of reaction time. When the reaction time was prolonged further to 20 h, no improvement (75 %) was obtained (Table 2, entry 3), as compared to that of 10 h (72 %). Also, it could be observed that the yield was significantly lower at room temperature (Table 2, entry 4). For different substituted benzaldehydes, under optimal conditions, as depicted in Table 3, **III-1-III-31** could be obtained in 53-89% yields at 78 °C in ethanol in presence of HOAc as the catalyst.

Table 2. The effect of reaction conditions on yield of title compound **III-1.**

Entry	Catalyst	Reaction temperature (°C)	Reaction time (h)	Yield (%)
1	HOAc	78	10	71.9
2	-	78	30	52.4
3	HOAc	78	20	74.8
4	HOAc	r.t	10	22.1

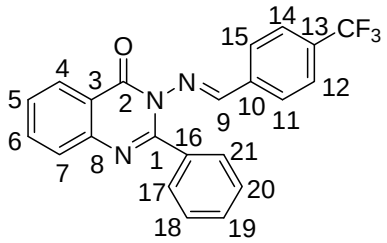
Table 3. Substituents and yields of title compounds **III-1-III-31** under optimized conditions.

Compound No.	R	R^1	R^2	Yields (%)
III-1	H	3,5-Cl	Ph	79
III-2	H	3-NO_2-4-Cl	Ph	74
III-3	H	4-CF_3	Ph	72
III-4	H	3-Cl	Ph	71
III-5	H	2,3-Cl	Ph	70
III-6	H	2,6-Cl	Ph	78
III-7	H	3,4-F	Ph	69
III-8	H	3-CF_3	Ph	74
III-9	H	H	4-Pyridinyl	67
III-10	H	2-Cl	4-Pyridinyl	69
III-11	H	4-F	4-Pyridinyl	89
III-12	H	2,3-Cl	4-Pyridinyl	89
III-13	6-Br	2-F	Me	59
III-14	6-Br	3-F	Me	63
III-15	6-Br	4-F	Me	68
III-16	6-Br	2-CF_3	Me	66
III-17	6-Br	3-Cl	Me	63
III-18	6-Br	2,4-Cl	Me	72
III-19	6-Br	2,6-Cl	Me	70
III-20	6-Br	3,4-F	Me	64
III-21	6-Br	2-Cl-5-NO_2	Me	70
III-22	6-Br	4-Cl-3-NO_2	Me	68
III-23	6-Br	2-F-3-CF_3	Me	64
III-24	6-Br	3,4-OMe	Me	66
III-25	6-Br	2,3-OMe	Me	61
III-26	6-Br	2,5-OMe	Me	63
III-27	6-Br	3-NO_2	Me	56
III-28	6-Br	2-OH	Me	53
III-29	6-Br	2,4-OMe	Me	62
III-30	6-Br	5-Cl-3-OH	Me	64
III-31	H	2,3-Cl	Me	87

Representative analysis of spectral data: IR and ^{1}H-NMR of compound **III-3.**

The IR spectrum of **III-3** (molecular formula $C_{22}H_{14}F_3N_3O$, m.w. 393.37, structure and carbon numbering in Figure 1) over the 3060-3032 cm^{-1} range showed multiple weak absorption peaks corresponding to Qu-H and Ar-H stretching vibration absorption peaks. The strong absorption at 1671 cm^{-1} is due to the C=O stretching vibration and the moderate intensity absorption at 1607 cm^{-1} corresponds to a C=N stretching vibration. The 1591~1474 cm^{-1} absorptions are due to the skeleton vibration of the aryl and heterocyclic rings. The series of bands in the 1319, 1174, 1125 cm^{-1} region correspond to the *para* C-F stretching of the imine-Ar-CF group, the one at 843 cm^{-1} is due to a methylene linked to the benzene ring at the substituted 4-position. The absorption peaks at 766 and 696 cm^{-1} arise due to the phenyl-substituted at the 2-position in the quinazolinone.

Figure 1. The structure and carbon atom numbering of compound **III-3**



It can be seen from the chemical structure that different pairs of carbons e.g. C-11 and C15, C-12 and C-14, C-17 and C-21, C-18 and C-20 are attached to chemically equivalent protons. The proton attached at C-5 position appears as a multiplet at δ 7.46-7.58 ppm due to mutual coupling with C4-H and C6-H. Chemical shift in the aromatic region with a multiplet centered at δ 7.63 ppm corresponds to C4-H and the multiplet in the region 7.80 - 7.94 ppm arises due to the five aromatic protons present in the phenyl ring directly attached to the quinazolinone ring. The multiplet at 7.70-7.73 ppm must be for the two equivalent protons at C-11 and C-15. The relatively downfield multiplet at 8.19-8.26 ppm which integrates up to 2H corresponds to the protons in the vicinity of trifluoromethyl group, i.e. attached to C-12 and C-14. The singlet at 9.25 ppm appears due to the proton attached to the imine carbon at C-9.

^{13}C-NMR of compound **III-8**

The chemical structure of compound **III-8** and the assignment of carbon atoms are shown in Figure 2. This compound **(**molecular formula $C_{22}H_{14}F_3N_3O$, m.w. 393.37) contains 22 carbon atoms. Among them, there are two equivalent pairs, C-17, C-21 and C-18,C-20. The ^{13}C chemical shift values of the 20 non equivalent carbons are assigned and shown in Table 4.

Figure 2. The structure and carbon atoms assignment of compound **III-8**.

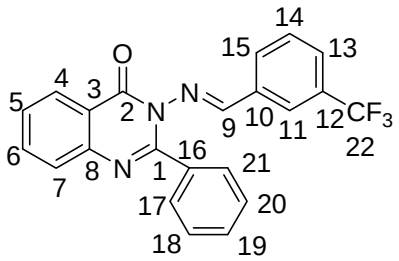


Table 4. ^{13}C-NMR analysis of compound **III-8.**

Carbon atom No.	**Chemical shift (δ, ppm)**
C-1	158.4
C-2	167.2
C-3	121.6
C-4	130.3
C-5	128.2
C-6	135.1

Table 4. Cont.

C-7	123.2
C-8	153.7
C-9	146.7
C-10	135.4
C-11	127.4
C-12	132.6
C-13	129.3
C-14	130.5
C-15	134.1
C-16	130.1
C-17	127.8
C-18	130.4
C-19	131.0
C-22	125.4

Compound **III-8** essentially has three parts. The chemical shifts of the quinazolinone ring carbons vary from δ = 167.2 to 121.6 ppm. The carbon nuclei under the influence of a strong electronegative environment appear downfield, e.g. the C-2 carbonyl, which is directly linked to the ring nitrogen has a chemical shift value of δ =167.2 ppm, whereas the C-1, linked to two ring nitrogen atoms, appears at 158.4 ppm. The chemical shift of the ring carbon at C-8 is affected by the presence of the directly attached ring nitrogen atom and appears at δ =153.7 ppm. The imine carbon at C-9, conjugated to the phenyl ring, records a downfield chemical shift at 146.7 ppm. The carbons of the phenyl ring conjugated to the imine functionality have chemical shifts in the 127.4 to 135.4 ppm range. The trifluoromethyl carbon present on the side chain appears at 125.4 ppm, under the strong electron withdrawing influence exerted by the three fluorine atoms. The carbons of the unsubstituted phenyl ring directly linked to the quinazolinone ring through the intervening carbon between two ring nitrogens appear upfield (127.8 ppm-131.0 ppm) compared to those of quinazolinone ring carbons at C1, C2 and C8. The equivalent C17,C-21 and C-18,C-20 have chemical shift values δ = 127.8 and 130.4 ppm respectively.

Antifungal activity

The antiviral bioassay results are given in Table 5. It could be seen that these newly synthesized derivatives exhibit weak to good antiviral activities. At 500 μg/mL, the title compounds exhibited weak activities against *Fusarium oxysporum*, *Valsa mali* and *Gibberella Zeae*, which are lower than that of a hymexazol standard.

Antiviral activity

The *in vivo* bioassay results against TMV are given in Table **6.** Ningnanmycin was used as reference antiviral agent. It was found that these compounds exhibit certain activities against TMV *in vivo*. The compounds **III-31**, **III-16**, **III-3**, **III-1** and **III-8** have relatively higher curative effect than

the other compounds **III-2, III-4, III-5, III-6, III-7, III-9-III-15, III-17** and **III-30**. The data listed in Table 6 indicate that antiviral activity depends on the nature of the substituents present in the title compound. When R was 4-CF_3, the title compounds **III-31** and **III-16** showed curative rates of 55 and 54%, which was slightly higher than that of reference (54%) against TMV at 500 μg/mL. The other compounds displayed a slightly lower antiviral activity than that of the reference.

Table 5. Inhibitory effects on phytopathogenic fungi.

Compd (50 μg/mL)	*Fusarium oxysporum*	*Gibberella zeae*	*Valsa mali*
III-1	17.5±0.77	11.3±0.29	6.75±0.84
III-2	6.14±0.50	1.07±0.32	1.23±0.51
III-3	1.59±0.68	-3.23±0.54	2.45±0.37
III-4	2.90±0.88	3.78±0.94	2.00±0.55
III-5	10.2±0.62	6.45±0.52	-0.31±1.49
III-6	5.68±0.91	0.00±0.55	9.51±1.15
III-7	1.90±0.38	3.09±0.94	5.66±0.30
III-8	5.68±0.69	2.69±0.94	5.52±0.52
III-9	4.09±0.51	4.77±0.71	0.90±0.12
III-10	5.90±0.77	7.12±0.99	1.09±0.33
III-11	9.54±0.95	6.18±0.26	1.23±0.81
III-12	8.89±0.37	4.77±0.55	7.98±0.86
III-13	17.5±3.25	7.55±1.70	-0.81±1.79
III-14	13.9±3.92	5.03±2.06	0±1.51
III-15	- 0.89±3.22	5.03±1.84	-1.35±2.50
III-16	12.4±3.69	1.41±1.74	1.89±1.59
III-17	14.2±3.40	3.14±1.7	4.04±1.45
III-18	12.4±3.80	6.60±1.53	3.50±1.60
III-19	16.3±3.14	11.0±1.31	2.43±1.54
III-20	17.0±1.42	9.00±1.0	8.09±1.09
III-21	20.11±2.99	15.22±2.11	6.78±2.09
III-22	9.00±3.11	24.21±1.99	3.09±1.01
III-23	19.91±2.88	26.01±6.43	10.32±4.92
III-24	19.00±2.77	17.70±3.44	6.77±1.99
III-25	16.9±1.11	19.0±1.22	4.93±1.09
III-26	21.1±1.89	9.99±0.77	7.68±0.96
III-27	26.7±7.55	3.97±1.18	4.99±1.40
III-28	28.8±3.09	34.1±10.1	7.77±2.10
III-29	39.7±3.01	22.30±1.55	6.66±1.08
III-30	29.4±9.99	10.4±2.90	20.5±3.33
III-31	12.8±1.19	3.12±0.84	3.04±2.19
Hymexazol	68.7±3.59	62.2±1.86	67.4±2.06

Table 6. The curative effects of the new compounds **III** against TMV *in vivo.*

Agents	**Ningnanmycin**	**III-1**	**III-2**	**III-3**	**III-4**	**III-5**
Concentration (mg/L)	500	500	50	500	500	500
Inhibition rate (%)	53.5	51.1	25.3	52.9	44.7	37.9
Agents	**III-6**	**III-7**	**III-8**	**III-9**	**III-10**	**III-11**
Concentration (mg/L)	500	500	500	500	500	500
Inhibition rate (%)	37.4	49.8	50.4	27.1	9.30	50.4
Agents	**III-12**	**III-13**	**III-14**	**III-15**	**III-16**	**III-17**
Concentration (mg/L)	500	500	500	500	500	500
Inhibition rate (%)	22.1	23.4	36.6	40.4	54.0	42.8
Agents	**III-18**	**III-19**	**III-20**	**III-21**	**III-22**	**III-23**
Concentration (mg/L)	500	500	500	500	500	500
Inhibition rate (%)	45.6	41.4	24.6	26.1	34.8	39.9
Agents	**III-24**	**III-25**	**III-26**	**III-27**	**III-28**	**III-29**
Concentration (mg/L)	500	500	500	500	500	500
Inhibition rate (%)	43.2	40.0	29.9	41.0	41.8	38.9
Agents	**III-30**	**III-31**				
Concentration (mg/L)	500	500				
Inhibition rate (%)	44.6	55.4				

Product of PCR in PR-1a and PR-5 and its sequence identification

By following RT-PCR, the PCR product produced in 35 cycles was resolved on a 1.5% agarose gel. The purified products of PCR were sequenced by model ABI 310 DNA sequencer and were identified as the target gene by BLAST searching in Genbank (Figure 3).

Gene expression analysis of PR-1a and PR-5 in III-31-treated tobacco leaf

In vitro synthesized single-stranded cDNA from RNA samples were isolated from leaves of water-treated tobacco and the TMV-treated tobacco and the **III-31**-and TMV-treated tobacco. The differential expression analysis of the PR-1a and PR-5 gene was determined by semi-quantitative PCR and the relative quantification real-time PCR analysis. The mRNAs of PR-1a and PR-5 gene accumulated to detectable levels in **III-31**-and TMV-treated tobacco leaf, while no-detectable levels were reached in water-treated tobacco and the TMV-treated tobacco. The mRNA content of **III-31**-treated tobacco leaf for PR-1a gene started to increase after five days and reached a peak at the end of the 5^{th} day before falling to the normal level. In contrast, in TMV-treated tobacco leaf, no significant increase in the levels of gene expression was noticed (Figures 4-A, 5-A). The expression levels of PR-5 gene in **III-31**-treated tobacco rapidly increased and reached a peak within 5^{th} day after the inoculation and then started to decrease gradually. As depicted in Figure 4-B and Figure 5-B, **III-31** treated tobacco leaves showed significant enhancement in the levels of gene expression as compared to TMV-treated tobacco

leaves within 5th day after the inoculation. In contrast, in TMV-treated tobacco leaf, no significant increase in the levels of gene expression was observed.

Figure 3. The clone of PR-1a and PR-5 genes

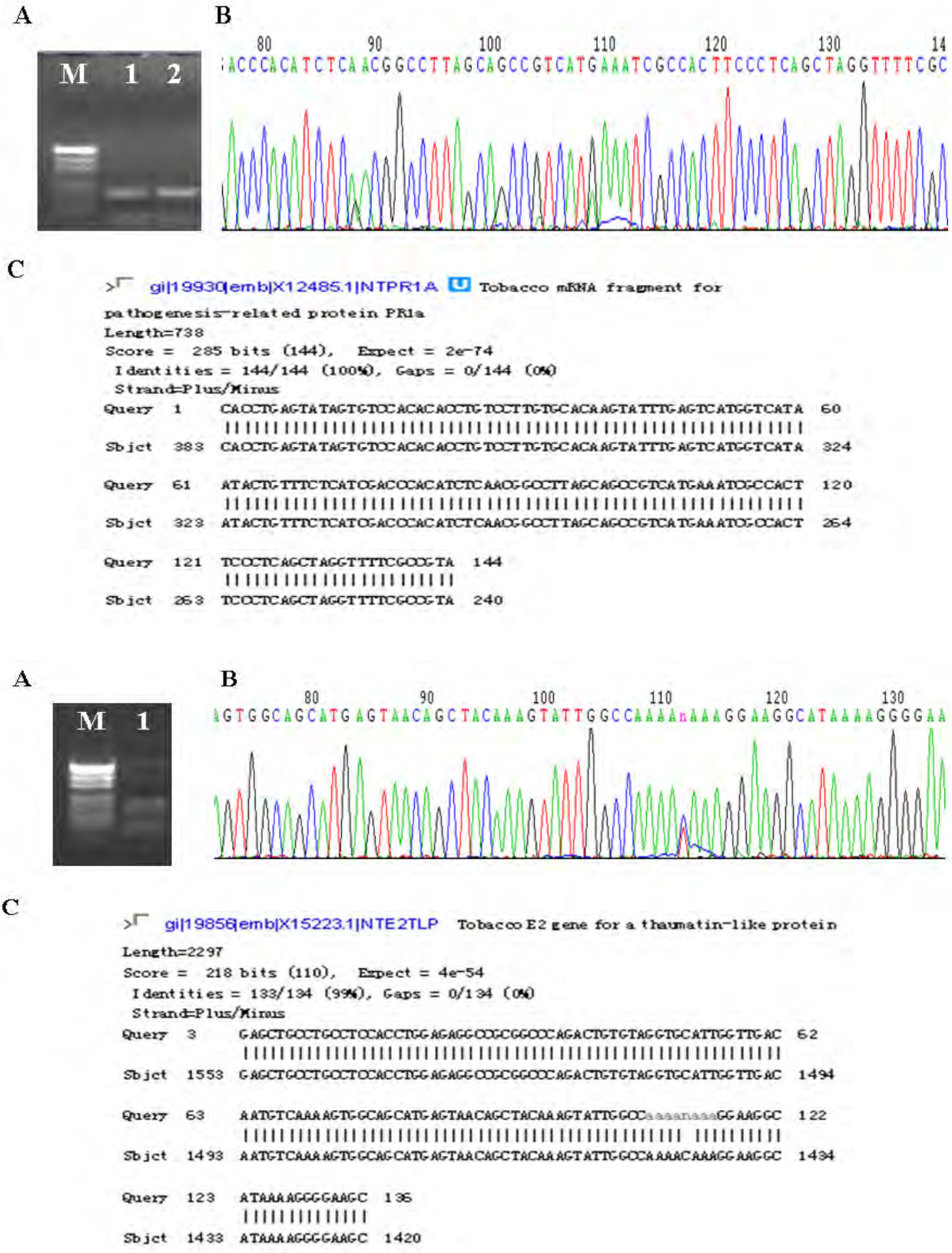
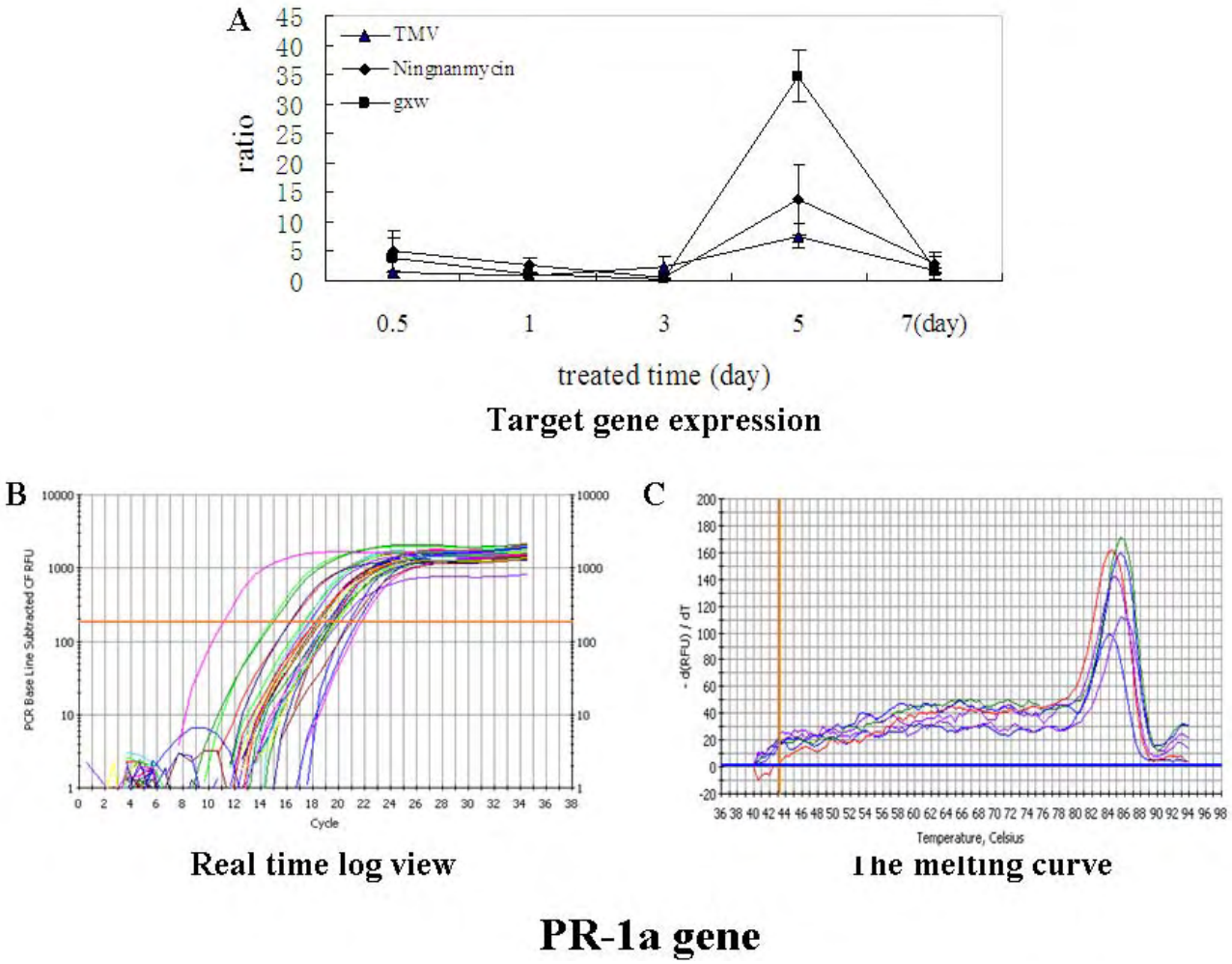


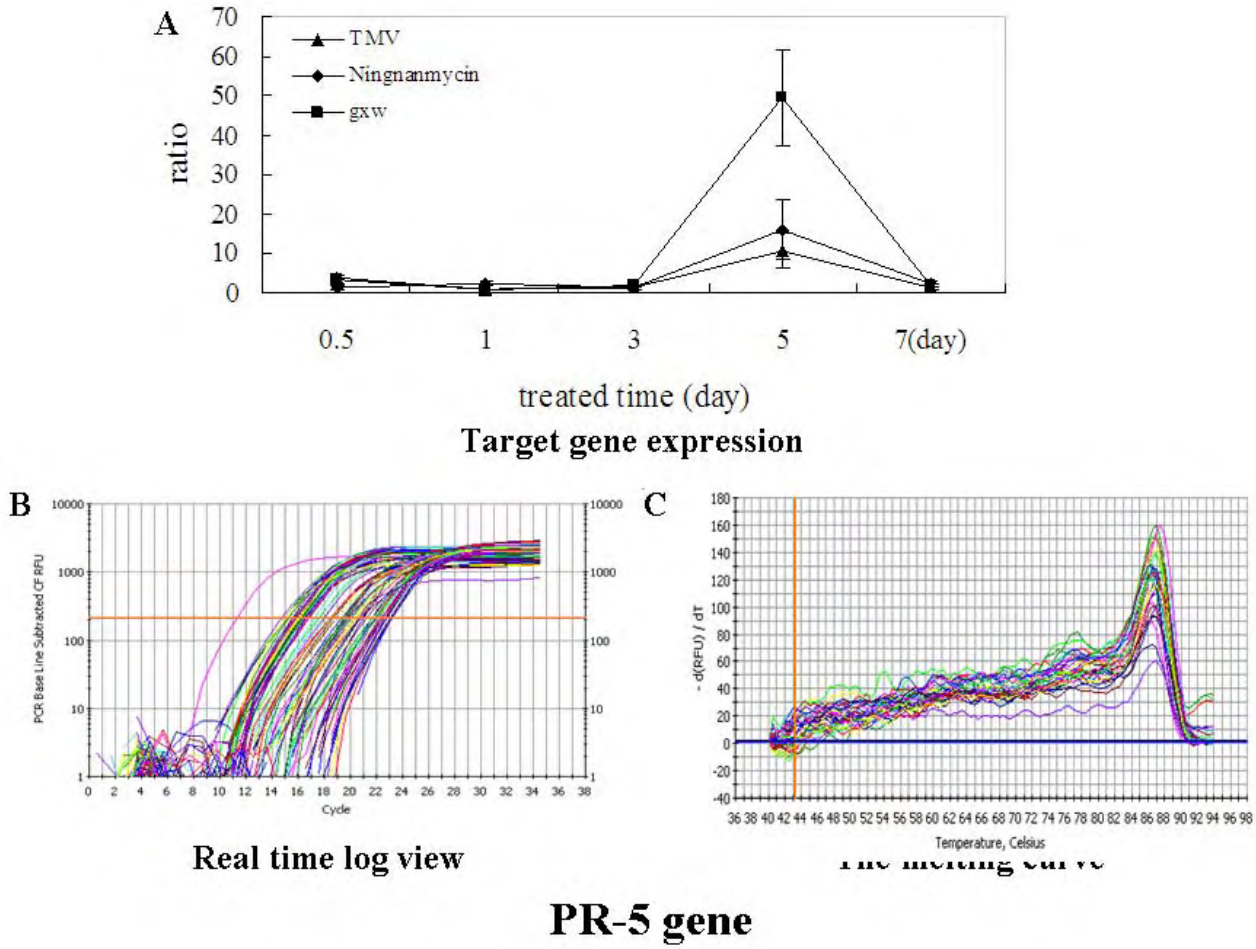
Figure 4. The real time PCR effects of PR-1a gene.



Conclusions

In summary, the presented new method of preparation of 4(3H)-quinazolinone Schiff base derivatives from appropriate 3-amino-2-aryl-4(3H)-quinazolinones and substituted benzaldehydes in ethanol is convenient, rapid and gives moderate yields. It was also found that the title compounds **III-31**, **III-1, III-3**, **III-8, III-16** displayed good antiviral activity. Semi-quantitative PCR and real time PCR assay were evaluated to ascertain the target of action of compound **III-31** against TMV. The studies suggest that **III-31** possesses antiviral activity by induction up-regulation of PR-1a, PR-5 thereby inhibiting proliferation and movement of virus, by enhancing activity of some defensive enzyme.

Figure 5. The real time PCR effects of PR-5 gene



Experimental

General

The melting points of the products were determined on a XT-4 binocular microscope (Beijing Tech Instrument Co., China) and were not corrected. The IR spectra were recorded on a Bruker VECTOR 22 spectrometer on KBr disks. ^{1}H- and ^{13}C-NMR experiments (solvent DMSO-d_6) were recorded at 500 and 125 MHz, respectively, on a JEOL-ECX 500 NMR spectrometer at room temperature using TMS as an internal standard. Elemental analysis was performed on an Elementar Vario-III CHN analyzer. All reagents employed were of analytical reagent grade or chemically pure. All solvents were dried, deoxygenated and redistilled before use.

Preparation of 2-aryl-4(3H)-3,1-benzoxazinone **I** (R=H, R^2=Ph)

To a stirred solution of anthranilic acid (0.05 mole) in pyridine (60 mL), Benzoyl chloride (0.05 mole) was added dropwise, maintaining the temperature near 0-5 °C for 1 hour. The reaction mixture was stirred for another 2 hours at room temperature until a solid product was formed. The reaction mixture was neutralized with saturated sodium bicarbonate solution and the pale yellow solid which separated was filtered, washed with water and recrystallised from ethanol. Yield 83 %, mp. 113-

115 °C (lit [20] 114 °C); IR: *v* 3030 (C-H, Ar-H), 1721 (C=O), 1595 (C=N), 1180 (C-O) cm^{-1}; ^{1}H-NMR: *δ* 7.61 (m, 9H, Ar-H, quinazolinone-H).

Preparation of 3-amino-2-aryl-4(3H)-quinazolinone **II-1** (R=H, R^2=Ph)

To a stirred solution of **I** (0.05 mole) in pyridine (20 mL), 80% N_2H_4•H_2O (0.15 mole) was added. The reaction mixture was stirred and refluxed for 20 minutes at 117 °C. After cooling, the crude product was obtained by filtration and recrystallized from ethanol to afford **II** as a white solid, yield 88%; m.p. 177-178 °C (lit [21] 178-179 °C); IR: *v* 3448 (NH_2), 3030 (C-H, Ar-H), 1685 (C=O), 1598 (C=N) cm^{-1}; ^{1}H-NMR: *δ* 7.92 (m, 9H, Ar-H, quinazolinone-H), 5.67 (s, 2H, 3-quinazolinone-NH_2), 7.48-8.19 (m, 9H, Ar-H, quinazolinone-H); ^{13}C-NMR: *δ* 161.7, 156.3, 147.2, 135.4, 134.8, 130.1, 130.0, 127.9, 127.3, 126.6, 120.6.

Preparation of Schiff base derivatives **III-1~III-31**

To a solution of the appropriate substituted benzaldehyde (1.0 mmol) in ethanol (15 mL), were added the 3-amino-2-aryl-4(3H)-quinazolinone (1.0 mmol) and a few drops of acetic acid (0.05 mmol). The reaction mixture was refluxed for 3-24h and the course of the reaction was monitored by TLC [petroleum ether/ethyl acetate (*V*/*V*=1:2)] to its completion. The reaction mixture was cooled. The crude product was recrystallized from 95% ethanol to give title compounds **III-1~III-31**.

2-Phenyl-3-(3,5-di-chlorobenzalamino-4(3H)-quinazolinone (**III-1**). White solid, yield 79%; m.p. 218~220 °C; IR (cm^{-1}): *v* 3088 (quinazolinone-H, Ar-H), 1678 (C=O), 1622, 1590 (C=N), 1551-1489 (C=C, benzene and quinazolinone rings), 875, 775, 688 (1,3,5-substituted benzene), 748, 700 (mono substituted benzene); ^{1}H-NMR: *δ* 7.45-8.25 (m, 12H, quinazolinone-H, Ar-H), 9.35 (s, 1H, N=CH); ^{13}C-NMR: *δ* 170.0, 158.3, 153.6, 146.9, 135.2, 135.1, 133.2, 132.8, 130.4, 130.3, 129.6, 129.1, 128.2, 128.1, 127.7, 127.3, 121.6; Anal. calcd. for $C_{21}H_{13}Cl_2N_3O$ (394.26): C, 63.98%; H, 3.32%; N, 10.66%. Found: C, 63.86%;, H, 3.22%; N, 10.58%.

2-Phenyl-3-(3-nitro-4-chlorobenzalamino)-4(3H)-quinazolinone (**III-2**)**.** White solid, yield 74%; m.p. 209~211 °C; IR (cm^{-1}): *v* 3057 (quinazolinone-H, Ar-H), 1681 (C=O), 1605, 1589 (C=N), 1568-1445 (C=C, benzene and quinazolinone rings), 889, 830 (1,2,4-trisubstituted benzene), 768, 692 (monosubstituted benzene); ^{1}H-NMR: *δ* 7.28-8.26 (m, 12H, quinazolinone-H and Ar-H), 9.31 (s, 1H, N=CH); ^{13}C-NMR: *δ* 163.3, 162.7, 161.2, 158.4, 146.8, 135.5 (J_3 = 8.8 Hz), 135.2 (J_3 = 6.5 Hz), 134.8, 130.3 (J_2 = 12.5 Hz), 130.1, 130.0, 128.2 (J_3 = 11.3 Hz), 127.9, 127.8, 127.7, 127.4, 127.3, 125.8, 121.6, 120.6 (J_2 = 10.0 Hz), 117.0 (J_2 = 8.8 Hz); Anal. calcd. for $C_{21}H_{13}ClN_4O_3$ (404.85): C, 42.25%; H, 3.21%; N, 13.83%. Found: C, 42.30%; H, 3.15%; N,13.75%.

2-Phenyl-3-(4-trifluoromethylbenzalamino)-4(3H)-quinazolinone (**III-3**). White solid, yield 72%; m.p. 179.4~181.0 °C; IR (cm^{-1}): *v* 3061 (quinazolinone-H, Ar-H), 1672 (C=O), 1605, 1591 (C=N), 1564-1445 (C=C, benzene and quinazolinone rings), 843 (1,4-disubstituted benzene), 766, 696 (monosubstituted benzene); ^{1}H-NMR: *δ* 5.68 (s, 2H), 7.42-8.28 (m, 13H, quinazolinone-H and Ar-H), 9.48 (s, 1H, N=CH); ^{13}C-NMR: *δ* 164.4, 157.8, 153.0, 146.1, 134.9, 134.6 (J_3 = 3.8 Hz), 133.9, 130.2,

129.9, 129.7 (J_2 = 12.5 Hz), 127.8, 127.6 (J_2 = 12.5 Hz), 127.1, 126.8, 121.0; Anal. Calcd. for $C_{22}H_{14}F_3N_3O$ (393.37): C, 67.17%; H, 3.59%; N, 10.68%. Found: C, 67.41%; H, 4.09%; N, 10.86%.

2-Phenyl-3-(3-chlorobenzalamino)-4(3H)-quinazolinone (**III-4**). White solid, yield 71%; m.p. 190.0~192.1 °C; IR (cm^{-1}): *v* 3059 (quinazolinone-H, Ar-H), 1680 (C=O), 1605, 1584 (C=N), 1559,1474 (C=C, benzene and quinazolinone rings), 903, 775, 690 (1,3-disubstituted benzene), 765, 692 (monosubstituted benzene); ^{1}H-NMR: δ 7.45-8.26 (m, 13H, quinazolinone-H and Ar-H), 9.12 (s, 1H, N=CH); ^{13}C-NMR: δ 167.2, 157.7, 153.0, 146.1, 134.7, 134.4 (J_2 = 7.5 Hz), 133.7, 132.1, 131.0, 129.8, 129.6, 127.6 (J_2 = 7.2 Hz), 127.5, , 127.1 (J_2 = 5.0 Hz), 126.7, 120.8; Anal. Calcd. for $C_{21}H_{14}ClN_3O$ (359.82): C, 70.10%; H, 3.92%; N, 11.68%. Found: C, 69.99%; H, 4.19%; N, 11.78%.

2-Phenyl-3-(2,3-dichlorobenzalamino)-4(3H)-quinazolinone (**III-5**). White solid, yield 70%; m.p. 207~208 °C; IR (cm^{-1}): *v* 3061 (quinazolinone-H, Ar-H), 1684 (C=O), 1603, 1587 (C=N), 1551-1445 (C=C, benzene and quinazolinone rings), 764, 696 (1,2,3-trisubstituted benzene), 768.0, 690.5 (monosubstituted benzene); ^{1}H-NMR: δ 7.42-8.28 (m, 13H, quinazolinone-H and Ar-H), 9.48 (s, 1H, N=CH); ^{13}C-NMR: δ 164.4, 157.8, 153.0, 146.1, 134.9, 134.6 (J_3 = 3.8 Hz), 133.9, 130.2, 129.9, 129.7 (J_2 = 12.5 Hz), 127.8, 127.6 (J_2 = 12.5 Hz), 127.1, 126.8, 121.0; Anal. Calcd. for $C_{21}H_{13}Cl_2N_3O$ (394.26): C, 63.98%; H, 3.32%; N, 10.66%. Found: C, 63.94%; H, 3.18%; N, 10.83%.

2-Phenyl-3-(2,6-dichlorobenzalamino)-4(3H)-quinazolinone (**III-6**). White solid, yield 78%; m.p. 179-180.3 °C; IR (cm^{-1}): *v* 3055 (quinazolinone-H, Ar-H), 1680 (C=O), 1607, 1587 (C=N), 1566-1472 (C=C, benzene and quinazolinone rings), 770, 690 (1,2,3-trisubstituted benzene), 766, 690 (monosubstituted benzene); ^{1}H-NMR: δ 7.43-8.28 (m, 12H, quinazolinone-H and Ar-H), 9.37 (s, 1H, N=CH); ^{13}C-NMR: δ 166.1, 158.1, 153.8, 146.7, 135.3, 135.0, 133.4, 130.2, 130.1, 130.0, 129.3, 128.3, 128.1, 127.8, 127.5, 121.8; Anal. Calcd. for $C_{21}H_{13}Cl_2N_3O$ (394.26): C, 63.98%; H, 3.32%; N, 10.66%. Found: C, 63.66%;, H, 3.62%; N, 10.58%.

2-Phenyl-3-(3,4-difluorobenzalamino)-4(3H)-quinazolinone (**III-7**). White needles, yield 69%; m.p. 137.6~139.6 °C; IR (cm^{-1}): *v* 3057 (quinazolinone-H, Ar-H), 1678 (C=O), 1603, 1591 (C=N), 1566-1445 (C=C, benzene and quinazolinone rings), 881, 830 (1,2,4-trisubstituted benzene), 770, 692 (monosubstituted benzene); ^{1}H-NMR δ: 7.45-8.25 (m, 13H, quinazolinone-H and Ar-H), 9.11 (s, 1H, N=CH); ^{13}C-NMR δ: 170.0, 161.7, 158.3, 156.3, 153.6, 151.4, 147.2, 146.7, 135.4 (J_2 = 13.8 Hz), 134.9 (J_2 = 28.9 Hz), 130.6-130.0 (m), 128.2-127.8 (m), 127.3 (J_2 = 10.0 Hz), 126.9 (J_3 = 7.5 Hz), 126.6, 121.5, 120.6, 119.1 (J_2 = 13.8 Hz), 117.3 (J_2 = 17.6 Hz); Anal. Calcd. for $C_{21}H_{13}F_2N_3O$ (361.35): C, 69.80%; H, 3.63%; N, 11.63%. Found: C, 69.38%; H, 3.28%; N, 11.09%.

2-Phenyl-3-(3-trifluoromethylbenzalamino)-4(3H)-quinazolinone (**III-8**). White solid, yield 74%; m.p. 184~186 °C; IR (cm^{-1}): *v* 3060 (quinazolinone-H, Ar-H), 1670 (C=O), 1603, 1590 (C=N), 1564, 1445 (C=C, benzene and quinazolinone rings), 903 (1,3-disubstituted benzene), 766, 696 (mono- substituted benzene); ^{1}H-NMR: δ 5.60 (s, 2H,), 7.38-8.19 (m, 13H, quinazolinone-H and Ar-H), 9.40 (s, 1H, N=CH); ^{13}C-NMR: δ 158.4, 157.8, 153.0, 146.1, 134.9, 134.6 (J_3 = 3.8 Hz), 133.9, 130.2, 129.9, 129.7 (J_2 = 12.5 Hz), 127.8, 127.6 (J_2 = 12.5 Hz), 127.1, 126.8, 121.0; Anal. calcd. for $C_{22}H_{14}F_3N_3O$ (393.37): C, 67.17%; H, 3.59%; N, 10.68%. Found: C, 67.22%; H, 3.60%; N, 10.80%.

2-(4-Pyridinyl)-3-(benzalamino)-4(3H)-quinazolinone (**III-9**). White solid, yield 67%; m.p. 225~226 °C; IR (cm^{-1}): *v* 3042 (ArH), 1682 (C=O), 1607, 1585 (C=N), 1566-1452 (C=C, benzene, quinazolinone and pyridine rings), 847 (4-substituted pyridine), 770, 691 (monosubstituted benzene); ^{1}H-NMR: δ 7.51-7.73 (t, 9H, Ar-H, Qu-H), 7.82 (d, 2H, 3-Py-H, *J* = 10 Hz), 7.93 (t, 2H, 2-Py-H, *J* = 10 Hz), 9.11 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 169.5, 158.2, 150.0, 146.6, 135.3, 133.3, 132.7, 129.7, 129.1 (C-4, C-12, C-14), 128.8, 128.2, 127.4, 124.3, 122.0 (C-3, C-17, C-20); Anal. calcd.for $C_{20}H_{14}N_4O$ (326.36): C 73.61%, H 4.32%, N 17.17%. Found: C 73.54%, H 4.48%, N 17.24%.

2-(4-Pyridinyl)-3-(2-chlorobenzalamino)-4(3H)-quinazolinone (**III-10**). White solid, yield 69%; m.p. 248.7~250.2 °C; IR (cm^{-1}): *v* 3046 (ArH), 1682 (C=O), 1611, 1584 (C=N), 1564-1458 (C=C, benzene, quinazolinone and pyridine rings), 835, 725 (4-substituted pyridine), 764 (1,2-disubstituted benzene); ^{1}H-NMR: δ 6.56 (d, 1H, *J* = 15 Hz), 7.37-7.87 (m, 8H, Ar-H, Qu-H), 7.91 (d, 2H, 3-Py-H, *J* = 10 Hz), 8.14 (d, 2H, 2-Py-H), 10.22 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 169.2, 163.8, 162.0 (J_3 = 10.0 Hz), 158.8, 152.0, 147.8, 135.3, 132.1 (J_3 = 8.8 Hz), 130.0 (J_2 = 28.0 Hz), 129.4, 127.9, 127.3, 127.0, 125.7, 123.6 (J_4 = 10.0 Hz), 121.2, 120.1, 116.7 (J_2 = 21.5 Hz); Anal. calcd. for $C_{20}H_{13}FN_4O$ (344.35): C 69.76%, H 3.81%, N 16.27%. Found: C 69.59%, H 3.75%, N 16.39%.

2-(4-Pyridinyl)-3-(4-fluorobenzalamino)-4(3H)-quinazolinone (**III-11**). White solid, yield 89%; m.p. 203~204 °C; IR (cm^{-1}) *v*: 3080 (ArH), 1678 (C=O), 1611, 1597 (C=N), 1570-1447 (C=C, benzene, quinazolinone and pyridine rings), 839 (1,4-disubstituted benzene), 825, 726 (4-subsituted pyridine); ^{1}H-NMR: δ 7.36-7.82 (m, 8H, Ar-H, Qu-H), 8.27 (d, 2H, 3-Py-H, *J* = 5 Hz), 8.70 (d, 2H, 2-Py-H, *J* = 5 Hz), 9.11 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 168.1, 166.0, 164.0, 158.2, 151.8, 150.0, 146.5, 142.6, 135.4, 131.7 (J_3 = 8.8 Hz), 129.4, 128.3 (J_4 = 2.5 Hz), 127.4, 124.3, 121.9, 117.1 (J_2 = 21.3 Hz); Anal calcd for $C_{20}H_{13}FN_4O$ (344.35): C 69.76%, H 3.81%, N 16.27%. Found: C 69.73% H 4.00% N 16.40%.

2-(4-Pyridinyl)-3-(2, 3-dichlorobenzalamino)-4(3H)-quinazolinone (**III-12**). White solid, yield 89%; m.p. 201~203°C; IR (cm^{-1}): *v* 3060 (ArH), 1681 (C=O), 1614 (C=N), 1577-1447 (C=C, benzene, quinazolinone and pyridine rings), 826, 727 (4-subsituted pyridine), 790, 696 (1,2,3-trisubstituted benzene); ^{1}H-NMR: δ 7.48-8.27 (m, 11H, Ar-H, Py-H, Qu-H), 9.56(s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 164.6, 158.4, 153.7, 146.6, 135.4, 135.2, 134.4, 133.4, 133.0, 130.4, 130.3, 129.3, 128.3, 128.1, 127.8, 127.5, 126.7, 121.6.

6-Bromo-2-methyl-3-(2-fluorobenzalamino)-4(3H)-quinazolinone (**III-13**). White solid, yield 59%; m.p. 179~181 °C; IR (cm^{-1}): *v* 3059 (Qu-H, Ar-H), 1680 (C=O), 1614, 1595 (C=N), 1571-1452 (C=C, benzene and quinazolinone rings), 760 (1,2-disubstituted benzene); 675 (Qu-Br), ^{1}H-NMR: δ 2.51 (s, 3H, 2-Qu-C*H*$_3$), 7.42-8.23 (m, 7H, Qu-H, Ar-*H*), 9.20 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 163.3 (*J* = 15 Hz), 161.3, 157.0 154.6, 146.7, 137.8, 135.7 (*J* = 10 Hz), 129.7, 129.2 (*J* = 10 Hz), 128.3, 125.9, 123.3, 120.4, 119.2, 117.0 (*J* = 15 Hz), 22.8; Anal. calcd. for $C_{16}H_{11}BrFN_3O$ (360.19): C 53.36% H 3.08% N 11.67%. Found: C 53.51%, H 3.28%, N 11.70%.

6-Bromo-2-methyl-3-(3-fluorobenzalamino)-4(3H)-quinazolinone (**III-14**). White solid, yield 63%; m.p. 212~214 °C; IR (cm^{-1}): *v* 3032 (Qu-H, Ar-H), 1670 (C=O), 1616, 1502 (C=N), 1581-1449 (C=C, benzene and quinazolinone rings), 874, 777, 695 (1,3-disubstituted benzene); 675 (Qu-Br); ^{1}H-NMR: δ

2.53 (s, 3H, 2-Qu-CH_3), 7.50—8.23 (m, 7H, Ar-H, Qu-H), 9.04 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 168.6, 163.9, 162.0, 157.0, 154.7, 145.8, 137.9, 135.1 (J = 10.0 Hz), 132.0 (J = 10.0 Hz), 129.8, 126.0, 123.2, 1203 (J = 25.0 Hz), 119.3, 115.0 (J = 25.0 Hz), 22.8; Anal. calcd. for $C_{16}H_{11}BrFN_3O$ (360.19): C 53.36%, H 3.08%, N 11.67%. Found: C 53.03%, H 3.49%, N 11.75%.

6-Bromo-2-methyl-3-(4-fluorobenzalamino)-4(3H)-quinazolinone (**III-15**). White solid, yield 68%; m.p. 228.4~229.0 °C; IR (cm^{-1}): v 3032 (Qu-H, Ar-H), 1674 (C=O), 1610, 1589 (C=N), 1568-1474 (C=C, benzene and quinazolinone rings), 860, 815 (1,2,4-trsubstituted benzene), 675 (Qu-Br); ^{1}H-NMR: δ 2.57 (s, 3H, 2-Qu-CH_3), 7.64-8.24 (m, 5H, Ar-*H*), 9.42 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 163.2, 158.2 154.1, 146.6, 136.4, 135.1, 130.1. 129.9, 129.8, 129.6, 128.9, 128.8, 127.3, 127.0, 121.6, 22.9; Anal. calcd. for $C_{16}H_{11}BrFN_3O$ (360.19): C 53.36%, H 3.08%, N 11.67%. Found: C 53.35%, H 3.29%, N 11.52%

6-Bromo-2-methyl-3-(2-trifluoromethylbenzalamino)-4(3H)-quinazolinone (**III-16)**. White solid, yield 66 %; m.p. 196~198 °C; IR (cm^{-1}): v 3032 (Qu-H, Ar-H), 2980 (Qu-CH_3), 1678 (C=O), 1616, 1602 (C=N), 1568-1469 (C=C, benzene and quinazolinone rings), 772 (1,2-disubstituted benzene); 675 (Qu-Br), ^{1}H-NMR: δ 2.57 (s, 3H, 2-Qu-CH_3), 7.64-8.24 (m, 5H, Ar-*H*), 9.42 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 164.2, 161.1, 158.2 146.5, 136.4, 135.1, 130.1. 131.3, 129.8, 129.6, 128.9, 128.8, 127.3, 117.0, 115.6, 22.9; Anal. calcd. for $C_{17}H_{11}BrF_3N_3O$ (410.20): C 49.78%, H 2.70%, N 10.24%. Found: C 49.27%, H 3.07%, N 10.26%.

6-Bromo-2-methyl-3-(3-chlorobenzalamino)-4(3H)-quinazolinone (**III-17**). White solid, yield 63%; m.p. 212~214 °C; ^{1}H-NMR: δ 2.57 (s, 3H, 2-Qu-CH_3), 7.64-8.24 (m, 5H, Ar-*H*), 9.42 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 163.2, 158.2 154.1, 146.6, 136.4, 135.1, 130.1. 129.9, 129.8, 129.6, 128.9, 128.8, 127.3, 127.0, 121.6, 22.9; Anal. calcd. for $C_{16}H_{11}BrClN_3O$ (376.64): C 51.02%, H 2.94%, N 11.16%; found: C 51.11%, H 2.88%, N 11.25%.

6-Bromo-2-methyl-3-(2,4-dichlorobenzalamino)-4(3H)-quinazolinone (**III-18**). White solid, yield 72%; m.p. 212.3~214.4 °C; IR (cm^{-1}): v 3061 (Qu-H, Ar-H), 1688 (C=O), 1605, 1587 (C=N), 1575-1468 (C=C, benzene and quinazolinone rings), 868, 827 (1,2,4-trisubstituted benzene); 675 (Qu-Br), ^{1}H-NMR: δ 2.55 (s, 3H, 2-Qu-CH_3), 7.60-8.24 (m, 6H, Ar-H, Qu-H), 9.39 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 164.0, 157.3, 154.8, 145.7. 147.0138.6, 137.8, 130.5, 129.8, 129.7, 129.4, 129.3, 129.0, 123.3, 119.3, 22.9; Anal. calcd. for $C_{16}H_{10}BrCl_2N_3O$ (411.09): C 46.75%, H 2.45%, N 10.22%. Found: C 46.68%, H 2.30%, N 10.22%.

6-Bromo-2-methyl-3-(2,6-dichlorobenzalamino)-4(3H)-quinazolinone (**III-19**). White solid, yield 70%; m.p. 222~224 °C; IR (cm^{-1}): v 3066 (Qu-H, Ar-H), 1678 (C=O), 1597 (C=N), 1579-1443 (C=C, benzene and quinazolinone rings), 781, 710 (1,2,3-trisubstituted benzene), 675 (Qu-Br); ^{1}H-NMR: δ 2.50 (s, 3H, 2-Qu-CH_3), 7.45-8.95 (m, 6H, Ar-H, Qu-H), 9.37 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 165.9, 157.0, 154.6, 145.7, 138.0, 135.0, 133.7, 130.2, 129.7, 129.4, 129.3, 123.4, 119.4, 23.4; Anal. calcd. for $C_{16}H_{10}BrCl_2N_3O$ (411.09): C 46.75%, H 2.45%, N 10.22%. Found: C 46.60%, H 2.60%, N 10.29%.

6-Bromo-2-methyl-3-(3,4-difluorobenzalamino)-4(3H)-quinazolinone (**III-20**). White solid, yield 64%; m.p. 187~189 °C; IR (cm^{-1}): *v* 3030 (Qu-H, Ar-H), 1678 (C=O), 1605, 1585 (C=N), 1517, 1470 (C=C, benzene and quinazolinone rings), 881, 829 (1,2,4-trisubstituted benzene), 675 (Qu-Br), ^{1}H-NMR: δ 2.50 (s, 3H, 2-Qu-C*H*$_3$), 7.61-8.23 (m, 6H, Qu-H, Ar-*H*), 9.02 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 167.6, 156.9, 154.8, 151.3, 145.8, 142.0, 137.9, 129.8, 129.1, 127.5, 123.1, 119.3, 119.0, 117.5, 22.8.

6-Bromo-2-methyl-3-(2-chloro-5-nitrobenzalamino)-4(3H)-quinazolinone (**III-21**). White solid, yield 70%; m.p. 277~279 °C; IR (cm^{-1}): *v* 3076 (Qu-H, Ar-H), 1682 (C=O), 1610, 1599 (C=N), 1568-1447 (C=C, benzene and quinazolinone rings), 1529, 1348 (Ar-NO_2), 880, 833 (1,2,4-trisubstituted benzene), 675 (Qu-Br); ^{1}H-NMR: δ 2.59 (s, 3H, 2-Qu-CH_3), 7.62-8.87 (m, 6H, Ar-H, Qu-H), 9.55 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 163.0, 155.7, 154.7, 147.4, 145.4, 141.5, 140.1, 137.9, 132.6, 129.8, 129.4, 123.4, 128.4, 122.9, 119.6, 22.7; Anal. calcd. for $C_{16}H_{10}BrClN_4O_3$ (421.64): C 45.58%, H 2.39%, N 13.29%. Found: C 45.75%, H 2.46%, N 13.58%.

6-Bromo-2-methyl-3-(4-chloro-3-nitrobenzalamino)-4(3H)-quinazolinone (**III-22**). White solid, yield 68%; m.p. 230~232 °C; IR (cm^{-1}): *v* 3062 (Qu-H, Ar-H), 1668 (C=O), 1612, 1597 (C=N), 1469 (C=C, benzene and quinazolinone rings), 1537, 1355 (Ar-NO_2), 870, 829 (1,2,4-trisubstituted benzene); 673 (Qu-Br); ^{1}H-NMR: δ 2.55 (s, 3H, 2-Qu-CH_3), 7.61-8.66 (m, 6H, Ar-H, Qu-H), 9.20 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 166.1, 157.0, 154.8, 148.6, 145.7, 138.0, 133.7, 133.3, 133.2, 129.8, 129.3, 129.2, 125.8, 123.1, 119.4, 22.9; Anal. calcd. for $C_{16}H_{10}BrClN_4O_3$ (421.64): C 45.58%, H 2.39%, N 13.29%; found: C 45.62%, H 2.17%, N 13.46%.

6-Bromo-2-methyl-3-(2-fluoro-3-trifluoromethylbenzalamino)-4(3H)-quinazolinone (**III-23**). White solid, yield 64%; m.p. 230.7~232.0 °C; IR (cm^{-1}) *v*: 3012 (Qu-H, Ar-H), 1670 (C=O), 1614, 1605 (C=N), 1566, 1431 (C=C, benzene and quinazolinone rings), 762, 696 (1,2,3-trisubstituted benzene); 675 (Qu-Br), ^{1}H-NMR: δ 2.53 (s, 3H, 2-Qu-CH_3), 7.61-8.39 (m, 6H, Ar-H, Qu-H), 9.02 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 168.5, 156.8, 154.7, 145.8, 137.9, 134.9, 134.5, 133.0, 131.7, 129.8, 129.1, 128.5, 128.2, 124.3, 123.1, 119.3, 22.8.

6-Bromo-2-methyl-3-(3,4-dimethoxybenzalamino)-4(3H)-quinazolinone (**III-24**). White solid, yield 66%; m.p. 216~218 °C; IR (cm^{-1}): *v* 3060 (Qu-H, Ar-H), 2934, 2837 (ArO-CH_3), 1674 (C=O), 1599 (C=N), 1571-1466 (C=C, benzene and quinazolinone rings), 1271, 1024 (Ar-O-Me), 880, 833 (1,2,4-trisubstituted benzene); ^{1}H-NMR: δ 2.51 (s, 3H, 2-Qu-C*H*$_3$), 7.14-8.37 (m, 6H, Qu-H, Ar-*H*), 8.78 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 170.2, 156.9, 154.7, 153.5, 149.7, 145.9, 139.9, 137.6, 129.7, 129.0, 125.2, 124.0, 123.2, 119.1, 112.0, 110.0, 56.2, 22.8; Anal. calcd. for $C_{18}H_{16}BrN_3O_3$ (402.25): C 53.75%, H 4.01%, N 10.45%; found: C 53.67%, H 4.17%, N 10.56%.

6-Bromo-2-methyl-3-(2,3-dimethoxybenzalamino)-4(3H)-quinazolinone (**III-25**). White solid, yield 61%; m.p. 194.5~196.5 °C; IR (cm^{-1}): *v* 3060 (Qu-H, Ar-H), 2965, 2935, 2837 (Ar-CH_3, ArO-CH_3), 1684 (C=O), 1614, 1597 (C=N), 1577-1468 (C=C, benzene and quinazolinone rings), 1275, 1088 (Ar-O-Me), 746, 711 (1,2,3-trisubstituted benzene), 675 (Qu-Br); ^{1}H-NMR: δ 2.51 (s, 3H, 2-Qu-C*H*$_3$), 7.26-8.24 (m, 6H, Qu-H, Ar-*H*), 9.13 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 165.8, 157.0, 154.7, 153.3, 150.4, 145.8, 137.7, 129.7, 129.2, 126.2, 125.2, 123.3, 119.1, 118.3, 117.6, 62.4, 56.5, 22.8; Anal.

calcd. for $C_{18}H_{16}BrN_3O_3$ (402.25): C 53.75%, H 4.01%, N 10.45%; found: C 53.94%, H 4.22%, N 10.35%.

6-Bromo-2-methyl-3-(2,5-dimethoxybenzalamino)-4(3H)-quinazolinone (**III-26**). White solid, yield 63%; m.p. 224~226 °C; IR (cm^{-1}): *v* 3060 (Qu-H, Ar-H), 2943, 2833 (Ar-CH_3, ArO-CH_3), 1682 (C=O), 1604 (C=N), 1575-1445 (C=C, benzene and quinazolinone rings), 1223, 1049 (Ar-O-Me), 875, 800 (1,2,4-trisubstituted benzene); 675 (Qu-Br); ^{1}H-NMR: δ 2.51 (s, 3H, 2-Qu-CH_3), 3.35-3.84 (m, 6H, ArO-CH_3), 6.98-8.36 (m, 6H, Ar-H, Qu-H), 9.13 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 166.0, 164.9, 156.8, 154.8, 154.4, 153.7, 145.8, 137.6, 129.7, 129.2, 121.6, 121.0, 119.0, 114.5, 110.4, 56.9, 56.1, 22.8; Anal. calcd. for $C_{18}H_{16}BrN_3O_3$ (402.25): C 53.75%, H 4.01%, N 10.45%; found: C 53.67%, H 3.89%, N 10.32%.

6-Bromo-2-methyl-3-(3-nitrobenzalamino)-4(3H)-quinazolinone (**III-27**). White solid, yield 56%; m.p. 227~230 °C; IR (cm^{-1}): *v* 3086 (Qu-H, Ar-H), 1670 (C=O), 1602 (C=N), 1535 1352 (Ar-NO_2), 1470 (C=C, benzene and quinazolinone rings), 843, 770, 700 (1,3-disubstituted benzene); 675 (Qu-Br); ^{1}H-NMR: δ 2.56 (s, 3H, 2-Qu-CH_3), 7.63-8.77 (m, 7H, Ar-H, Qu-H), 9.23 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 167.8, 156.8, 154.7, 148.9, 145.8, 138.0, 135.2, 134.5, 131.5, 129.8, 129.2, 127.5, 123.5, 123.1, 119.4, 22.9; Anal. calcd. for $C_{16}H_{11}BrN_4O_3$ (387.20): C 49.63%, H 2.86%, N 14.47%. Found: C 49.42%, H 2.75%, N 14.32%.

6-Bromo-2-methyl-3-(2-hydroxybenzalamino)-4(3H)-quinazolinone (**III-28**). White solid, yield 53%; m.p. 212.4~212.9 °C; IR (cm^{-1}): *v* 3364 (Ar-OH), 3072 (Qu-H, Ar-H), 1682 (C=O), 160 (C=N), 1464, 1447 (C=C, benzene and quinazolinone rings), 1273 (Ar-O-H), 750 (1,2-disubstituted benzene); 675 (Qu-Br), ^{1}H-NMR: δ 2.50 (s, 3H, 2-Qu-C*H*$_3$), 6.97-8.22 (m, 7H, Qu-H, Ar-*H*), 9.10 (s, 1H, Qu-N=CH-Ar), 10.56 (Ar-OH); ^{13}C-NMR: δ 167.6, 159.3, 157.0, 154.6, 145.9, 140.0, 137.6, 135.1, 129.7, 129.1, 128.6, 123.3, 120.2, 118.7, 117.3, 22.8; Anal. calcd. for $C_{16}H_{12}BrN_3O_2$ (358.20); C 53.65%, H 3.38%, N 11.73%. Found: C 53.79%, H 3.66%, N 11.72%.

6-Bromo-2-methyl-3-(2,4-dimethoxybenzalamino)-4(3H)-quinazolinone (**III-29**). White solid, yield 62 %; m.p. 216~218 °C; IR (cm^{-1}): *v* 3032 (Qu-H, Ar-H), 2968, 2941, 2833 (Ar-CH_3, ArO-CH_3), 1674 (C=O), 1599 (C=N), 1570-1468 (C=C, benzene and quinazolinone rings), 1277, 1107 (Ar-O-Me), 876, 813 (1,2,4-trisubstituted benzene); 675 (Qu-Br); ^{1}H-NMR: δ 2.49 (s, 3H, 2-Qu-C*H*$_3$), 3.88 (Ar-O-Me), 6.58-8.20 (m, 6H, Qu-H, Ar-*H*), 8.97 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 165.5, 165.0, 161.6, 157.1, 154.8, 145.9, 137.5, 129.7, 129.1, 128.7, 123.3, 119.0, 113.4, 107.6, 98.7, 56.5, 56.3, 22.8; Anal. calcd. for $C_{18}H_{16}BrN_3O_3$ (402.25): C 53.75%, H 4.01%, N 10.45%. Found: C 53.99%, H 4.19%, N 10.27%.

6-Bromo-2-methyl-3-(5-chloro-2-hydroxybenzalamino)-4(3H)-quinazolinone (**III-30**). Yellow solid, yield 64 %; m.p. 273.2~225.0 °C; IR (cm^{-1}): *v* 3064 (Qu-H, Ar-H), 1682 (C=O), 1610 (C=N), 1474 (C=C, benzene and quinazolinone rings), 860, 815 (1,2,4-trisubstituted benzene); 665 (Qu-Br); ^{1}H-NMR: δ 2.50 (s, 3H, 2-Qu-C*H*$_3$), 7.49-8.21 (m, 6H, Qu-H, Ar-*H*), 9.11 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 165.1, 157.9, 157.0, 154.7, 145.8, 137.7, 134.4, 129.7, 129.1, 126.7, 123.9, 123.2, 120.3, 119.2, 119.1, 22.9; Anal. calcd. for $C_{16}H_{11}BrClN_3O_2$ (392.64): C 48.94%, H 2.82%, N 10.70%. Found: C 48.80%, H 2.96%, N 10.75%.

2-Methyl-3-(2,3-dichlorobenzalamino)-4(3H)-quinazolinone (**III-31**). White needles, yield 87%; m.p. 209~210 °C; IR (cm^{-1}) *v*: 3034 (Qu-H, Ar-H), 2982 (Ar-CH_3), 1680 (C=O), 1612, 1593 (C=N), 1550-1474 (C=C, benzene and quinazolinone rings), 766, 691 (1,2,3-trisubstituted benzene); ^{1}H-NMR: δ 2.51 (s, 3H, 2-Qu-C*H*$_3$), 7.53-8.19 (m, 7H, Qu-H, Ar-*H*), 9.42 (s, 1H, Qu-N=CH-Ar); ^{13}C-NMR: δ 164.0, 158.2, 154.1, 146.7, 135.1, 134.6, 133.4, 133.0, 129.4, 127.4, 127.3, 127.2, 127.0, 121.6, 22.9; Anal. calcd. for $C_{16}H_{11}Cl_2N_3O$ (332.19): C 57.85%, H 3.34%, N 12.65%. Found: C 57.78%, H 3.52%, N 12.53%.

Bioassays: Antifungal Bioassays

The antifungal activity of all synthesized compounds **III-1-III-31** was tested against three pathogenic fungi, namely *Fusarium oxysporum*, *Gibberella zeae,* and *Valsa mali*, by the poison plate technique [22]. Compounds **III-1-III-31** were dissolved in acetone (10 mL) before mixing with Potato Dextrose Agar (PDA, 90 mL). The final concentration of compounds **III-1-III-31** in the medium was fixed at 500 μg/mL. Three kinds of fungi were incubated in PDA at 25±1 °C for 5 days to get new mycelium for antifungal assay, then a mycelia disk of approximately 0.45 cm diameter cut from the culture medium was picked up with a sterilized inoculation needle and inoculated in the center of PDA plate. The inoculated plates were incubated at 25±1°C for 5 days. Acetone in sterilized distilled water served as control, while hymexazole was used as positive control For each treatment, three replicates were carried out. The radial growth of the fungal colonies was measured on the sixth day and the data were statistically analyzed. The *in vitro* inhibiting effects of the test compounds on the fungi were calculated by the formula CV = $\frac{A-B}{A}$, where A represents the diameter of fungi growth on untreated PDA, B represents the diameter of fungi on treated PDA, and CV represents the rate of inhibition.

Antiviral Bioassays

The leaves of *Nicotiana tabacum. L* growing at the same ages were selected. The tobacco mosaic virus with the concentration of 6×10^{-3} mg/mL was dipped and inoculated on the whole leaves. Then the leaves were washed with water and dried. The compound solution was smeared on the left side and the solvent was smeared on the right side for control. The local lesion numbers were then recorded 3-4 days after inoculation [23]. For each compound, three times of repetition were conducted to ensure the reliability of the results.

$$\text{Inhibition rate (\%)} = \frac{\text{av local lesion numbers of control (not treated with compound)} - \text{av local lesion numbers smeared with drugs}}{\text{av local lesion numbers without drugs}}$$

Isolation of total RNA from tobacco leaf

Trizol kit was used according to the standard protocol for total RNA isolation. Prior to RT-PCR, the total RNA samples were treated with DNase I for 10 min and quantified by spectrophotometry and identified by agarose gel electrophoresis [24].

Reagents

RNA isolation kit, MMLV reverse transcriptase and RT-PCR kit were purchased from TAKARA Biotechnology (Dalian) CO., LTD. DNA Marker pUC/Msp I was purchased from MBI Company, Ltd. Primer was designed with Beacon Designer software. Oligo $(dT)_{18}$ and primers were synthesized by Shanghai Sangon Biotechnology Company, Ltd.

RT-PCR assay

cDNA was synthesized with oligo $(dT)_{18}$ complementary at the 3' end of mRNA as a primer. Total RNA (1μg) was used for temple of first-strand cDNA synthesis using extend reverse transcriptase. Reverse transcription was carried out at 37 ^{o}C for 1 h. The single-stranded DNA mixture was used as template in PCR. The primers for PCR amplification are shown in Figure 3. The PCR were performed in Tris-HCl buffer (10 mM, pH 8.3), KCl (50 mM), $MgCl_2$ (1.8-2.0 μl), dNTPs (0.02 μM), primers (0.04 μM), DNA polymerase (1 U). PCR amplification steps consisted of a preliminary denaturation step at 94 ^{o}C for 1 min, followed by 35 cycles at 94 ^{o}C for 40 seconds, at 58 ^{o}C for 40 seconds and at 72 ^{o}C for 50 seconds on icycle of BioRad. PCR products were separated on 1.5% agarose gel in 0.5×TBE buffer and visualized under UV light after staining with ethidium bromide [25]

Products of PCR sequencing

The purified PCR products were sequenced on an automated DNA sequencer by model ABI 310 DNA sequencer. The sequencer analysis was carried out using the chromas223 software and BLAST network services at the National Center for Biotechnology information (NCBI) Genbank.

Semi-quantitative PCR for gene expression

In order to assess relative expression levels of target-gene in water-treated tobacco and (*R*)-4p- and TMV-treated tobacco and tobacco by inoculated TMV only, semi-quantity PCR consisting of 20 cycles (within the logarithmic range of amplification of gene) with putting primer of *β*-actin serving as an internal reference gene was employed for the study. The amplified products were analyzed on a 1.5% agarose gel by the method of F. Mohamed [26].

The relative quantification real-time PCR for expression of the target gene

The relative quantification real-time PCR was carried out with iCycler IQ according to manufacturer's protocol with primer of *β*-actin serving as an internal reference gene. Precautions were taken to ascertain reliable quantitative results: Log-linear dilution curves were performed with primers for the target gene as well as with primers for the *β*-actin. Reactions performed without reverse transcriptase or without template did not result in any product. Reactions were conducted in 50 μL final volumes with IQ Supermix (25 μL) containing SYBR Green and cDNA as template (2μg), and primer mixture (40 pM) for each gene. PCR cycles were as follows: 2 min at 94 ^{o}C, followed by 40 cycles each of 40 seconds duration at 94 ^{o}C, 40 seconds at 58 ^{o}C, and 50 seconds at 72 ^{o}C. By

following PCR, 110 steps for melt curve analysis were completed in 10 seconds at temperature ranging from 40 ^{o}C to 95 ^{o}C. The amplification efficiency was 95-99% for PR-1a and PR-5 gene, respectively (The figure of standard curve are not shown). Each target gene peak was assigned an arbitrary quantitative value correlated to the *β*-actin gene peak, according to the formula:

$$\Delta\Delta C_T = \Delta C_{T\,(test)} - \Delta C_{T\,(calibrator)}$$

C_T being the cycle threshold. Rates of stimulation of RNA expression were calculated from the ΔC_T values at various time points [27].

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References and Notes

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Sample Availability: Samples of the compounds are available from authors.