

In Vitro Antiviral Activity of the 6-Substituted 2-(3',4'-Dichlorophenoxy)-2H-Pyrano[2,3-b]Pyridines MDL 20,610, MDL 20,646, and MDL 20,957

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The 6-substituted 2-(3',4'-dichlorophenoxy)-2H-pyrano[2,3-b]pyridines MDL 20,610 (6-SO₂CH₃), MDL 20,646 (6-Br), and MDL 20,957 (6-Cl) are potent antirhinovirus compounds with median plaque 50% inhibitory concentrations (IC_{1/2}s) of 0.03, 0.006, and 0.006 µg/ml, respectively, against the 32 serotypes evaluated. The 6-halogenated analogs produced 99% reductions in progeny virion yields at concentrations as low as 0.004 µg/ml. However, these analogs perturbed HeLa cell metabolism at lower concentrations (at or above 5 µg/ml) than did the 6-methylsulfonyl analog (at or above 20 µg/ml). Compound MDL 20,610 was also active against human, simian, and bovine rotaviruses (cytopathic effect IC_{1/2}s of 0.8 to 1.5 µg/ml) and possessed variable enterovirus and paramyxovirus activity.

Synthesis and preliminary evaluation of a series of 18 2-phenyl-2H-pyrano[2,3-b]pyridines showed that a 3',4'-dichloro substitution coupled with a 6-methylsulfonyl (MDL 20,610), 6-bromo (MDL 20,646), or 6-chloro (MDL 20,957) (Fig. 1) is necessary for strong (median plaque 50% inhibitory concentrations [IC_{1/2}s] of 0.01 to 0.2 µg/ml) in vitro activity against rhinoviruses (RVs) 1A, 9, and 64 (T. M. Bargar, J. K. Dulworth, M. T. Kenny, R. Massad, J. K. Daniel, T. Wilson, and R. N. Sargent, *J. Med. Chem.*, in press). These studies also showed mean peak plasma levels of 17.0 and 4.2 µg of parent compound per ml after mice were given a single oral dose of 200 mg of MDL 20,610 or MDL 20,646 per kg, respectively. The current studies evaluated the in vitro activity of these 6-substituted analogs against a larger number of RVs and their effects on HeLa cells. Based on these data, one compound, MDL 20,610, was further evaluated for its activity against selected enteroviruses and nonpicornaviruses.

To assess in vitro antiviral efficacy, 2.0 mg of each compound was mixed with 0.02 ml of dimethyl sulfoxide followed by the addition of 10 ml of test medium to give a stock concentration of 200 µg of compound per ml. RV plaque assays were performed in quadruplicate on HeLa cells in 6-well microtiter plates (9). The concentration of the test compound necessary to reduce the number of plaques by 50% when compared to untreated controls was considered the IC_{1/2}. All other viruses were evaluated in triplicate by using cytopathic effect or hemadsorption endpoint assays. Coxsackieviruses A7, A21, B3, and B4, echoviruses 6, 12, and 30, enteroviruses 70 and 71, poliovirus 2, parainfluenzaviruses 2 and 3, respiratory syncytial virus, measles virus, and adenoviruses 4 and 7 were assayed in HeLa cells. Echovirus 19, coxsackievirus A9, and coronavirus 229E were assayed in MRC-5 cells; adenovirus 11, reovirus 1, and parainfluenzavirus 4 were assayed in secondary rhesus monkey kidney cells; and influenza viruses were assayed in MDCK cells (2, 8). For rotavirus assays,

MA-104 monolayers were washed three times with a test medium composed of Eagle minimal essential medium supplemented with 1% PSN (GIBCO Laboratories, Grand Island, N.Y.) and 0.1 to 0.2 µg of trypsin per ml. The monolayers were then refed with 1.0 ml of compound-containing or compound-free test medium. The cultures were examined for rotavirus cytopathic effect 5 days after incubation at 36 to 37°C. For the cytopathic effect (or hemadsorption) endpoint assays, the lowest dilution of compound inhibiting viral cytopathic effect (or hemadsorption) by 50% or more was considered the IC_{1/2}.

In the yield reduction studies, HeLa cell monolayers in 6-well microtiter plates each received 2.0 ml of Eagle minimal essential medium supplemented with 1% heat-inactivated fetal bovine serum and 1% PSN and containing 0 (virus control) or 0.0015 to 2.0 µg of test compound per ml. After 3 h of incubation at 36 to 37°C each culture received 0.2 ml of virus suspension containing 2 × 10² to 3 × 10⁴ PFU (multiplicity of infection of 0.0001 to 0.03). All variables were tested in triplicate. The cell cultures were incubated at 33°C for 72 to 96 h (until the virus control exhibited 50 to 75% cell destruction), and the titers of the pooled cell-supernatant harvests were determined by plaque assay (9) after three freeze (-50°C)-thaw cycles.

The effect of the compounds on the development of isolated HeLa cell colonies was determined as previously described (9). The cytotoxic endpoint was considered to be the concentration of the compound which reduced the colony number by 50% or more. After this initial evaluation, the crystal violet was extracted from the stained colonies by the technique described by Schellekens and Stitz (6). The A₅₉₀ of the fluids harvested from each well was then determined with a spectrophotometer (model 25; Beckman Instruments, Inc., Fullerton, Calif.). Preliminary regression analysis (data not shown) showed that the optical density was linear (correlation coefficient of >0.99) at cell concentrations of 100 to 300,000 per microtiter plate well. The concentration of compound required to reduce the A₅₉₀ by 50% when

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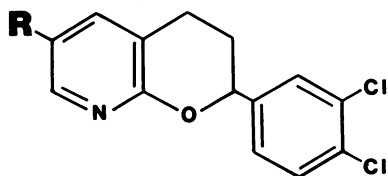


FIG. 1. Structure of 6-substituted 2-(3',4'-dichlorophenoxy)-2H-pyrano[2,3-b]pyridines. MDL 20,610, R = SO₂CH₃; MDL 20,646, R = Br; MDL 20,957, R = Cl.

compared to the compound-free controls was considered the cell growth inhibitory concentration.

The effects of the compounds on host cell DNA, RNA, and protein synthesis were determined by techniques described previously (3, 9). Briefly, cellular DNA synthesis was measured indirectly by the incorporation of [6-³H]thymidine into a trichloroacetic acid-insoluble cell fraction. Cellular RNA and protein synthesis were measured indirectly by the incorporation of [5-³H]uridine and a U-¹⁴C-labeled L-amino acid mixture, respectively, into a trichloroacetic acid-insoluble cell fraction.

The plaque reduction data (Table 1) show that the 6-halogen analogs MDL 20,646 (6-Br) and MDL 20,957 (6-Cl) were approximately fivefold more active by weight against RVs than was the 6-methylsulfonyl analog MDL 20,610. This difference was mirrored in the reduced production of infectious RV virions by compound-pretreated HeLa cells (Table 2). HeLa cell growth studies, however, showed that MDL 20,957 was cytotoxic at concentrations as low as 10 μg/ml. In contrast, MDL 20,610 and MDL 20,646 were found to be noncytotoxic at concentrations up to 20 μg/ml. The 6-halogen analogs MDL 20,646 and MDL 20,957 also inhibited the growth of HeLa cell colonies at concentrations of 12.6 and 11.0 μg/ml, respectively, whereas MDL 20,610 only showed some inhibition at a test concentration of 20 μg/ml. These findings were confirmed in studies of the effects of the compounds on HeLa cell macromolecular synthesis (Fig. 2).

TABLE 1. In vitro activity of 6-substituted 2-(3',4'-dichlorophenoxy)-2H-pyrano[2,3-b]pyridines against 32 RV serotypes

| IC _{1/2} range (μg/ml) ^a | Serotype(s) for which IC _{1/2} was in indicated range ^b | | |
|--|---|---|---|
| | MDL 20,610 | MDL 20,646 | MDL 20,957 |
| 0.0001-0.0009 | 30, 49 (6.3) ^c | 29, 31 (6.3) | 29, 30, 31, 81 (12.6) |
| 0.001-0.009 | 2, 19, 25, 33, 50, 61, 81, Hanks (31.3) | 1B, 2, 10, 12, 19, 25, 30, 32, 33, 40, 44, 49, 50, 56, 74, 81, Hanks (59.4) | 1B, 2, 10, 12, 19, 25, 32, 33, 40, 44, 50, 56, 61, 74, Hanks (59.4) |
| 0.01-0.09 | 1B, 10, 12, 15, 29, 32, 40, 44, 56, 64, 74, 89 (68.8) | 1A, 9, 13, 15, 21, 39, 61, 64, 89 (87.5) | 1A, 9, 13, 15, 21, 49, 64, 89 (84.4) |
| 0.1-0.9 | 1A, 9, 13, 21, 31, 39 (87.5) | | 39 (87.5) |
| >4.0 | 5, 8, 14, 45 (100) | 5, 8, 14, 45 (100) | 5, 8, 14, 45 (100) |

^a Determined by plaque reduction.

^b The median IC_{1/2}s (μg/ml) were as follows: MDL 20,610, 0.03; MDL 20,646, 0.006; MDL 20,957, 0.006.

^c Cumulative percentage of serotypes inhibited.

TABLE 2. Concentrations of 6-substituted 2-(3',4'-dichlorophenoxy)-2H-pyrano[2,3-b]pyridines required to reduce yield of infectious RV from compound-pretreated HeLa cells by 99%^a

| RV serotype | Concn (μg/ml) of compound which reduced yield by 99% | | |
|-------------|--|------------|------------|
| | MDL 20,610 | MDL 20,646 | MDL 20,957 |
| 9 | >2.0 | 0.8 | 0.7 |
| 13 | 0.7 | 0.2 | 0.1 |
| 32 | 0.5 | 0.1 | 0.1 |
| 39 | 1.3 | 0.3 | 0.3 |
| 44 | 0.08 | 0.005 | 0.004 |
| Hanks | 0.6 | 0.2 | 0.2 |

^a Virus yields in infected control cultures (no compound) were 2 log₁₀ to 5 log₁₀ higher than the initial infecting dose.

The overall spectrum of MDL 20,610 antiviral activity is shared with the 4',6-dichloroflavan BW683C (1), suggesting a common mechanism of action. Recent studies (7) showed that BW683C binds directly to infecting virions, inhibiting subsequent uncoating. This is a property shared with the arylalkyldiketone arildone (5) and with the antipicornavirus isoxazole WIN 51711 (4). Studies of the antiviral mechanism of action of MDL 20,610 are currently in progress.

Since MDL 20,610 is a potent anti-RV compound while producing little effect on HeLa cells, additional studies were conducted to determine its activity against representative enteroviruses and nonpicornaviruses. Compound MDL 20,610 was not active (cytopathic effect or hemadsorption IC_{1/2} of >5.0 μg/ml) against coxsackieviruses A7, A9, A21, and B3, echovirus 19, enterovirus 71, parainfluenzaviruses 2 and 3, respiratory syncytial virus, measles virus, influenza viruses A₁/Ann Arbor/1/57 and B/Lee/40, adenoviruses 4, 7, and 11, herpes simplex virus, and human coronavirus 229E. The compound was moderately active (cytopathic effect IC_{1/2} of 1.3 to 2.5 μg/ml) against coxsackievirus B4, poliovirus 2, parainfluenzavirus 4, reovirus 1, and simian rotavirus. Echoviruses 6, 12, and 30, enterovirus 70, and human and bovine rotaviruses were inhibited by less than 1.0 μg of MDL 20,610 per ml. The activity of MDL 20,610 against human, simian, and bovine rotaviruses demonstrated in our studies warrants further in vitro and in vivo investigation.

In summary, the potency of MDL 20,610 against the RVs,

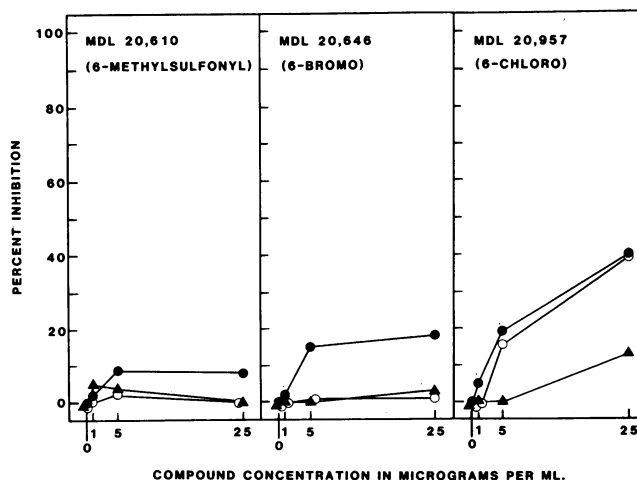


FIG. 2. Effect of 6-substituted 2-(3',4'-dichlorophenoxy)-2H-pyrano[2,3-b]pyridines on HeLa cell DNA (●), RNA (○), and protein (▲) synthesis.

enterovirus 70, and the rotaviruses, coupled with low cell toxicity and good systemic bioavailability, makes this compound a good candidate for clinical evaluation.

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