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Synthesis and anti-measles virus activity of new isoquinolin-4-one derivatives

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Abstract

Despite intense efforts to increase vaccine coverage, measles virus (MV) still causes significant morbidity and mortality in the world sometimes as a results of severe, chronic and lethal diseases. In an effort to develop therapies to supplement immunization strategies a number of 1-oxo-2-{[(1*E*)-phenylmethylene]amino}-1,2-dihydroisoquinoline-4-carboxylic acid derivatives were synthesized and evaluated for anti-measles activity. The substituents on the aromatic ring were chosen in order to evaluate the influence of electron-withdrawing or electron-donating effects on the electronic density of the aromatic moiety. We also evaluated the introduction of a vinyl chain between the exocyclic nitrogen and phenyl moiety. The biological results allow to outline some preliminary considerations on structure–activity relationship.

Keywords: Isoquinolin-4-one derivatives; Synthesis; Structure–activity relationship; Anti-viral activity; Measles virus

1. Introduction

Despite the availability of efficacious vaccines and eradication efforts measles virus (MV) is one of leading causes of infectious disease-induced morbidity and mortality in childhood in the world. Currently 30–40 million measles infections occurs worldwide each year lead to 1 million deaths due to MV-related infections [1]. In addition to causing an acute respiratory infection, measles is also correlated with an important, but transient suppression of cell-mediated immunity that can lead to secondary infections, and complications such as pneumonia and diarrhoea.

There are highly effective vaccines available, although their safety has been occasionally questioned because of tenuous and questionable link to Crohn disease and to autism [2,3].

Of interest for the eradication of MV outbreaks is that virus isolates are not of single geno- or serotype, hence MV demonstrates substantial heterogeneity [4]. In the last decade, genotyping studies have indicated that the RNA sequences of the MV strains currently circulating have changed considerably from those isolated previously. This suggests that MV may contribute considerably to worldwide morbidity and mortality as it evolves consequently to the elimination efforts. Therefore, to treat unexpected outbreak infections rapidly and efficiently may be useful to supplement global immunization with non-toxic treatments.

No chemotherapeutic agents are currently approved for use for the prophylaxis or treatment of measles, although ribavirin has reportedly been efficacious when administered intravenously and orally for treatment of MV infections [5–7] alone or in combination with immune serum globulin in patients [8,9]. Regardless of their clinical efficacies, both ribavirin and immune serum globulin are costly [10,11] and require hospitalization for administration [12], limiting the use of either of these agents for the treatment of measles, particularly in developing countries where this disease is most preponderant. To date no other clinical trials have been done that support these findings of efficacy; on

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the contrary, a number of studies have also been done showing that ribavirin has no obvious clinical efficacy in MV infections when given as small-particle aerosol or intravenously [13]. The continued prevalence of measles and the paucity of available agents for the prevention or treatment of MV infections make the elucidation and development of new chemotherapeutic and/or biologic agents effective against this virus highly desirable.

Several compounds with antiviral activity versus myxovirus and paramyxovirus such as UK 2054 (famotine), 1-[(*p*-clorofenoxymethyl]-3,4-dihydroquinoline, the corresponding *p*-methoxy analogous UK 2371 (memotine), and DIQA, 2-(3,4-dihydroisoquinolin-1-yl)acetamide, have an isoquinoline nucleus in their structure [14,15] (Fig. 1). As part of our ongoing interest in the area of isoquinoline derivatives [16], in this paper, we disclose our synthetic efforts and structure–activity relationship (SAR) studies that led to the identification of a novel group of analogues with isoquinoline scaffold (**A**) having antiviral activity against MV (Fig. 1).

2. Experimental procedures

2.1. Chemistry

Reagents used for synthesis were purchased from Sigma Aldrich (Milano, Italy) unless otherwise specified. The course of the reaction was monitored by thin-layer chromatography (TLC) on precoated silica gel 60 F_{254} aluminium sheets (Merck, Darmstadt, Germany), visualisation was provided under UV light. Melting points were obtained in open capillary tubes with a Büchi apparatus (Büchi Italia, Assago, Italy) and are uncorrected. Infrared spectra (IR) were recorder in KBr disks using a 1600 FT-IR Perkin–Elmer (Perkin–Elmer Italia, Monza, Italy) spectrophotometer and are consistent with the assigned structures. Nuclear magnetic resonance (NMR) spectra were recorded with a Varian Inova 200 spectrometer (Varian, Leini, Italy). NOE experiments were carried out using the standard CYCLENOE pulse sequence on the Varian spectrometer. Log D and solubility were calculated using ACD/Labs software (version 4.5, 2000, ACD, Toronto, ON, Canada). Elemental analyses (C, H, N) were determined on an elemental analyser Carlo Erba Model 1106 (Carlo Erba, Milano, Italy), and were within $\pm 0.4\%$ of the theoretical values.

2.2. Synthesis of 2-amino-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**2**)

A solution of **1** (2.0 g, 10 mmol) and hydrazine monohydrate (15 ml, 309 mmol) in 40 ml of absolute ethanol was refluxed for 1 h. The solution was allowed to cool to room temperature (r.t.) and subsequently diluted hydrochloric acid was added until to pH 4. The precipitate obtained was collected by filtration and washed with water until to neutral pH. Finally the white solid was crystallised from ethyl alcohol to give 1.32 g of desired acid (65%). ^{1}H NMR (DMSO-d_6): δ, 12.92 (s broad, 1H, COOH), 8.67 (d, 1H, ArH), 8.15 (d, 1H, ArH), 8.14 (s, 1H, ArH), 7.72 (t, 1H, ArH), 7.46 (t, 1H, ArH), 6.43 (s broad, 2H, $-NH_2$).

2.3. General procedure for synthesis of N-arylenamine derivatives (**3**–**16**)

The appropriate aldehydes (3 mmol), was added to a solution of **2** (2 mmol) in 25 ml of acetic acid, and was heated for 30 min under reflux. The mixture obtained was cooled to r.t. and the precipitate was collected by filtration. Afterward the solid was washed with water and was crystallised by opportune solvents. Yields, melting points and crystallization solvents are reported in Table 1.

2.3.1. 1-oxo-2-{[(1E)-phenylmethylene]amino}-1,2-dihydroisoquinoline-4-carboxylic acid (**3**)

^{1}H NMR (DMSO-d_6): δ 13.02 (s broad, 1H, COOH), 9.12 (s, 1H, CH=N), 8.67 (d, 1H, ArH), 8.41 (s, 1H, ArH), 8.31 (d, 1H, ArH), 7.93–7.78 (m, 3H, ArH), 7.68–7.42 (m, 4H, ArH).

2.3.2. 2-{[(1E)-(4-chlorophenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**4**)

^{1}H NMR (DMSO-d_6): δ 13.02 (s broad, 1H, COOH), 9.44 (s, 1H, CH=N), 8.89 (d, 1H, ArH), 8.43 (s, 1H,

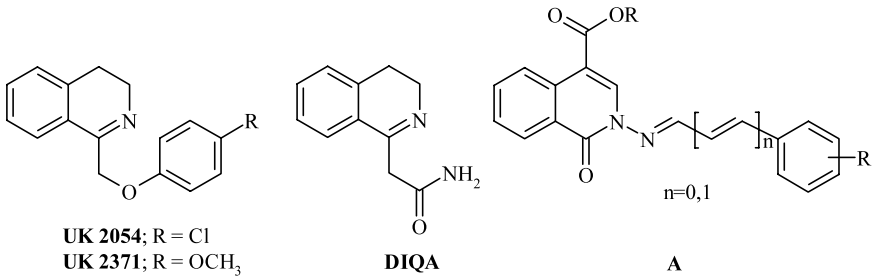
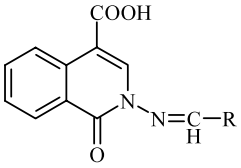


Fig. 1. UK 2054 (Famotine), 1-[(*p*-clorofenoxymethyl]-3,4-dihydroquinoline, the corresponding *p*-methoxy analogous UK 2371 (memotine), DIQA, 2-(3,4-dihydroisoquinolin-1-yl)acetamide and (**A**) isoquinoline derivatives synthesized in this study.

Table 1
Structures, yields and melting points of the prepared quinazolinones derivatives



Compound	R	Yield (%)	Melting point (°C)	Formula
3 [a]	$-C_6H_5$	73	262–4	$C_{17}H_{12}N_2O_3$
4 [a]	$-C_6H_4$-4-Cl	78	293–4	$C_{17}H_{11}N_2O_3Cl$
5 [a]	$-C_6H_4$-2-Cl	83	295–7	$C_{17}H_{12}N_2O_3$
6 [a]	$-C_6H_4$-4-Br	85	295–6	$C_{17}H_{11}N_2O_3Br$
7 [a]	$-C_6H_4$-2-Br	90	298–9	$C_{17}H_{12}N_2O_3$
8 [b]	$-C_6H_4$-4-NO_2	58	> 300	$C_{17}H_{11}N_3O_5$
9 [b]	$-C_6H_4$-2-COOH	75	287–9	$C_{17}H_{12}N_2O_3$
10 [b]	$-C_6H_4$-4-OH	64	280–1	$C_{17}H_{12}N_2O_4$
11 [b]	$-C_6H_4$-4-OCH_3	79	253–5	$C_{17}H_{12}N_2O_3$
12 [a]	$-CH=CH-C_6H_5$	87	264–6	$C_{19}H_{13}N_2O_3$
13 [a]	$-CH_2CH_2-C_6H_5$	81	261–2	$C_{17}H_{12}N_2O_3$
14 [a]	$-C(CH_3)=CH-C_6H_5$	65	256–8	$C_{17}H_{12}N_2O_3$
15 [b]	$-CH=CH-C_6H_4$-4-NO_2	86	272–4	$C_{17}H_{12}N_2O_3$
16 [b]	$-CH=CH-C_6H_4$-4-$N(CH_3)_2$	68	268–9	$C_{17}H_{12}N_2O_3$

[a] Recrystallized from acetic acid.
[b] Recrystallized from dimethylformamide.

ArH), 8.38 (d, 1H, ArH), 7.88 (d, 2H, ArH), 7.96–7.83 (m, 1H, ArH), 7.67 (t, 1H, ArH), 7.52 (d, 2H, ArH).

*2.3.3. 2-{[(1E)-(2-chlorophenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**5**)*

^{1}H NMR (DMSO-d_6): δ 13.27 (s broad, 1H, COOH), 9.91 (s, 1H, CH=N), 8.89 (d, 1H, ArH), 8.55 (s, 1H, ArH), 8.39 (d, 1H, ArH), 8.23–7.59 (m, 6H, ArH).

*2.3.4. 2-{[(1E)-(4-bromophenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**6**)*

^{1}H NMR (DMSO-d_6): δ 13.09 (s broad, 1H, COOH), 9.64 (s, 1H, CH=N), 8.91 (d, 1H, ArH), 8.47 (s, 1H, ArH), 8.41 (d, 1H, ArH), 7.82 (d, 2H, ArH), 7.98–7.85 (m, 1H, ArH), 7.71 (t, 1H, ArH), 7.67 (d, 2H, ArH).

*2.3.5. 2-{[(1E)-(2-bromophenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**7**)*

^{1}H NMR (DMSO-d_6): δ 13.31 (s broad, 2H, COOH), 9.96 (s, 1H, CH=N), 8.92 (d, 1H, ArH), 8.59 (s, 1H, ArH), 8.43 (d, 1H, ArH), 8.29–7.63 (m, 6H, ArH).

*2.3.6. 2-{[(1E)-(4-nitrophenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**8**)*

^{1}H NMR (DMSO-d_6): δ 13.25 (s broad, 1H, COOH), 9.71 (s, 1H, CH=N), 8.92 (d, 1H, ArH), 8.46 (s, 1H, ArH), 8.39 (d, 1H, ArH), 8.11 (d, 2H, ArH), 7.99–7.83 (m, 1H, ArH), 7.69 (t, 1H, ArH), 8.23 (d, 2H, ArH).

*2.3.7. 2-{[(1E)-(2-carboxyphenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**9**)*

^{1}H NMR (DMSO-d_6): δ 13.24 (s broad, 2H, COOH), 9.86 (s, 1H, CH=N), 8.86 (d, 1H, ArH), 8.50 (s, 1H, ArH), 8.35 (d, 1H, ArH), 8.19–7.57 (m, 6H, ArH).

*2.3.8. 2-{[(1E)-(4-hydroxyphenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**10**)*

^{1}H NMR (DMSO-d_6): δ 12.97 (s broad, 1H, COOH), 9.82 (s broad, 1H, Ar–OH), 9.03 (s, 1H, CH=N), 8.85 (d, 1H, ArH, 8 Hz), 8.40 (s, 1H, ArH), 8.33 (d, 1H, ArH, 8.4 Hz), 7.89 (d, 2H, ArH, 9 Hz), 7.92–7.78 (m, 1H, ArH), 7.61 (t, 1H, ArH, 7.2 Hz), 7.11 (d, 2H, ArH, 8.6 Hz).

*2.3.9. 2-{[(1E)-(4-methoxyphenyl)methylene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**11**)*

^{1}H NMR (DMSO-d_6): δ 12.97 (s broad, 1H, COOH), 9.09 (s, 1H, CH=N), 8.87 (d, 1H, ArH, 8 Hz), 8.41 (s, 1H, ArH), 8.35 (d, 1H, ArH, 8.4 Hz), 7.91 (d, 2H, ArH, 9 Hz), 7.94–7.79 (m, 1H, ArH), 7.63 (t, 1H, ArH, 7.2 Hz), 7.12 (d, 2H, ArH, 8.6 Hz), 3.86 (s, 3H, OCH_3)

*2.3.10. 1-oxo-2-{[(1E,2E)-3-phenylprop-2-enylidene]amino}-1,2-dihydroisoquinoline-4-carboxylic acid (**12**)*

^{1}H NMR (DMSO-d_6): δ 12.95 (s broad, 1H, COOH), 8.85 (d, 1H, CH=N, 9.27 Hz), 8.63 (d, 1H, ArH), 8.25 (s, 1H, ArH), 8.23 (dd, 1H, ArH, 8.6, 1.2 Hz), 7.80–7.20 (m, 1H, ArH), 7.66–7.57 (m, 2H, ArH), 7.54–7.43 (m,

1H, ArH), 7.35–7.24 (m, 3H, ArH), 7.35 (d, 1H, ArCH=C, 15.62 Hz), 7.10 (dd, 1H, ArCH=*CH*–, 15.62, 9.27 Hz).

*2.3.11. 1-oxo-2-{[(1E)-3-phenylpropylidene]amino}-1,2-dihydroisoquinoline-4-carboxylic acid (**13**)*

^{1}H NMR (DMSO-d_6): δ 12.96 (s broad, 1H, COOH), 8.84 (d, 1H, ArH), 8.48 (t, 1H, CH=N), 8.30 (d, 1H, ArH), 8.21 (s, 1H, ArH), 7.81 (t, 1H, ArH), 7.58 (t, 1H, ArH), 7.28–7.14 (m, 5H, ArH), 3.02–2.91 (m, 2H, Ar–CH_2–), 2.86–2.74 (m, 2H, –CH_2–C=N).

*2.3.12. 2-{[(1E,2E)-2-methyl-3-phenylprop-2-enylidene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**14**)*

^{1}H NMR (DMSO-d_6): δ 12.99 (s broad, 1H, COOH), 8.90 (s, 1H, CH=N), 8.86 (d, 1H, ArH), 8.35 (s, 1H, ArH), 8.33 (d, 1H, ArH), 7.83 (t, 1H, ArH), 7.57–7.35 (m, 6H, ArH), 7.25 (s, 1H, ArH), 2.21 (s, 3H, CH_3–C=).

*2.3.13. 2-{[(1E,2E)-3-(2-nitrophenyl)prop-2-enylidene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**15**)*

^{1}H NMR (DMSO-d_6): δ 13.01 (s broad, 1H, COOH), 9.10 (d, 1H, CH=N, 9.4 Hz), 8.84 (d, 1H, ArH), 8.36 (s, 1H, ArH), 8.33 (d, 1H, ArH), 8.12–8.02 (m, 2H, ArH), 7.80 (d, 1H, ArCH=C, 15.8 Hz), 7.85–7.75 (m, 2H, ArH), 7.70–7.60 (m, 3H, ArH), 7.24 (dd, 1H, ArCH=*CH*–, 15.8, 9.4 Hz).

*2.3.14. 2-({(1E,2E)-3-[4-(dimethylamino)phenyl]prop-2-enylidene}amino)-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**16**)*

^{1}H NMR (DMSO-d_6): δ 12.94 (s broad, 1H, COOH), 8.86 (d, 1H, ArH), 8.76 (d, 1H, CH=N, 9.4 Hz), 8.31 (d, 1H, ArH), 8.29 (s, 1H, ArH), 7.81 (t, 1H, ArH), 7.64–7.50 (m, 3H, ArH), 7.28 (d, 1H, ArCH=C, 15.8 Hz), 6.92 (dd, 1H, ArCH=*CH*–, 15.8, 9.4 Hz), 6.72 (d, 2H, ArH), 2.97 (s, 6H, N(CH_3)$_2$).

*2.4. Synthesis of 4-acethyl-2-aminoisoquinolin-1-(2H)-one (**17**)*

A solution of **2** (10.2 g, 50 mmol) in 100 ml of methanol 10% H_2SO_4 was refluxed for 2 h. After cooled to r.t. an aqueous solution of saturated Na_2CO_3 was added and the white precipitate was collected and washed with water until to neutral pH. The solid was finally crystallised from absolute ethanol to yield 7.74 g (71%) of **17**. ^{1}H NMR (DMSO-d_6): δ 8.79 (d, 1H, ArH), 8.29 (d, 1H, ArH), 8.26 (s, 1H, ArH), 7.79 (t, 1H, ArH), 7.57 (t, 1H, ArH), 6.49 (s broad, 2H, –NH_2), 3.87 (s, 3H, –$COOCH_3$).

*2.5. General procedure for synthesis of N-arylenamine ester derivatives (**18**–**31**)*

The appropriate aldehydes (3 mmol), was added to a solution of **2** (2 mmol) in 25 ml of acetic acid, and was heated for 30 min under reflux. The mixture obtained was cooled to r.t. and the precipitate was collected by filtration. Afterward the solid was washed with water and was crystallised from the opportune solvents. Yields, melting points and crystallization solvents are reported in Table 2.

*2.5.1. Methyl 1-oxo-2-{[(1E)-phenylmethylene]-amino}-1,2-dihydroisoquinoline-4-carboxylate (**18**)*

^{1}H NMR (DMSO-d_6): δ 9.22 (s, 1H, CH=N), 8.75 (d, 1H, ArH), 8.48 (s, 1H, ArH), 8.36 (d, 1H, ArH), 7.98–7.81 (m, 3H, ArH), 7.94–7.79 (m, 3H, ArH), 7.77–7.50 (m, 4H, ArH), 3.85 (s, 3H, –$COOCH_3$).

*2.5.2. Methyl 2-{[(1E)-(4-chlorophenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**19**)*

^{1}H NMR (DMSO-d_6): δ 9.57 (s, 1H, CH=N), 9.03 (d, 1H, ArH), 8.57 (s, 1H, ArH), 8.52 (d, 1H, ArH), 8.01 (d, 2H, ArH), 8.01–7.96 (m, 1H, ArH), 7.79 (t, 1H, ArH), 7.66 (d, 2H, ArH), 3.85 (s, 3H, –$COOCH_3$).

*2.5.3. Methyl 2-{[(1E)-(2-chlorophenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**20**)*

^{1}H NMR (DMSO-d_6): δ 10.03 (s, 1H, CH=N), 9.02 (d, 1H, ArH), 8.69 (s, 1H, ArH), 8.53 (d, 1H, ArH), 8.36–7.73 (m, 6H, ArH), 3.87 (s, 3H, –$COOCH_3$).

*2.5.4. Methyl 2-{[(1E)-(4-bromophenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**21**)*

^{1}H NMR (DMSO-d_6): δ 9.78 (s, 1H, CH=N), 9.03 (d, 1H, ArH), 8.61 (s, 1H, ArH), 8.55 (d, 1H, ArH), 7.94 (d, 2H, ArH), 8.11–7.98 (m, 1H, ArH), 7.85 (t, 1H, ArH), 7.80 (d, 2H, ArH), 3.87 (s, 3H, –$COOCH_3$).

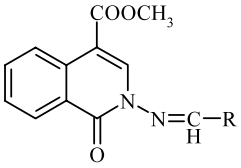
*2.5.5. Methyl 2-{[(1E)-(2-bromophenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**22**)*

^{1}H NMR (DMSO-d_6): δ 10.09 (s, 1H, CH=N), 9.04 (d, 1H, ArH), 8.72 (s, 1H, ArH), 8.57 (d, 1H, ArH), 8.43–7.75 (m, 6H, ArH), 3.87 (s, 3H, –$COOCH_3$).

*2.5.6. Methyl 2-{[(1E)-(4-nitrophenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**23**)*

^{1}H NMR (DMSO-d_6): δ 9.85 (s, 1H, CH=N), 9.06 (d, 1H, ArH), 8.60 (s, 1H, ArH), 8.54 (d, 1H, ArH), 8.27 (d, 2H, ArH), 8.13–7.96 (m, 1H, ArH), 7.83 (t, 1H, ArH), 8.37 (d, 2H, ArH), 3.88 (s, 3H, –$COOCH_3$).

Table 2
Structures, yields and melting points of the prepared quinazolinones derivatives



Compound	R	Yield (%)	Melting point (°C)	Formula
18 [a]	$-C_6H_5$	78	135–7	$C_{18}H_{14}N_2O_3$
19 [a]	$-C_6H_4$-4-Cl	82	198–9	$C_{18}H_{13}N_2O_3Cl$
20 [a]	$-C_6H_4$-2-Cl	85	183–4	$C_{17}H_{12}N_2O_3$
21 [a]	$-C_6H_4$-4-Br	86	206–7	$C_{18}H_{13}N_2O_3Br$
22 [a]	$-C_6H_4$-2-Br	82	176–7	$C_{17}H_{12}N_2O_3$
23 [a]	$-C_6H_4$-4-NO_2	83	257–9	$C_{18}H_{13}N_3O_5$
24 [a]	$-C_6H_4$-2-COOH	77	272–4	$C_{17}H_{12}N_2O_3$
25 [a]	$-C_6H_4$-4-OH	74	263–4	$C_{18}H_{14}N_2O_4$
26 [a]	$-C_6H_4$-4-OCH_3	81	222–4	$C_{17}H_{12}N_2O_3$
27 [a]	$-CH=CH-C_6H_5$	95	158–60	$C_{20}H_{15}N_2O_3$
28 [a]	$-CH_2CH_2-C_6H_5$	84	162–3	$C_{17}H_{12}N_2O_3$
29 [a]	$-C(CH_3)=CH-C_6H_5$	69	158–9	$C_{17}H_{12}N_2O_3$
30 [a]	$-CH=CH-C_6H_4$-4-NO_2	88	212–4	$C_{17}H_{12}N_2O_3$
31 [a]	$-CH=CH-C_6H_4$-4-$N(CH_3)_2$	69	147–9	$C_{17}H_{12}N_2O_3$

[a] Recrystallized from dimethylformamide.

*2.5.7. 2-((E)-{[4-(methoxycarbonyl)-1-oxoisoquinolin-2(1H)-yl]imino}methyl)benzoic acid (**24**)*

^{1}H NMR (DMSO-d_6): δ 13.26 (s broad, 1H, COOH), 9.99 (s, 1H, CH=N), 8.97 (d, 1H, ArH), 8.62 (s, 1H, ArH), 8.49 (d, 1H, ArH), 8.33–7.69 (m, 6H, ArH), 3.87 (s, 3H, $-COOCH_3$).

*2.5.8. Methyl 2-{[(1E)-(4-hydroxyphenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**25**)*

^{1}H NMR (DMSO-d_6): δ 9.96 (s broad, 1H, Ar–OH), 9.17 (s, 1H, CH=N), 8.97 (d, 1H, ArH, 8 Hz), 8.54 (s, 1H, ArH), 8.47 (d, 1H, ArH, 8.4 Hz), 8.03 (d, 2H, ArH, 9 Hz), 8.05–7.91 (m, 1H, ArH), 7.75 (t, 1H, ArH, 7.2 Hz), 7.25 (d, 2H, ArH, 8.6 Hz), 3.86 (s, 3H, $-COOCH_3$).

*2.5.9. Methyl 2-{[(1E)-(4-methoxyphenyl)methylene]-amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**26**)*

^{1}H NMR (DMSO-d_6): δ 9.22 (s, 1H, CH=N), 8.98 (d, 1H, ArH, 8 Hz), 8.52 (s, 1H, ArH), 8.45 (d, 1H, ArH, 8.4 Hz), 8.04 (d, 2H, ArH, 9 Hz), 8.04–7.93 (m, 1H, ArH), 7.77 (t, 1H, ArH, 7.2 Hz), 7.25 (d, 2H, ArH, 8.6 Hz), 3.89 (s, 3H, $-COOCH_3$), 3.86 (s, 3H, $-OCH_3$)

*2.5.10. Methyl 1-oxo-2-{[(1E,2E)-3-phenylprop-2-enylidene]amino}-1,2-dihydroisoquinoline-4-carboxylate (**27**)*

^{1}H NMR (DMSO-d_6): δ 8.96 (d, 1H, CH=N, 9.27 Hz), 8.76 (d, 1H, ArH), 8.39 (s, 1H, ArH), 8.36 (dd, 1H, ArH, 8.6, 1.2 Hz), 7.92–7.82 (m, 1H, ArH), 7.79–7.70 (m, 2H, ArH), 7.69–7.59 (m, 1H, ArH), 7.53–7.42 (m, 3H, ArH), 7.48 (d, 1H, ArCH=C, 15.62 Hz), 7.23 (dd, 1H, ArCH=*CH*–, 15.62, 9.27 Hz), 3.87 (s, 3H, $-COOCH_3$).

*2.5.11. Methyl 1-oxo-2-{[(1E)-3-phenylpropylidene]-amino}-1,2-dihydroisoquinoline-4-carboxylate (**28**)*

^{1}H NMR (DMSO-d_6): δ 8.96 (d, 1H, ArH), 8.60 (t, 1H, CH=N, 4.6/9.2 Hz), 8.41 (d, 1H, ArH), 8.32 (s, 1H, ArH), 7.93 (t, 1H, ArH), 7.70 (t, 1H, ArH), 7.40–7.27 (m, 5H, ArH), 3.87 (s, 3H, $-COOCH_3$), 3.11–3.01 (m, 2H, Ar–CH_2–), 2.95–2.82 (m, 2H, $-CH_2-C=N$)

*2.5.12. Methyl 2-{[(1E,2E)-2-methyl-3-phenylprop-2-enylidene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**29**)*

^{1}H NMR (DMSO-d_6): δ 9.01 (s, 1H, CH=N), 8.99 (d, 1H, ArH), 8.47 (s, 1H, ArH), 8.45 (d, 1H, ArH), 7.96 (t, 1H, ArH), 7.69–7.47 (m, 6H, ArH), 7.36 (s, 1H, ArH), 3.86 (s, 3H, $-COOCH_3$), 2.31 (s, 3H, $CH_3-C=$).

*2.5.13. Methyl 2-{[(1E,2E)-3-(2-nitrophenyl)prop-2-enylidene]amino}-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**30**)*

^{1}H NMR (DMSO-d_6): δ 9.22 (d, 1H, CH=N, 9.4 Hz), 8.95 (d, 1H, ArH), 8.48 (s, 1H, ArH), 8.46 (d, 1H, ArH), 8.24–8.15 (m, 2H, ArH), 7.92 (d, 1H, ArCH=C, 15.8 Hz), 7.98–7.87 (m, 2H, ArH), 7.82–7.71 (m, 3H, ArH),

7.35 (dd, 1H, ArCH=*CH*–, 15.8, 9.4 Hz), 3.85 (s, 3H, –$COOCH_3$).

*2.5.14. Methyl 2-({(1E,2E)-3-[4-(dimethylamino)-phenyl]prop-2-enylidene}amino)-1-oxo-1,2-dihydroisoquinoline-4-carboxylate (**31**)*

^{1}H NMR (DMSO-d_6): δ 8.98 (d, 1H, ArH), 8.87 (d, 1H, CH=N, 9.4 Hz), 8.43 (d, 1H, ArH), 8.42 (s, 1H, ArH), 7.94 (t, 1H, ArH), 7.76–7.61 (m, 3H, ArH), 7.40 (d, 1H, ArCH=C, 15.8 Hz), 7.04 (dd, 1H, ArCH=*CH*–, 15.8, 9.4 Hz), 6.84 (d, 2H, ArH), 3.84 (s, 3H, –$COOCH_3$), 3.02 (s, 6H, $N(CH_3)_2$).

2.6. Virology

2.6.1. Viruses and cells

ECHO virus 9 (Hill strain), Poliovirus 1 (Brunhilde strain), Coxsackievirus B1, measles (Edmonston strain) and adenovirus type 2 were purchased from the American Type Culture Collection (ATCC) and propagated in human epidermoid carcinoma larynx cells (Hep-2). Encephalomyocarditis (EMC strain) and Herpes simplex type 1 (F strain) were purchased from the ATCC and propagated in mouse connective tissue cells (L-929) and African green monkey kidney cells (Vero), respectively. Cells were kept in a humidified 5% carbon dioxide atmosphere at 37 °C and grown in Dulbecco modified Eagle's Minimum Essential medium (DMEM) supplemented with 6% heat inactivated fetal calf serum (FCS), 200 μg ml^{-1} of streptomycin and 200 units ml^{-1} of penicillin G. For all viruses tested working stocks were prepared as cellular lysates using DMEM without FCS (maintenance medium).

2.6.2. Test compounds

All compounds were dissolved in dimethylsulphoxide (DMSO) and diluted in maintenance medium to achieve the final concentration need. Dilution of the test compounds contained a maximal concentration of 0.01% DMSO, which was not toxic to cell lines.

2.6.3. Cell viability

The cytotoxicity of the test compounds was evaluated by measuring the effect produced on cell morphology and cell growth. Cell monolayers were prepared in 24-well tissue culture plates and exposed to various concentrations (μM) of the compounds. Plates were checked by light microscopy after 12, 24 and 48 h. Cytotoxicity was scored as morphological alterations (rounding up, shrinking and detachment). The viability of the cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method. Briefly, Hep-2, L929 and Vero cells were prepared in 96-well tissue culture plates and serial concentrations of the compounds were added. After incubation for 48 h at 37 °C, MTT (0.5 mg ml^{-1}) in DMEM without phenol red was replaced in each well. After 90 min incubation at 37 °C, the overlay was removed and isopropanol (100 μl) was added; plates were then mixed twice to dissolve the dark blue crystals. The optical density (OD) was read at 540 and 690 nm on a Titertek Multiscan MCC/340, within 15 min of adding the isopropanol [17,18]. The absorbance at 690 nm was automatically subtracted from the absorbance at 540 nm, so as to eliminate the effect of non-specific absorption. Cell viability values obtained in the presence of the compounds were expressed as the percentage of those obtained in untreated controls and were calculated by the following formula:

$$\frac{(OD)_c - (OD)_t}{(OD)_c} \times 100$$

where $(OD)_c$ and $(OD)_t$ indicated the absorbances of the untreated cell control and the test sample, respectively. The 50% cytotoxic dose (CC_{50}), calculated by dose–response curves and linear regression, was expressed as the concentration of the compound that reduced the absorbance of the control sample by 50%.

2.6.4. Antiviral activity

The antiviral activity was estimated using a plaque reduction assay. Confluent cells were grown in 6-well tissue culture plates and infected with 300 plaque forming units (PFU) of the virus stock for well. During and after 1 h of virus adsorption at 37 °C (half hour for picornavirus), overlay medium containing 1% of methylcellulose with or without the test compound at doses below CC_{50} was added. After 24 h of incubation at 37 °C, when the plaques appeared clearly in virus controls, the overly was removed and cells were stained with 1% crystal violet in methanol. The number of visible plaques was then counted under light microscopy. The antiviral activity of each compound was determined as the percentage decrease in the number of plaques, which was calculated by the following formula:

$$\frac{\text{no. of plaques (control)} - \text{no. of plaques (test)}}{\text{no of plaques (control)}} \times 100$$

The compound concentration required to inhibit virus plaque formation by 50% was expressed as IC_{50} and calculated by dose–response curves and linear regression.

3. Results and discussion

3.1. Chemistry

Twenty-eight new isoquinolin-4-one derivatives, reported in Tables 1 and 2, were synthesized and their

inhibition of MV replication were evaluated. The synthetic pathway (Scheme 1) starts from the isocoumarin-4-carboxylic acid (**1**) previously described [15]. Reaction of **1** with hydrazine monohydrate in absolute ethanol under reflux for 1 h yielded the corresponding 2-amino-1-oxo-1,2-dihydroisoquinoline-4-carboxylic acid (**2**). The enamines **3**–**8** were obtained by treating compound **2** with the suitable aldehydes, commercially available, in acetic acid. Compounds **10**–**15** were prepared in a similar way from the compound **9**, obtained by esterification of **2** with methanol in 10% H_2SO_4.

All the synthesized compounds were obtained in the E or E,E configurations. The stereochemistry of the propenylideneamino chain for compounds **12**–**16** and **27**–**31** (Tables 1 and 2) was assigned as all trans on the basis of ^{1}H NMR coupling constants analysis as well as nuclear Overhauser effects (NOE) enhancement experiments. The coupling constant between hydrogen atom H-2′ and H-3′ (15–16 Hz) are indicative of 2′ trans geometry. The geometry of the 1′ double bond is clearly established by NOE. In these studies, NOE cross peaks were observed between the H-1′ proton and the proton H-3 of the dihydroisoquinoline ring with values in the range 8–12%. In addition, the irradiation of H-1′ proton resonances intensified the H-3′ signal (16–19%), identifying unequivocally the dominance of planar or near-planar s-trans conformations of the propenylideneamino chain.

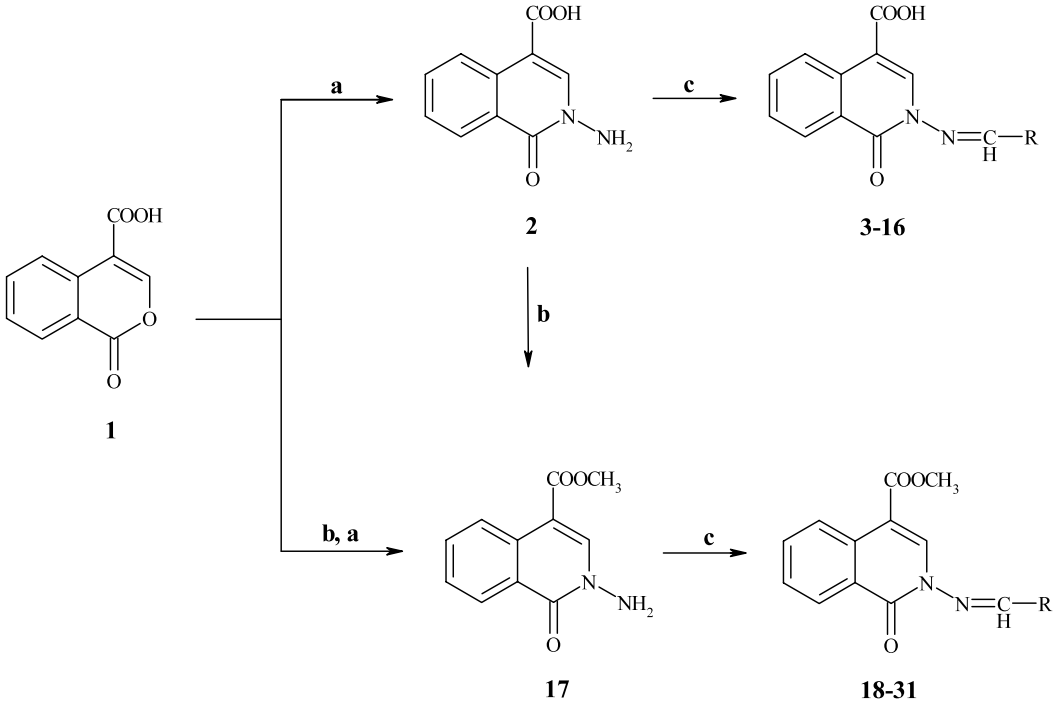
The geometry of the 1′ double bond in the derivative **3**–**11** and **18**–**26** (Tables 1 and 2) was clearly assigned by interpretation of NOE data. Irradiation of the singlet due to the H-1′ produced NOE signals for H-3 proton of the dihydroisoquinoline ring and for the aromatic H-2′/H-6′ protons with values in the range 2–5 and 16–21%, respectively.

3.2. Antiviral activity and SAR studies

A series of new isoquinoline analogues were evaluated for anti-measles activity using Edmonston strain of MV. All compounds were evaluated with respect to the inhibition of MV in human epidermoid carcinoma larynx cells (Hep-2). Table 3 shows the 50% cytotoxic dose values (CC_{50}), the compound concentrations required to inhibit virus plaque formation by 50% (IC_{50}) and the selectivity indexes (SI) calculated dividing CC_{50} by IC_{50}.

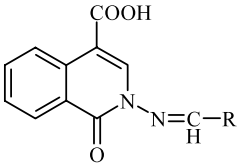
The appearance of anti-MV activity was obtained with the introduction of phenylmethanimine side chain on the exocyclic nitrogen of the inactive derivative **2**, to give 1-oxo-2-{[(1*E*)-phenylmethylene]amino}-1,2-dihydroisoquinoline-4-carboxylic acid (**3**) with an IC_{50} of 45 μM and a CC_{50} of 100 μM. Therefore, our medicinal chemistry effort was directed toward the effect of substituents on the phenyl ring of phenylmethanimine moiety with the intention to examine the effect of electron-donating and electron-withdrawing groups on the antiviral activity.

An inspection of data reported in Table 3 reveals that the introduction of electron-withdrawing group in 4-position of the phenyl ring increased the anti-MV activity. The most active compound was the 4-bromo



Scheme 1. (a) H_2NNH_2; (b) MeOH, H_2SO_4; (c) R-phenyl-CHO.

Table 3
Antiviral activity and SI of isoquinoline derivatives



Compound	R	CC_{50} (μM) [a,c] HEp-2 L-929/VERO	IC_{50} (μM) [b,c] Measles virus	SI [d]
3	$-C_6H_5$	100	48.0	2
4	$-C_6H_4$-4-Cl	100	25.0	4
5	$-C_6H_4$-2-Cl	30	>30	1
6	$-C_6H_4$-4-Br	80	15.0	5
7	$-C_6H_4$-2-Br	30	>30	1
8	$-C_6H_4$-4-NO_2	250	42.0	6
9	$-C_6H_4$-2-COOH	600	>600	1
10	$-C_6H_4$-4-OH	300	>300	1
11	$-C_6H_4$-4-OCH_3	500	>500	1
12	$-CH=CH-C_6H_5$	100	12.5	8
13	$-CH_2CH_2-C_6H_5$	750	>750	1
14	$-C(CH_3)=CH-C_6H_5$	500	10.0	50
15	$-CH=CH-C_6H_4$-4-NO_2	150	25.0	6
16	$-CH=CH-C_6H_4$-4-$N(CH_3)_2$	150	>150	1

[a] CC_{50}: concentration which inhibited cell growth by 50% as compared with control cultures.
[b] IC_{50}: concentration which inhibited virus plaque formation by 50%.
[c] Values are mean ± 0.5 SD (maximal standard deviation estimated) of three separate assays.
[d] SI was determined for the effective compounds dividing CC_{50} by IC_{50}.

derivative **6**. In this series the increase of the anti-MV activity follow the order 4-Br > 4-Cl (**4**) > 4-NO_2 (**8**) with IC_{50} of 15, 25 and 42 μM, respectively. For these latter three derivatives the activity is well separated from their cytotoxicity, as underlined by their respective SI. The SI value followed the order 4-NO_2 (**8**) > -4-Br (**6**) > 4-Cl (**4**). Interestingly, the meta-analogues (**5**, **7**) showed an increase in the cytotoxicity and a loss of activity at concentrations lower than their respective the CC_{50} values.

Despite their lower cytotoxicity, derivatives with electron-donor groups -4-OH (**10**) -4-OCH_3 (**11**) at the position 4 in the phenyl ring showed no activity against MV. It is interesting to note that the inactive -4-OCH_3 derivative (**11**), has a calculated log D value comprised between the one of the active phenyl (**3**) and that of 4-NO_2-phenyl (**8**) derivatives (log D at pH 7.4 = 0.0099, −0.13 and −0.22, respectively). Therefore, for the above mentioned compounds (**3**–**11**), the electronic factor seems to be more important when compared to lipophilicity in improving the recognition at the biological target. The correlation between anti-MV activity and the electron-withdrawing effect of the substituents on the phenyl ring outlined in this study is in agreement with literature data. In fact, the same trend was observed for the aromatic substituents of the phenoxymethyl moiety of the 4-chloro-phenyl-derivative UK2054, that was reported active against MV, whereas, the corresponding 4-methoxy-phenyl- analogous UK 2371 was totally inactive (Fig. 1) [19].

With the aim to further explore the structural requirements for an optimum interaction with the viral target, we synthesized the vinylogous compounds **12**–**16** listed in Table 1. The insertion of a vinyl group is beneficial for the activity, as is demonstrated by vinylogous compound **12**, **14** and **15**. Compound **12**, vinylogous of compound **3**, maintain the same CC_{50} (100 μM) of the parent compound and showed a twofold increase in the anti-MV activity (from 25 to 12.5 μM) with a resulting doubling of the SI from 4 to 8. Compound **15**, vinylogous of compound **8**, showed a 1.68-fold increase in the anti-MV activity as highlighted by the IC_{50} reduction from 42 to 25 μM. The vinylogous **15** was more cytotoxic with respect to the parent compound **8** with a 60% decrease of CC_{50} (from 250 to 150 μM).

Regarding the electronic effect of substituents at the 4-position of aromatic ring in the vinylogous derivatives **12**–**16**, we observed the same trend as the analogues **3**–**11**, in fact, the non-substituted compounds **12** and **14** together with the electron-withdrawing substitute derivative **15** were active. On the contrary, the introduction of an electron donor group in the 4-position like in the

4-N(CH_3)$_2$ derivative **16** determined a loss of antiviral activity. In addition, as above discussed for the compound **11**, the 4-N(CH_3)$_2$ derivative **16**, despite of similar calculated log D value with respect to non-substituted compounds **12** (log D at pH 7.4 = 0.98 and 0.85, respectively), is inactive. Therefore, also for vinylogous **12**–**16** the electronic factor seems to be more relevant with respect to lipophilicity. Moreover, the saturated derivative **13**, despite its lowest cytotoxicity (CC_{50} 750 μM) was inactive. Interestingly, the insertion of a $-CH_3$ group in the double bound in position 1 of (1*E*,2*E*)-3-phenylprop-2-enylidene moiety seemed to optimize the antiviral activity, since the compound **14** showed the highest anti-MV activity (10 μM) and exhibited highest SI value of 50.

Taken together these experimental data for the series **12**–**16**, support two implications: the importance of the transmission of electronic effects between the 1-oxo-2-dihydroisoquinoline nucleus and the aromatic ring, and/or the relevance planar or near-planar s-trans conformation of the propenylideneamino chain.

All ester derivatives **18**–**31** were not active against MV. The loss of activity upon esterification could be due to the strong reduction of water solubility at pH 7.4 of these compounds that, in turn, can reduce the intracellular bioavailability. In fact, the calculated water solubility values for the acid (**3**–**16**) and the esters (**18**–**31**) derivatives were in the ranges 0.13–3.53 and 9.2×10^{-4}–1.4×10^{-2} g l^{-1}, respectively, with a solubility reduction for ester compounds of 140–250 fold.

Exceptions for both series were represented by the 2-COOH-phenyl derivatives (**9** and **24**) that have good calculated water solubility but are both inactive. Alternatively, the lack of activity of the esters derivatives could also suggests that the presence of a carboxylic acid group is essential for the antiviral activity.

All the compounds (**3**–**16** and **18**–**31**) were also tested for their antiviral activity against RNA (polio 1, ECHO 9, Coxsackie Bl, EMC) and DNA (adeno 2, HSV-1) viruses in Hep-2 cells or mouse connective tissue cells (L-929) and African green monkey kidney cells (Vero). The compounds synthesized in this study displayed an interesting selectivity against MV since only compound **12** was active against EMC strain with IC_{50} of 25 μM and a selectively index of 4.

In conclusion, the facile synthesis and the information obtained from the structure–activity relationship may constitute the basis for the future development of more active compounds.

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