Short communication

Synthesis, antiviral activity and cytotoxicity evaluation of Schiff bases of some 2-phenyl quinazoline-4(3)H-ones

Krishnan Suresh Kumar [a,*], Swastika Ganguly [b], Ravichandran Veerasamy [c], Erik De Clercq [d]

[a] Medicinal chemistry research Laboratory, KMCH College of Pharmacy, Coimbatore, India
[b] Medicinal chemistry research Laboratory, Department of Pharmaceutical Sciences, Birla Institute of Technology, Ranchi, India
[c] Faculty of Pharmacy, AIMST University, Malaysia
[d] Rega Institute for Medical research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium

ABSTRACT

A new series of 3-(benzylideneamino)-2-phenylquinazoline-4(3H)-ones were prepared through Schiff base formation of 3-amino-2-phenyl quinazoline-4(3)H-one with various substituted carbonyl compounds. Their chemical structures were elucidated by spectral studies. Cytotoxicity and antiviral activity were evaluated against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK- KOS ACVr, para influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, feline corona virus (FIPV), feline herpes virus, respiratory syncytial virus, influenza A H1N1 subtype, influenza A H3N2 subtype, and influenza B virus. Compound **2a** showed better antiviral activity against the entire tested virus.

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1. Introduction

Viral infections caused by the rapid emergence of antiviral drug resistant strains have become a serious threat globally and have fostered the search for new antiviral agents directed against unexplored drug targets. Various nucleoside analogues have been currently used as antiviral agents these derivatives often inhibit viral polymerases. However, only few non-nucleoside antiviral agents are currently marketed.

Quinazoline is an interesting molecule and its pharmacological activities are well documented. It has been reported as antimicrobial [1–7], antiviral [8], anti-HIV [9], anticonvulsant [10,11], anti-inflammatory [12–14], antihistaminic [15], anti-tubercular [16,17], and anticancer [18,19] activity, etc. A huge number of quinazolines had been synthesized and evaluated for various activities however their antiviral activity was not fully investigated to a greater extent. We have synthesized a simple 3-(benzylideneamino)-2-phenyl quinazoline-4(3H)-ones and tested in vitro cytotoxicity and antiviral activity, against viruses representative of herpes simplex virus-1 (KOS),herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK- KOS ACVr, para influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus, feline corona virus (FIPV), feline herpes virus, respiratory syncytial virus, influenza A H1N1 subtype, influenza A H3N2 subtype, influenza B and vesicular stomatitis virus.

2. Chemistry

The synthetic approach for the title compounds were shown in Scheme 1. We utilized the method reported by Anjani et al. for synthesis of 2-phenyl– 4H-3, 1-benzoxazin-4-one [20]. Anthranilic acid (0.02 mol) was dissolved in 30 mL of anhydrous pyridine by stirring slowly at room temperature. The solution was cooled to 0 °C and a solution of benzoyl chloride (0.02 mol) in anhydrous pyridine 30 mL was added to this solution slowly with constant stirring. When the addition was complete the reaction mixture was stirred for half an hour mechanically at room temperature and set

* Corresponding author. Tel.: +91 9486371828; fax: +91 4222628645.
E-mail address: pharmsuki@gmail.com (K.S. Kumar).
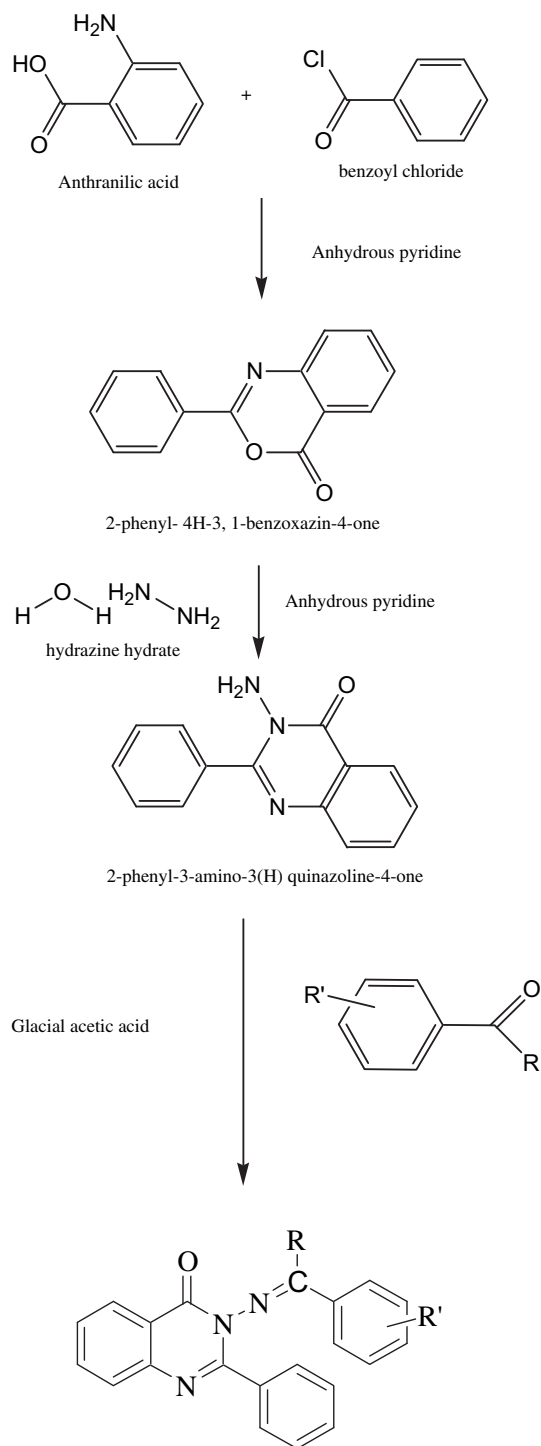
Scheme 1. Synthetic scheme for the title compounds.

aside for 1 h. The pasty mass obtained was diluted with water and treated with aqueous sodium bicarbonate to remove the unreacted acid. When effervescence ceased, the solid material was filtered off and washed with water to remove the inorganic materials and the adhered pyridine. The crude benzoxazine thus obtained was dried and re-crystallized from diluted ethanol.

Subsequently the crystallized material was converted to 2-phenyl-3-amino-3(H) quinazoline-4-one by adding dropwise a solution of hydrazine hydrate (0.1 mol) in anhydrous pyridine (25 mL) to cold solution of 2-phenyl-4H-3, 1-benzoxazin-4-one

(0.05 mol) in anhydrous pyridine (25 mL), with constant stirring [20]. When the addition was complete, the resultant reaction mixture was stirred vigorously for 30 min at room temperature and subsequently heated under reflux for 6 h under anhydrous reaction conditions. It was allowed to cool at room temperature and poured into ice cold water containing dilute hydrochloric acid on standing for 1 h, solidification occurred which was allowed to settle down. It was filtered off, washed repeatedly with water and dried in vacuum and purified by HPLC.

Title compounds were prepared through Schiff's reaction. An equimolar quantity of 2-phenyl-3-amino-3(H)-quinazoline-4-one and aldehyde/ketone were dissolved in ethanol and its pH was adjusted to 4.0–4.5 with glacial acetic acid. The content of the mixture were refluxed for 30–150 min and poured into ice cooled water thus the solid obtained was filtered and purified by HPLC (Compound **2a–l**, Table 1). Their structures are in agreement with elemental and spectral data.

3. Pharmacology

Cytotoxicity and antiviral activity of all compounds were evaluated against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK- KOS ACVr in HEL cell culture, vero cell culture, CRFK, Hela & MDCK cell cultures. Cytotoxic concentration, required to cause a microscopically detectable alteration of normal cell morphology, was measured. Cytopathogenicity concentration required to reduce virus-induced cytopathogenicity by 50%, was determined.

4. Results and discussion

Anthranilic acid reaction with benzoyl chloride yielded 2-phenyl-4H-3, 1-Benzoxazin-4 one by N-acylation via dehydrative cyclization mechanism. Subsequently which was converted to 2-phenyl-3-amino-3(H) quinazoline-4 one with hydrazine hydrate. A series of novel 3-(benzylideneamino)-2-phenylquinazoline-4 (3H)-ones were synthesized by reaction with 2-phenyl-3-amino-3 (H) quinazoline-4 one and compounds containing aldehyde and ketones to afford 2,3disubstituted quinazoline-4(3)H one derivatives (Table 1). The spectral data of synthesized compounds were

Table 1
Structure of the synthesized compounds.
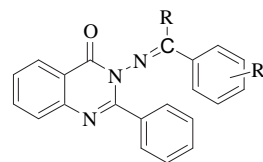
Compd.	R	R′
2a	H	2-OH
2b	H	3-NO$_2$
2c	H	4-OCH$_3$
2d	H	4-N(CH$_3$)$_2$
2e	CH$_3$	4-Cl
2f	H	H
2g	H	4-OH
2h	CH$_3$	H
2i	CH$_3$	4-OH
2j	H	4-Cl
2k	H	3-OH & 4-OCH$_3$
2l	H	2-OCH$_3$

Table 2
Cytotoxicity and antiviral activity of compounds in HEL cell cultures.

Compd.	Minimum cytotoxic concentration[a] (μg/mL)	EC$_{50}$[b] (μg/mL)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK- KOS ACVr
2a	100	1	1	0.8	15	1
2b	≥20	>20	>20	>20	>20	>20
2c	>100	9	8	9	>100	9
2d	100	>20	>20	>20	>20	>20
2e	100	>20	>20	>20	>20	>20
2f	>100	7	9	9	>100	9
2g	100	>20	>20	12	>20	>20
2h	>100	>100	>100	>100	>100	>100
2i	>100	>100	>100	>100	>100	>100
2j	100	>20	>20	20	>20	>20
2k	100	>20	>20	20	>20	>20
2l	100	>20	>20	>20	>20	>20
Brivudin (μM)	>250	0.04	50	4	>250	250
Ribavirin (μM)	>250	>250	>250	>250	146	>250
Cidofovir (μM)	>250	2	2	8	>250	3
Ganciclovir (μM)	>100	0.05	0.07	>100	>100	2

Data represent mean values for three independent determinations. Variation among duplicate samples was less than 15%.
  [a] Required to cause a microscopically detectable alteration of normal cell morphology.
  [b] Required to reduce virus-induced cytopathogenicity by 50%.

consistent with the assigned structures and the compounds were obtained in 68–78% yield.

Title compounds were tested against representative members of the virus includes herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK- KOS ACVr (Table 2), para influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus (Table 3), feline corona virus (FIPV), feline herpes virus (Table 4), respiratory syncytial virus (Table 5) and influenza A H1N1 subtype, influenza A H3N2 subtype, influenza B (Table 6).

Compound **2a** exhibited antiviral activity against herpes simplex virus-1 (KOS), herpes simplex virus-2(G), herpes simplex virus-1 (TK- KOS ACV) and vaccinia virus in HEL cell culture (Table 2) at selectivity index of 100, 100, 100 and 125 respectively, whereas cytotoxicity was found at 100 μg/mL. Compound **2a** also inhibited the replication of reovirus, Sindbis virus, Coxsackie virus B4 and Punta Toro virus in Vero cell culture (Table 3) at selectivity index of 10, 20, 10 and 33, whereas cytotoxicity was found 100 μg/mL. It also showed inhibitory to vesicular stomatitis virus, respiratory syncytial virus in HeLa cells (Table 5) albeit at a concentration closely related to the cytotoxicity (4 μg/mL).

Compounds **2c** and **f** were found to be active against herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, herpes simplex virus-1 TK- KOS ACVr in HEL cell culture (Table 2) at selectivity index of 11, 13, 11, 11 and 14, 11, 11, 11 respectively, where they were not cytotoxic at 100 μg/mL none of the compounds tested was active against influenza A H1N1, influenza A H3N2 and influenza B in MDCK cell Cultures.

Of the compounds tested, compound **2a** clearly proved the most promising in terms of antiviral activity (potency). This lead molecule **2a** could be further utilized for designing newer non-nucleoside antiviral agents especially for activity against HSV and vaccinia virus.

The synthetic procedure reported in this article allows a rapid synthesis of derivative of the title compounds to be tested for their antiviral effects.

5. Experimental

Melting points of the synthesized compounds were determined by using an open capillary tube method and are uncorrected. Microanalyses were carried out on a Carlo Erba 1106 elemental

Table 3
Cytotoxicity and antiviral activity of compounds in Vero cell cultures.

Compd.	Minimum cytotoxic concentration[a] (μg/mL)	EC$_{50}$[b] (μg/mL)				
		Para influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
2a	100	>20	10	5	10	3
2b	20	>4	>4	>4	>4	>4
2c	100	>20	>20	>20	>20	>20
2d	100	>20	>20	>20	>20	>20
2e	100	>20	>20	>20	>20	>20
2f	20	>4	>4	>4	>4	>4
2g	100	>20	>20	>20	>20	>20
2h	100	>20	>20	>20	>20	>20
2i	>100	>100	>100	>100	>100	>100
2j	20	>4	>4	>4	>4	>4
2k	100	>20	>20	>20	>20	>20
2l	100	>20	>20	>20	>20	>20
DS-5000	>100	>100	>100	20	20	20
(S)-DHPA (μM)	>250	>250	>250	>250	>250	>250
Ribavirin (μM)	>250	146	>250	>250	>250	146

  [a] Required to cause a microscopically detectable alteration of normal cell morphology.
  [b] Required to reduce virus-induced cytopathogenicity by 50%.

Table 4
Anti-Feline Corona Virus (FIPV) and anti-Feline Herpes Virus activity and cytotoxicity in CRFK cell cultures.

Compd.	CC$_{50}$[a] (µg/mL)	EC$_{50}$[b] (µg/mL)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
2a	11.2	>4	>4
2b	13.8	>4	>4
2c	13.7	>4	>4
2d	>100	>100	>100
2e	>100	>100	>100
2f	3.7	>0.8	>0.8
2g	59.5	>20	>20
2h	2.6	>0.8	>0.8
2i	>100	>100	>100
2j	42.2	>20	>20
2k	42.7	>20	>20
2l	14.4	>4	>4
HHA	>100	2.6	1.6
UDA	>100	11.7	53.1
Ganciclovir (µM)	>100	>100	0.8

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay. CRFK cells: Crandell-Rees Feline Kidney cells.

analyzer. The results of elemental analysis were within ±0.3% for C and ±0.1% for H and N of the theoretical value. ^{1}H NMR spectra were performed on a Varian Gemini 200 (200 MHz) spectrometer using tetramethylsilane (TMS) as internal standard. IR spectra were recorded on an FTIR-JASCO 4100 Spectrophotometer. GC–MS spectra were performed with HP 6890–5973. GC parameters: injector temperature 250 °C; capillary column HP5 poly (methylphenylsiloxane) 30 m, 0.35 mm, 0.25 mm; temperature program: from 100 to 300 °C at 10 °C/min. MS parameters: mode SCAN 40–600 amu.

5.1. 3-[(2-Hydroxy-benzilidine)-amino]-2-phenyl-3H-quinazolin-4one (**2a**)

Yield: 78%; m.p 144–146 °C; IR (KBr, in cm^{-1}): 3200–3100 (O–H str. for −OH), 1683 (C=O str.), 1604 (C=N str.), 1465 (ring C=C str.),

Table 5
Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compd.	Minimum cytotoxic concentration[a] (µg/mL)	EC$_{50}$[b] (µg/mL)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
2a	4	>0.8	>0.8	>0.8
2b	20	>4	>4	>4
2c	20	>4	>4	>4
2d	100	>20	>20	>20
2e	100	>20	>20	>20
2f	20	>4	>4	>4
2g	100	>20	>20	>20
2h	>100	>100	>100	>100
2i	>100	>100	>100	>100
2j	20	>4	>4	>4
2k	20	>4	>4	>4
2l	20	>4	>4	>4
DS-5000	>100	>100	7	0.8
(S)-DHPA (µM)	>250	>250	>250	>250
Ribavirin (µM)	>250	22	146	22

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

1362 (Ar C−N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 7.28–7.46 (m, 6H, ArH), 7.66–7.92 (m, 7H, ArH), 8.68 (s, 1H, H−C=N).

5.2. 3-[(3-Nitro-benzilidine)-amino]-2-phenyl-3H-quinazolin-4one (**2b**)

Yield: 74%; m.p 246–248 °C; IR (KBr, in cm^{-1}): 1678 (C=O str.), 1645.4 (C=N str.), 1502 (ring C=C str.), 1458 (Ar C−N Str), 1345 (N=O str. for ArNO$_2$); ^{1}H NMR (CDCl$_3$, δ in ppm): 7.25–7.52 (m, 6H, ArH), 7.62–7.80 (m, 4H, ArH), 8.0–8.21 (m, 3H, ArH), 8.67 (s, 1H, H−C=N).

5.3. 3-[(4-Methoxy-benzilidine)-amino]-2-phenyl-3H-quinazolin-4one (**2c**)

Yield: 68%; m.p 242–244 °C; IR (KBr, in cm^{-1}): 1680 (C=O str.), 1644.6 (C=N str.), 1502 (ring C=C str.), 1446 (Ar C−N Str), 1026 (C−O−C str. for −C−O−CH$_3$); ^{1}H NMR (CDCl$_3$, δ in ppm): 3.73 (s, 3H, −OCH$_3$), 7.25–7.49 (m, 6H, ArH), 7.58–7.80 (m, 4H, ArH), 8.10–8.22 (m, 3H, ArH), 8.60 (s, 1H, H−C=N).

5.4. 3-[(4-Dimethylamino-benzilidine)-amino]-2-phenyl-3H-quinazolin-4one (**2d**)

Yield: 72%; m.p 175–177 °C; IR (KBr, in cm^{-1}): 1676 (C=O str.), 1640 (C=N str.), 1510 (ring C=C str.), 1460 (Ar C−N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 3.12 (s, 6H, -NCH$_3$), 7.23–7.42 (m, 6H, ArH), 7.62–7.80 (m, 7H, ArH), 8.56 (s, 1H, H−C=N).

5.5. 3-[1-(4-Chloro-phenyl)-ethylideneamino-]-2-phenyl-3H-quinazolin-4one (**2e**)

Yield: 74%; m.p 243–245 °C; IR (KBr, in cm^{-1}): 1670 (C=O str.), 1646 (C=N str.), 1515 (ring C=C str.), 1455 (Ar C−N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 2.46 (s, 3H, -CH$_3$), 7.20–7.48 (m, 6H, ArH), 7.57–7.78 (m, 7H, ArH).

5.6. 3-(Benzilidine-amino)-2-phenyl-3H-quinazolin-4one (**2f**)

Yield: 75%; m.p 194–196 °C; IR (KBr, in cm^{-1}): 1665 (C=O str.), 1642 (C=N str.), 1510 (ring C=C str.), 1449 (Ar C−N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 7.25–7.44 (m, 6H, ArH), 7.62–7.80 (m, 8H, ArH), 8.58 (s, 1H, H−C=N).

5.7. 3-[(4-Hydroxy-benzilidine)-amino]-2-phenyl-3H-quinazolin-4one (**2g**)

Yield: 78%; m.p 164–166 °C; IR (KBr, in cm^{-1}): 3304–3212 (O−H str. for −OH), 1668 (C=O str.), 1645 (C=N str.), 1554 (ring C=C str.), 1372 (Ar C−N Str); ^{1}H NMR (DMSO-d$_6$, δ in ppm): 5.10 (s, 1H, −OH), 7.42–7.48 (m, 6H, ArH), 7.70–7.79 (m, 5H, ArH), 8.12–8.29 (m, 3H, ArH), 8.62 (s, 1H, H−C=N).

5.8. 2-Phenyl-3(1-phenyl-ethylideneamino)-3H-quinazolin-4one (**2h**)

Yield: 73%; m.p 194–196 °C; IR (KBr, in cm^{-1}): 1685 (C=O str.), 1614 (C=N str.), 1464.2 (ring C=C str.), 1363.8 (Ar C−N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 2.44 (s, 3H, CH$_3$), 7.28–7.46 (m, 6H, ArH), 7.66–7.92 (m, 7H, ArH).

5.9. 3-[1-(4-Hydroxy-phenyl)-ethylideneamino]-2-phenyl-3H-quinazolin-4one (**2i**)

Yield: 68%; m.p 166–168 °C; IR (KBr, in cm^{-1}): 3200–3150 (O−H str. for −OH), 1686.4 (C=O str.), 1614 (C=N str.), 1468 (ring C=C

Table 6
Anti-influenza virus activity and cytotoxicity in MDCK cell cultures.

Compd.	Concentration	Cytotoxicity		Antiviral EC_{50}[c]					
		CC_{50}[a]	Minimum cytotoxic concentration[b]	Influenza A H1N1 subtype		Influenza A H3N2 subtype		Influenza B	
				Visual CPE score	MTS	Visual CPE score	MTS	Visual CPE score	MTS
2a	μg/mL	2.6	≥0.8	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2b	μg/mL	>100	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2c	μg/mL	8.2	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2d	μg/mL	>100	100	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2e	μg/mL	>100	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2f	μg/mL	15.0	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2g	μg/mL	10.2	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2h	μg/mL	>100	100	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2i	μg/mL	>100	>100	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2j	μg/mL	76.4	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2k	μg/mL	47.4	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
2l	μg/mL	12.4	20	N.A.	N.A.	N.A.	N.A	N.A.	N.A.
Oseltamivir carboxylate	μM	>100	>100	0.2	0.1	4	3.8	2	1.5
Ribavirin	μM	>100	≥100	9	12.9	9	7.8	9	6.0
Amantadin	μM	>1000	>1000	101	87	117	115	N.A.	N.A.
Rimantadin	μM	472	1000	68	58	40	53	N.A.	N.A.

N.A − not active at the highest concentration tested, or at subtoxic concentration.
[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] Minimum compound concentration that causes a microscopically detectable alteration of normal cell morphology.
[c] 50% Effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay. MDCK cells: Madin Darby canine kidney cells.

str.), 1372 (Ar C–N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 2.47 (s, 3H, CH$_3$), 6.84–6.92 (m, 3H, ArH), 7.29–7.51 (m, 8H, ArH), 7.64–7.82 (m, 3H, ArH).

5.10. 3-[(4-Chloro-benzylidine)-amino]-2-phenyl-3H-quinazolin-4one (2j)

Yield: 70%; m.p 160–162 °C; IR (KBr, in cm^{-1}): 1680 (C=O str.), 1587 (C=N str.), 1552 (ring C=C str.), 1374 (Ar C–N Str); ^{1}H NMR (CDCl$_3$, δ in ppm): 7.42–7.58 (m, 6H, ArH), 7.66–7.82 (m, 7H, ArH), 8.76 (s, 1H, H–C=N).

5.11. 3-[(3-Hydroxy-4-methoxy-benzylidine)-amino]-2-phenyl-3H-quinazolin-4one (2k)

Yield: 75%; m.p 180–182 °C; IR (KBr, in cm^{-1}): 3206–3178 (O–H str. for −OH), 1686 (C=O str.), 1624 (C=N str.), 1460 (ring C=C str.), 1364 (Ar C–N Str), 1025 (C–O–C str. for −C–O–CH$_3$); ^{1}H NMR (DMSO-d_6, δ in ppm): 3.72 (s, 3H, −OCH$_3$), 5.02 (s, 1H, −OH), 6.64 (d, 1H, ArH), 7.27–7.46 (m, 8H, ArH), 7.62–7.75 (m, 3H, ArH), 8.72 (s, 1H, H–C=N).

5.12. 3-[(2-Methoxy-benzylidine)-amino]-2-phenyl-3H-quinazolin-4one (2l)

Yield: 75%; m.p 212–214 °C; IR (KBr, in cm^{-1}): 1680 (C=O str.), 1644.6 (C=N str.), 1502 (ring C=C str.), 1446 (Ar C–N Str), 1024.37 (C–O–C str. for −C–O–CH$_3$); ^{1}H NMR (CDCl$_3$, δ in ppm): 3.74 (s, 3H, -OCH$_3$), 6.78–6.81 (m, 2H, ArH), 7.26–7.43 (m, 7H, ArH), 7.58–7.80 (m, 4H, ArH), 8.59 (s, 1H, H–C=N).

5.13. Antiviral activity

Activity of compounds against para influenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus was measured by plaque reduction assays in Vero cell monolayer. To this end, Vero cells were seeded in 24-well plates at a density of 2×10^5 cells/well

and were allowed to form confluent monolayers by incubating overnight in growth medium at 37 °C in humidified CO$_2$ (5%) atmosphere. Then, monolayers were infected with 250 μL of proper virus dilutions to give 50–100 PFU/well. Following removal of unadsorbed virus, 500 μL of Dulbecco's modified Eagle's medium supplemented with 1% inactivated FCS and 0.75% methyl cellulose, without or with serial dilutions of test compounds were added. Cultures were incubated at 37 °C for 2 days and then fixed with PBS containing 50% ethanol and 0.8% crystal violet, washed and air-dried. Plaques were then counted, and 50% effective concentrations (EC$_{50}$) values were calculated by the linear regression technique.

Cytotoxicity and antiviral activity of compounds were also tested against vesicular stomatitis virus, Coxsackie virus B4 and respiratory syncytical virus in HeLa cell cultures. Minimum cytotoxic concentration was the concentration required to cause a microscopically detectable alteration of normal cell morphology. Effective concentration was the concentration required to reduce the virus-induced cytopathogenicity by 50%.

Compounds were also evaluated against influenza A HIN1, influenza A H3N2 and influenza B in Madin Darby canine kidney cells (MDCK) Cell cultures and 50% cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay similarly 50% effective concentration or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by visual scoring of the CPE, or by measuring the cell viability with the colorimetric formazan-based MTS assay protocol.

Activity of compounds against feline corona virus and feline herpes virus was based on 50% effective concentration, or concentration producing 50% inhibition of virus-induced cytopathic effect as determined by measuring cell viability with the colorimetric formazan-based MTS assay in Crandell-Rees Feline Kidney cells (CRFK Cells).

Compounds which are potentially active in the earlier screening were evaluated against herpes simplex virus-1(KOS), Herpes simplex virus-2(G), vaccina virus and herpes simplex virus-1 TK-KOS ACV in HEL cell cultures and analyses for their cytotoxic

concentration and effective concentration. Selective compounds were screened against para influenza-3 virus, reovirus-1, Sindbis virus B4 and Punta Toro virus in Vero cell cultures.

Acknowledgements

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