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Synthesis and *evaluation of in vitro* antiviral activity of novel phenoxy acetic acid derivatives

M. SHAHAR YAR[1], M. AFROZ BAKHT[1], A. A. SIDDIQUI[1], M. M. ABDULLAH[1], & ERIK DE CLERCQ[2]

[1]*Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110062, India, and* [2]*Rega Institute for Medical Research, Katholieke Universiteit Leuven, B-3000 Leuven, Belgium*

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Abstract

Several substituted phenoxy acetic acid derived pyrazolines were synthesized by the reaction between 2-{4-[3-(2,4-dihydroxyphenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy} acetic acid and substituted acid hydrazides and were tested for their *in vitro* cytotoxicity and antiviral activity. None of the compounds showed any specific antiviral activity [50% antivirally effective concentration (EC_{50}) ≥ 5-fold lower than minimum cytotoxic concentration]. The most cytotoxic of the series was 2-{4-[3-(2,4-dihydroxyphenyl)-1-(2-hydroxybenzoyl-4,5-dihydro-1*H*-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3j**), with a minimum cytotoxic concentration of 0.16 μg/mL in human embryonic lung (HEL) cells.

Keywords: *Pyrazoline, antiviral, cytotoxic*

Introduction

While great progress has been made in the development of antibiotics for the treatment of bacterial infections, there are only a limited number of antiviral drugs that can be used clinically. The strength of antiviral therapy raises a number of public health concerns regarding the optimal use of drugs. Specificity against virus replication is the key issue in antiviral chemotherapy. Because of the close interaction between virus replication and normal cellular metabolism, it was originally too difficult to interrupt the virus replicative cycle without adversely affecting the host cell metabolism. It is now clear, however, that several events in the virus replicative cycle either do not occur in normal uninfected cells or are controlled by virus-specified enzymes that differ structurally and functionally from the corresponding host cell enzymes.

The intrinsic property of viruses as obligate intracellular parasites has been major stumbling block in developing antiviral drugs because chemicals that inhibit viral replication also inhibit host cellular functions that leads to drug toxicity. The expanding list of emerging or re-emerging viral infections that requires treatment has invoked significant attention; in addition, resistance to antiviral drugs has also become a serious concern.

Earlier we have reported pyrazoline derivatives as anti-mycobacterial [1,2], antiproliferative and cytotoxic [3–7] activity. Others have reported on the wide variety of biological activities of pyrazoline derivatives [8–11]. Here, we report on the synthesis and *in vitro* antiviral activity evaluation of a series of novel phenoxy acetic acid derivatives.

Materials

All the chemicals used were laboratory grade and procured from E. Merck (Germany) and S.D. Fine Chemicals (India). Melting points were determined by the open tube capillary method and are uncorrected. The thin layer chromatography (TLC) plates

Correspondence: M. Shahar Yar, Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Hamdard University, New Delhi 110062, India. Tel: 91 9811811033. Fax: 91 11 26059666. E-mail: yarmsy@rediffmail.com

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(silica gel G) were used to confirm the purity of commercial reagents used, compounds synthesized and to monitor the reactions as well. Two different solvent systems: toluene: ethyl acetate: formic acid (5:4:1) and petroleum ether: toluene: acetic acid (5:4:1), were used to run the TLC and spots were located under iodine vapors/UV light. IR spectra were obtained on a Perkin-Elmer 1720 FT-IR spectrometer (KBr Pellets). ^{1}H-NMR spectra were recorded on a Bruker AC 300 MHz, spectrometer using TMS as internal standard in DMSO-d_6.

Methods

Chemistry

*2-{4-[3-(2,4-Dihydroxyphenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy} acetic acid (**2**)*. A mixture of 2-(4-formyl-2-methoxyphenoxy) acetic acid (**1**) (0.01 mole) and 1-(2,4-dihydroxyphenyl) ethanone (0.01 mole) in oxygen-free methanol were stirred at room temperature in the presence of base (aqueous solution of potassium hydroxide 30%; 5 mL) till completion of the reaction.The reaction mixture was allowed to stand overnight and then poured into ice-cold water followed by neutralization with HCl. The solid separated was filtered, dried and crystallized from ethanol. The purity of the chalcone was checked by TLC.

IR: (KBr) cm^{-1}: 3033 (COOH), 1682 (C=O), 1572 (C=C); ^{1}H-NMR (DMSO-d_6) ppm: 10.1 (1H, s, COOH), 9.2 (2H, s, 2 × OH), 6.5-8.3 (6H, m, aromatic), 6.1-6.5 (2d, 1H each, J = 8.4, 6.9Hz, CH=CH), 3.6(3H, s, OCH_3), m/z: 345(M^{+1}); Anal.Calcd. for $C_{18}H_{16}O_7$: C, 62.79; H, 4.68; O, 32.53%. Found: C, 62.80; H, 4.67; O, 32.54%.

*General procedure for synthesis of compound (**3$_{a-m}$**)*. A solution of 2-{4-[3-(2,4-dihydroxyphenyl)-3-oxo-1-propenyl]-2-methoxyphenoxy} acetic acid (0.001 mole) in 15 mL of glacial acetic acid and the appropriate acid hydrazides (0.001 mole) was refluxed for 12 h. Excess of solvent was removed under reduced pressure and the reaction mixture was poured onto crushed ice (20 gm). The product so obtained was filtered, washed with water and recrystalized from methanol.

*2-{4-[1-benzoyl-3-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxy phenoxy} acetic acid (**3$_a$**)*. *IR*: (KBr) cm^{-1}: 3245(COOH), 1682(C=O), 1563(C=N), 1318(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 11.4(1H, s, COOH), 8.8(2H, s, 2 × OH), 7.4–7.9 (11H, m, aromatic), 4.6(1H, t, CH), 3.9(3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 463 (M^{+1}); Anal. Calcd. for $C_{25}H_{22}N_2O_7$: C, 64.93; H, 4.80; N, 6.06; O, 24.22%. Found: C, 64.92; H, 4.81; N, 6.05; O, 24.24%.

*2-{4-[1-(2-chlorobenzoyl)-3-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3$_b$**)*. *IR*: (KBr) cm^{-1}: 3190(COOH), 1685(C=O), 1545(C=N), 1325(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.2(1H, s, COOH), 9.4(2H, s, 2 × OH), 6.8–8.1(10H, m, aromatic), 4.8(1H, t, CH), 3.6(3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 497 (M^{+1}); Anal.Calcd. for $C_{25}H_{21}ClN_2O_7$: C, 60.43; H, 4.26; Cl, 7.13; N, 5.64; O, 22.54%. Found: C, 60.44; H, 4.27; Cl, 7.12; N, 5.62; O, 22.53%.

*2-{4-[1-(4-chlorobenzoyl)-3-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3$_c$**)*. *IR*: (KBr) cm^{-1}: 3048(COOH), 1714(C=O), 1555(C=N), 1315(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.3(1H, s, COOH), 9.9(2H, s, 2 × OH), 7.7–7.8(10H, m, aromatic), 4.5(1H, t, CH),3.3(3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 497 (M^{+1}); Anal.Calcd. for $C_{25}H_{21}ClN_2O_7$: C, 60.43; H, 4.26; Cl, 7.13; N, 5.64; O, 22.54%. Found: C, 60.45; H, 4.27; Cl, 7.12; N, 5.62; O, 22.55%.

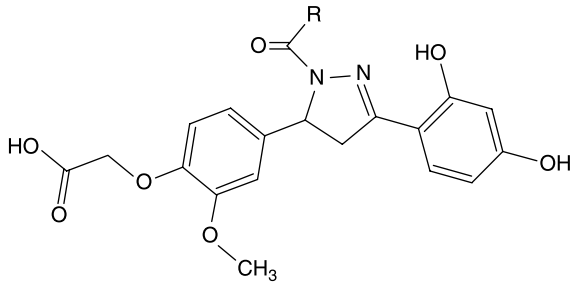
*2-{4-[1-(4-bromobenzoyl)-3-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3$_d$**)*. *IR*: (KBr) cm^{-1}: 3173(COOH), 1654(C=O), 1560(C=N), 1317(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 11.4(1H, s, COOH), 9.7(2H, s, 2 × OH), 6.7–8.1(10H, m, aromatic), 4.6(1H, t, CH),3.8 (3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 542 (M^{+1}); Anal.Calcd. for $C_{25}H_{21}BrN_2O_7$: C, 55.47; H, 3.91; Br, 14.76; N, 5.17; O, 20.69%. Found: C, 55.49; H, 3.93; Br, 14.78; N, 5.18; O, 20.72%.

*2-{4-[1-(4-nitrobenzoyl)-3-(2,4-dihydroxyphenyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3$_e$**)*. *IR*: (KBr) cm^{-1}: 3219(COOH), 1740(C=O), 1551(C=N), 1381(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.4(1H, s, COOH), 9.8(2H, s, 2 × OH), 8.0–8.03(10H, m, aromatic), 4.7(1H, t, CH), 3.9 (3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 508 (M^{+1}); Anal.Calcd. for $C_{25}H_{21}N_3O_9$: C, 59.17; H, 4.17; N, 8.28; O, 28.38%. Found: C, 59.16; H, 4.18; N, 8.27; O, 28.40%.

*2-{4-[3-(2,4-dihydroxyphenyl)-1-(4-methylbenzoyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (**3$_f$**)*. *IR*: (KBr) cm^{-1}: 3255(COOH), 1654(C=O), 1541(C=N), 1324(C—N), ^{1}H-NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH),9.0 (2H, s, 2 × OH), 6.8–8.0(10H, m, aromatic), 5.71 (1H, dd, J = 6.6, 6.3Hz), 4.42(1H, dd, J = 12.0, 12.0Hz), 3.92 (1H, dd, J = 6.3, 6.3Hz), 3.8 (3H, s, OCH_3), m/z: 477 (M^{+1}); Anal.Calcd. for $C_{26}H_{24}N_2O_7$: C, 65.54; H, 5.08; N, 5.88; O, 23.50%. Found: C, 65.53; H, 5.09; N, 5.90; O, 23.49%.

*2-{4-(3-(2,4-dihydroxyphenyl)-1-[2-(2-methylphenoxy)acetyl]-4,5-dihydro-1H-5-pyrazolyl(-2-methoxyphenoxy}acetic acid (**3$_g$**)*. *IR*: (KBr) cm^{-1}: 3304 (COOH), 1685(C=O), 1540(C=N), 1382(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.8(1H, s, COOH), 9.4(2H, s, 2 × OH), 6.7–8.2(10H, m, aromatic),

Table I. Physical constants of the synthesized compounds.



(3_{a-m})

Compound	R	Molecular Formula	Molecular Weight	% yield	Melting Point °C
3_a	C_6H_5	$C_{25}H_{22}N_2O_7$	462	82	115-17
3_b	2-Cl-C_6H_4	$C_{25}H_{21}ClN_2O_7$	496	86	190-92
3_c	4-Cl-C_6H_4	$C_{25}H_{21}ClN_2O_7$	496	82	208-09
3_d	4-Br-C_6H_4	$C_{25}H_{21}BrN_2O_7$	541	78	210-12
3_e	4-NO_2-C_6H_4	$C_{25}H_{21}N_3O_9$	507	84	224-26
3_f	4-CH_3-C_6H_4	$C_{26}H_{24}N_2O_7$	476	83	180-82
3_g	2-CH_3-C_6H_4–O-CH_2	$C_{27}H_{26}N_2O_8$	506	87	142-44
3_h	4-CH_3-C_6H_4–O-CH_2	$C_{27}H_{26}N_2O_8$	506	87	100-02
3_i	C_6H_5-O-CH_2	$C_{26}H_{24}N_2O_8$	492	83	100-02
3_j	2-OH-C_6H_4	$C_{25}H_{22}N_2O_8$	478	89	120-22
3_k	C_6H_5-CH_2	$C_{26}H_{24}N_2O_7$	476	85	145-47
3_l	α-$C_{10}H_7$-O-CH_2	$C_{30}H_{26}N_2O_8$	542	88	142-44
3_m	β-$C_{10}H_7$-O-CH_2	$C_{30}H_{26}N_2O_8$	542	85	126-30

4.8(1H, t, CH),3.4(3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 507 (M^{+1}); Anal.Calcd. for $C_{27}H_{26}N_2O_8$: C, 64.02; H, 5.17; N, 5.53; O, 25.27%. Found: C, 64.03; H, 5.17; N, 5.54; O, 25.27%.

2-{4-(3-(2,4-dihydroxyphenyl)-1-[2-(4-methylphenoxy)acetyl]-4,5-dihydro-1H-5-pyrazolyl(-2-methoxyphenoxy}acetic acid (3_h). *IR*: (KBr) cm^{-1}: 3220(COOH), 1680(C=O), 1559(C=N), 1378 (C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH), 9.0(2H, s, 2 × OH), 6.5–8.4(10H, m, aromatic), 4.9(1H, t, CH), 3.3(3H, s, OCH_3), 2.5(2H, d,CH_2), m/z: 507(M^{+1}); Anal.Calcd. for $C_{27}H_{26}N_2O_8$: C, 64.02; H, 5.17; N, 5.53; O, 25.27%. Found: C, 64.02; H, 5.17; N, 5.53; O, 25.27%.

2-{4-[3-(2,4-dihydroxyphenyl)-1-(2-phenoxyacetyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (3_i). *IR*: (KBr) cm^{-1}: 3180(COOH), 1692(C=O), 1550(C=N), 1342(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.3(1H, s, COOH), 8.7(2H, s, 2 × OH), 6.6—8.03 (10H, m, aromatic), 4.6 (1H, t,CH), 3.5 (3H, s, OCH_3), 2.5(2H, d, CH_2); m/z: 493 (M^{+1}); Anal.Calcd. for $C_{26}H_{24}N_2O_8$: C, 63.41; H, 4.91; N, 5.69; O, 25.99%. Found: C,63.40; H, 4.91; N, 5.70; O, 25.97%.

2-{4-[3-(2,4-dihydroxyphenyl)-1-(2-hydroxybenzoyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy} acetic acid (3_j). *IR*: (KBr) cm^{-1}: 3260(COOH), 1740(C=O), 1559(C=N), 1386(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH), 9.3(2H, s, 2 × OH), 6.8–8.1(10H, m, aromatic), 4.6(1H, t, CH),3.4(3H, s, OCH_3), 2.5(2H, d, CH_2), m/z: 479(M^{+1}); Anal.Calcd. for $C_{25}H_{22}N_2O_8$: C, 62.76; H, 4.63; N, 5.86; O, 26.75%. Found: C, 62.77; H, 4.64; N, 5.86; O, 26.77%.

2-{4-[3-(2,4-dihydroxyphenyl)-1-(2-phenylacetyl)-4,5-dihydro-1H-5-pyrazolyl]-2-methoxyphenoxy}acetic acid (3_k). *IR*: (KBr) cm^{-1}: 3199(COOH), 1654(C=O), 1560(C=N), 1317 (C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.5(1H, s, COOH), 8.9(2H, s, 2 × OH), 6.8–8.3(10H, m, aromatic), 5.82(1H, dd, J = 5.7, 5.7 Hz), 4.71 (1H, dd, J = 12.3, 12.3 Hz), 3.91 (1H, dd, J = 6.0, 5.7 Hz), 3.6(3H, s, OCH_3), m/z: 477(M^{+1}); Anal.Calcd. for $C_{26}H_{24}N_2O_7$: C, 65.54; H, 5.08; N, 5.88; O, 23.50%. Found: C, 65.54; H, 5.09; N, 5.88; O, 23.51%.

2-{4-(3-(2,4-dihydroxyphenyl)-1-[2-(1-naphthyloxy)acetyl]-4,5-dihydro-1H-5-pyrazolyl(-2-methoxyphenoxy}acetic acid (3_l). *IR*: (KBr) cm^{-1}: 3304 (COOH), 1760(C=O), 1560(C=N), 1380(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH), 8.2(2H, s, 2 × OH), 7.1–7.8(13H, m, aromatic), 4.8(1H, t, CH) 3.4(3H, s, OCH_3), 2.5(2H, d, CH_2); m/z: 543(M^{+1}); Anal.Calcd. for $C_{30}H_{26}N_2O_8$: C, 66.41; H, 4.83; N, 5.16; O, 23.59%. Found: C, 66.42; H, 4.83; N, 5.17; O, 23.60%.

*2-{4-(3-(2,4-dihydroxyphenyl)-1-[2-(2-naphthyl-oxy)acetyl]-4,5-dihydro-1H-5-pyrazolyl(-2-methoxy-phenoxy}acetic acid (**3m**). IR*: (KBr) cm^{-1}: 3304 (COOH), 1760 (C═O), 1560(C═N), 1380(C—N); ^{1}H-NMR (DMSO-d_6) ppm: 10.1(1H, s, COOH), 8.2(2H, s, 2 × OH), 7.1–7.8(13H, m, aromatic), 4.8(1H,t,CH); 3.4(3H, s, OCH_3), 2.5(2H, d, CH_2); m/z: 543 (M^{+1}); Anal.Calcd. for $C_{30}H_{26}N_2O_8$: C, 66.41; H, 4.83; N, 5.16; O, 23.59%. Found: C, 66.41; H, 4.83; N, 5.17; O, 23.59%.

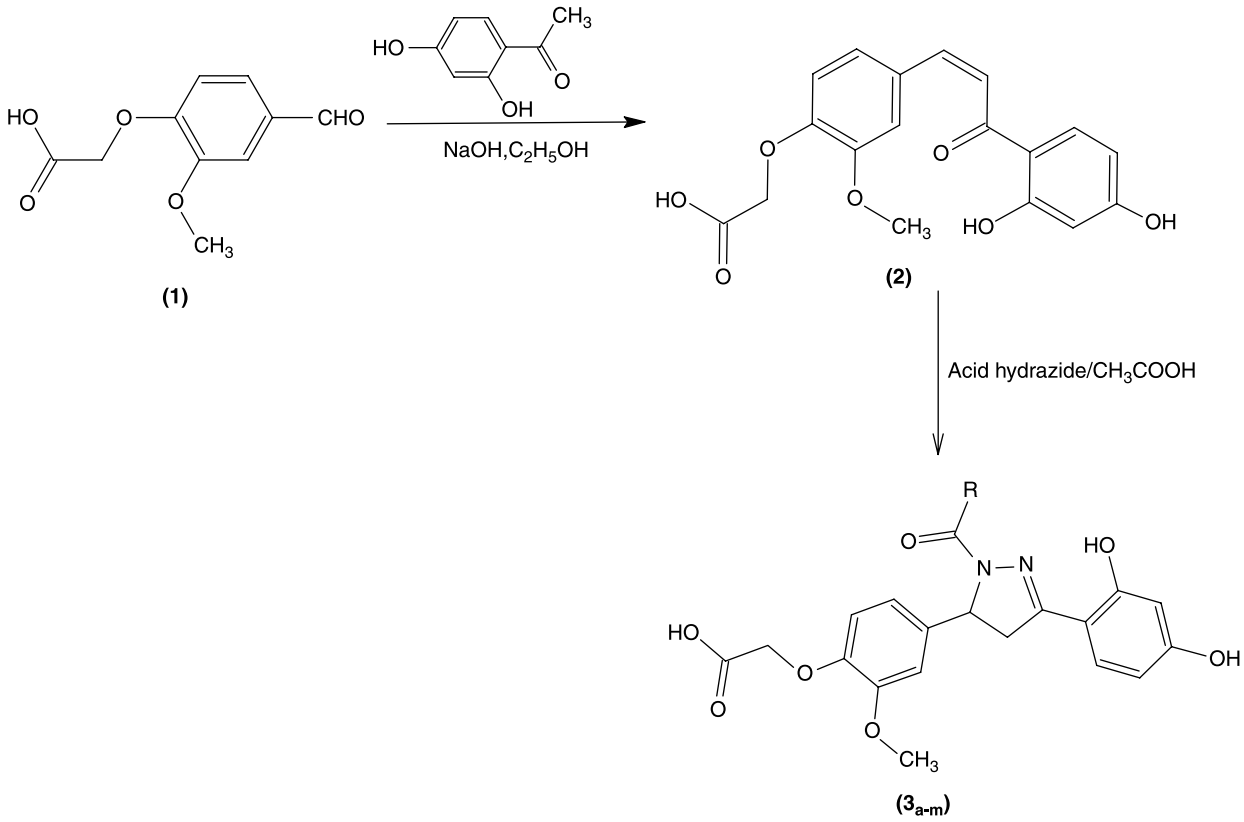
Antiviral activity assay

Confluent cell cultures in micro titer trays were inoculated with 100 $CCID_{50}$ (1 $CCID_{50}$ corresponding to the virus stock dilution that proved infective for 50% of the cell cultures). After 1 h of virus adsorption to the cells, residual virus was removed and replaced by cell culture medium (Eagle's minimal essential medium) containing 3% foetal calf serum and various concentrations of the test compounds. Viral cytopathogenicity was recorded as soon as it reached completion in the

Table II. Cytotoxicity and antiviral activity of compounds in Crandell-Rees Feline Kidney (CRFK) cell cultures.

Compound	CC_{50}* (μg/mL)	EC_{50}† (μg/mL) Feline Corona Virus (FIPV)	EC_{50}† (μg/mL) Feline Herpes Virus
3a	>100	>100	>100
3b	62.1	>20	>20
3c	>100	>100	>100
3d	>100	>100	>100
3e	>100	>100	>100
3f	>100	>100	>100
3g	>100	>100	>100
3h	>100	>100	>100
3i	>100	>100	>100
3j	13.5	>4	>4
3k	>100	>100	>100
3l	5.3	>4	>4
3m	8.8	>4	>4
Ganciclovir	>100	>100	4.0

* 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay; † 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.



Scheme 1. Synthesis of pyrazolines.

Table III. Cytotoxicity and antiviral activity of compounds in human embryonic lung (HEL) cell cultures.

Compound	Minimum cytotoxic concentration* (μg/mL)	EC_{50}† (μg/mL)				
		Herpes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Vesicular stomatitis virus	Herpes simplex virus-1 TK- KOS ACV^r
3_a	>100	>100	>100	>100	>100	>100
3_b	>100	100	>100	>100	>100	>100
3_c	>100	>100	>100	>100	>100	>100
3_d	>100	>100	>100	>100	>100	>100
3_e	>100	>100	>100	>100	>100	>100
3_f	>100	>100	>100	>100	>100	>100
3_g	>100	>100	>100	>100	>100	>100
3_h	>100	>100	>100	>100	>100	>100
3_i	>100	>100	>100	>100	>100	>100
3_j	0.16	>0.032	>0.032	>0.032	>0.032	>0.032
3_k	>100	>100	>100	>100	>100	>100
3_l	≥20	>20	>20	>20	>20	>20
3_m	20	>4	>4	>4	>4	>4
Brivudin (μM)	>250	0.08	250	50	>250	>250
Ribavirin (μM)	>250	50	>250	>250	>250	>250
Cidofovir (μM)	>250	2	2	10	>250	2
Ganciclovir (μM)	>100	0.16	0.16	>100	>100	20

* Required to cause a microscopically detectable alteration of normal cell morphology; † Required to reduce virus-induced cytopathogenicity by 50%.

untreated virus-infected cell cultures, i.e., at 1 to 2 days for vesicular stomatitis; at 2 days for Coxsackie and herpes simplex virus types 1 and 2 and Sindbis virus; and 6 to 7 days for reo- and parainfluenza viruses. The antiviral activity of the compounds is expressed as the concentration required to inhibit viral cytopathogenicity by 50%.

Cytotoxicity assays

Cytotoxicity was monitored by direct microscopical inspection of the cell monolayers which had not been infected but were treated by the compounds at the same concentration as used in the antiviral activity assays [12].

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

Compound	Minimum cytotoxic concentration* (μg/mL)	EC_{50}† (μg/mL)		
		Vesicular stomatitis virus	Coxsackie virus B4	Respiratory syncytial virus
3_a	>100	>100	>100	>100
3_b	100	>20	>20	>20
3_c	>100	>100	>100	>100
3_d	>100	>100	>100	>100
3_e	>100	>100	>100	>100
3_f	>100	>100	>100	>100
3_g	>100	>100	>100	>100
3_h	>100	>100	>100	>100
3_i	100	>20	>20	>20
3_j	20	>4	>4	>4
3_k	>100	>100	>100	>100
3_l	100	>20	>20	>20
3_m	20	>4	>4	>4
DS-5000	>100	4	>100	1
(*S*)-DHPA (μM)	>250	>250	>250	>250
Ribavirin (μM)	>250	22	146	10

* Required to cause a microscopically detectable alteration of normal cell morphology; † Required to reduce virus-induced cytopathogenicity by 50.

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

Compound	Minimum cytotoxic concentration* (μg/mL)	EC_{50}† (μg/mL)				
		Para-influenza-3 virus	Reovirus-1	Sindbis virus	Coxsackie virus B4	Punta Toro virus
$\mathbf{3_a}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_b}$	100	>20	>20	>20	>20	>20
$\mathbf{3_c}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_d}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_e}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_f}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_g}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_h}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_i}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_j}$	≥0.8	>0.8	>0.8	>0.8	>0.8	>0.8
$\mathbf{3_k}$	>100	>100	>100	>100	>100	>100
$\mathbf{3_l}$	100	>20	>20	>20	>20	>20
$\mathbf{3_m}$	100	>20	>20	>20	>20	>20
DS-5000	>100	>100	>100	>100	>100	100
(*S*)-DHPA (μM)	>250	250	>250	>250	>250	>250
Ribavirin (μM)	>250	45	146	146	>250	146

* Required to cause a microscopically detectable alteration of normal cell morphology; † Required to reduce virus-induced cytopathogenicity by 50%.

Results and discussion

Chemistry

The physical constants of pyrazoline derivatives ($\mathbf{3_{a-m}}$) are shown in Table I, whereas results obtained from the antiviral testing is described in this study are shown in Tables II–V, and a reaction sequence for the preparation is outlined in Scheme 1. The desired chalcone was obtained, by Claisen-Schmidt condensation, by reacting 2-(4-formyl-2-methoxyphenoxy) acetic acid with 1-(2,4-dihydroxyphenyl)ethanone in the presence of a base. Reaction between chalcone, 2-{4-[3-(2,4-dihydroxyphenyl)-3-oxoprop-1-enyl]-2-methoxyphenoxy} acetic acid and the appropriate acid hydrazide in the presence of glacial acetic acid, afforded after a reaction time varying from 6–12 h, the pyrazolines ($\mathbf{3_{a-m}}$) in 78–89% yield. TLC and elemental analyses was done to confirm the purity of the compounds. Analytical and spectral data [IR & ^{1}H-NMR] of all the synthesized compounds were found in full agreement with the proposed structures, which was further confirmed by mass fragmentation of the selected compounds. The IR spectra of chalcone revealed C=O, C=C, stretching at 1682, 1572 cm^{-1} where as in general pyrazoline ($\mathbf{3_b}$) revealed C=N, C–N and C=O band at 1545, 1325 and 1685 cm^{-1}, respectively. In the ^{1}H-NMR spectra the signals of the respective protons of the compounds were verified on the basis of their chemical shifts, multiplicities and coupling constants. The spectra showed a singlet at δ10.2 ppm corresponding to the COOH group. The pyrazoline ring formation showed a characteristic doublet and triplet for C-4 methylene and C-5 protons at a shift at 2.5 and 4.8 respectively, a singlet at δ3.6 ppm for methoxy group, a multiplet at δ6.8–8.1 ppm for aromatic protons and a singlet at δ9.4 ppm for OH proton. It was found interesting that only two compound of the series 3f and 3k have shown usual pyrazoline splitting.

Antiviral activity

Antiviral activity was tested against Feline Corona Virus (FIPV) and Feline Herpes Virus (in Crandell-Rees Feline Kidney (CRFK) cell cultures), herpes simplex virus-1 (KOS), herpes simplex virus-2 (G), vaccinia virus, vesicular stomatitis virus, herpes simplex virus-1 TK-KOS ACV^r (in HEL cell cultures); vesicular stomatitis virus, Coxsackie virus B4, Respiratory syncytial virus (in HeLa cell cultures); parainfluenza-3 virus, reovirus-1, Sindbis virus, Coxsackie virus B4, Punta Toro virus (in Vero cell cultures). For each compound, the Minimum Cytotoxic Concentration (MCC) [or 50% cytotoxic concentration (CC_{50}) for CRFK] was determined. Ribavirin, Brivudin, Acyclovir, Ganciclovir, Dextran sulfate (DS-5000) and (S)-DHPA were used as the reference compounds.

The results of the antiviral assays are shown in Tables II–V. Cytotoxicity towards uninfected host cells was determined under same conditions as antiviral activity i.e microscopic evaluation of the cell morphology of the confluent cell monolayer which either had or had not been inoculated with virus. As the criterion for specific antiviral activity was taken the inhibition of virus-induced cytopathogenicity at a concentration that was at least 5-fold lower than the

concentration required to alter the morphology of the uninfected host cells.

As per this criterion none of the compounds tested ($\mathbf{3_a}$-$\mathbf{3_m}$) showed any specific antiviral activity (Tables II–V). The most cytotoxic of the series was $\mathbf{3_j}$, with a minimum cytotoxic concentration of 0.8 μg/mL in Vero cell cultures and 0.16 μg/mL in HEL (human embryonic lung) cells.

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