from 2-butanone as the HCl salt (7·HCl), mp 190–192 °C (lit.[20] mp 189–191 °C).

**Neurotoxicological Evaluations.** Each of the six test compounds (2–7) and MPTP were injected intraperitoneally into male Swiss mice (CDL, Groningen) weighing 25–40 g. Each animal was dosed with $0.72 \times 10^{-4}$ mol/kg of each of the compounds on days 1 and 2 followed by $1.44 \times 10^{-4}$ mol/kg on days 3–12. Failure to give the "half-dose" injections of MPTP resulted in an $LD_{70}$ in this strain of mouse. The injected drugs were all dissolved as their HCl salts, except for 5 which was a fumarate salt, in normal saline solution. In each case, the concentration of compound in the injection solution was 0.14 M. Control animals were injected daily with an equivalent amount (based on body weight) of normal saline solution. Before and during the experiment the animals were maintained in a temperature- and light-controlled animal room and given free access to food and water. The drug solutions used for the injections were prepared at the beginning of the experiment and stored at 5 °C except during the daily injection periods. At the end of the experiment, TLC analyses of each of the solutions vs. pure crystalline materials showed no traces of impurities.

One day after the 12th day of injections, animals were sacrificed by spinal severance and rapidly decapitated, the brains removed, and the striata dissected out and immediately frozen on dry ice. The striatal tissues were weighed, homogenized, and subjected to separation on Sephadex columns, and the DA, DOPAC, and HVA concentrtions were determined by HPLC–EC detection as previously described by Westerink and Mulder.[29] Additional groups of mice were sacrificed 9 days after the last day of injections, and the striata of these animals were analyzed for DA content by the same procedure.

**Acknowledgment.** We are indebted to Drs. P. S. Protoghese and D. L. Larson of the University of Minnesota for providing the α-prodinol used in this study. This work was funded in part by a grant from the Faculty Research Committee of the University of the Pacific to D.S.F.

**Registry No.** 1, 28289-54-5; 1·HCl, 23007-85-4; 2, 13515-63-4; 2·HCl, 6672-58-8; 3, 6672-51-1; 3·HCl, 13299-60-0; 4, 3009-16-3; 4·HCl, 36224-30-3; 5, 4986-03-2; 5·fumarate, 99838-20-7; 6, 99156-44-2; 6·HCl, 99838-21-8; 7, 75712-94-6; 7·HCl, 99838-22-9; DA, 51-61-6; Dopac, 102-32-9; PhLi, 591-51-5; 1-methyl-4-piperidinone, 1445-73-4; 1-methyl-4-phenyl-4-piperidinol, 4972-68-3; α-prodinol, 15217-63-7; β-prodinol, 468-59-7; δ-promedol, 36260-34-1; 1,3,3-trimethyl-4-piperidinone, 18436-83-4; 1,3,3-trimethyl-4-phenyl-4-piperidinol, 18436-89-0; cinnamyl bromide, 4392-24-9; dimethylamine, 124-40-3; homovanillic acid, 306-08-1.

(29) Westerink, B. H. C.; Mulder, T. B. A. *J. Neurochem.* 1981, *36*, 1449.

# Antipicornavirus Activity of Substituted Phenoxybenzenes and Phenoxypyridines

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Phenoxybenzenes and phenoxypyridines were prepared and tested for the effect of substituents on antipicornavirus activity. The most active compound, 2-(3,4-dichlorophenoxy)-5-nitrobenzonitrile (8), demonstrated broad-spectrum antipicornavirus activity. Compound 8 and several analogues each given orally prior to and during infection protected mice against an otherwise lethal challenge with coxsackievirus A21.

The picornaviruses represent a large family of 20–30-nm single-stranded RNA viruses possessing similar physicochemical properties. They are implicated as the causative agents of several human diseases, including the common cold (rhinoviruses)[1] and primary myocardial disease (coxsackieviruses).[2] The widespread nature of picornavirus diseases, the economic consequences, and the impracticality of vaccine development have stimulated the search for broad-spectrum orally active chemotherapeutic agents.[3] We report here that many simple diphenyl and phenyl pyridyl ethers show considerable activity in cell culture against three representative picornaviruses. Several demonstrated broad-spectrum activity against 20 human rhinovirus serotypes and enhanced the survival rate of mice given the compounds orally prior to an otherwise lethal challenge with coxsackievirus A21.

**Chemistry.** Five series of diaryl ethers were evaluated and are listed in Tables I–V. All of the compounds were readily available through straightforward nucleophilic aromatic substitution reactions of the appropriate phenol with the appropriate aryl halide in the presence of base. Subsequent functional group transformations, where necessary, gave the desired substituent patterns. All of the starting aryl halides are known in the literature or were available commercially. The (methylsulfonyl)pyridines of Table V were prepared by reaction of phenols with the appropriate bis(methylsulfonyl)pyridine. Preparation of bis(alkylsulfonyl)pyridines is reported separately.[4]

**Biological Evaluation.** Compounds were evaluated initially for potency by standard tissue culture methods in which the approximate concentration of compound needed to reduce viral cytopathic effect on HeLa cells by 50% ($MIC_{50}$ in the tables) was determined by serial two-fold dilutions in culture medium.[5] The concentration at which compound induced cytotoxicity (Tox) became apparent, as evidenced by cell death or a change in cell morphology or growth rate, is also reported. Rhinovirus

†The Dow Chemical Co., Midland, MI.
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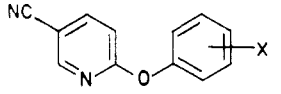
(1) Douglas, R. G., Jr. In "Antiviral Agents and Viral Diseases of Man"; Galasso, G. J., Merigan, T. C., Buchanan, R. A., Eds. Raven Press: New York, 1979; pp 385–459.
(2) Jawatz, E.; et al. "Review of Medical Microbiology", 13th ed.; Lange Medical Publications: Los Altos, CA, 1978; p 550.
(3) For recent work and leading references see: Diana, G. D.; et al. *J. Med. Chem.* 1985, *28*, 748. Ishitsuka, H.; et al. *Antimicrob. Agents Chemother.* 1982, *22*, 611, 617. Selway, J. W. T.; et al. *Nature* 1981, *292*, 369. Wikel, J. H.; et al. *J. Med. Chem.* 1980, *23*, 368. Galabov, A. S. *Arzneim.-Forsch.* 1979, *29* (II), 1863.
(4) Wood, S. G.; Matyas, B. T.; Vinogradoff, A. P.; Tong, Y. C. *J. Heterocycl. Chem.* 1984, *21*, 97.
(5) Torney, H. L.; Dulworth, J. K.; Steward, D. L. *Antimicrob. Agents Chemother.* 1982, *22*, 635.

0022-2623/86/1829-0427$01.50/0 

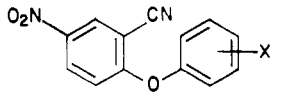
**Table I.** Antipicornavirus Activity of 2-Phenoxy-5-cyanopyridines



| compd | X | Tox,[a] μM | $MIC_{50}$,[b] μM | | | rel act.[c] | | |
|---|---|---|---|---|---|---|---|---|
| | | | RV-1A | RV-2 | coxsackie A21 | RV-1A | RV-2 | coxsackie A21 |
| 2 | 4-Cl | >433 | 54 | 54 | 54 | 1.9 | 2.0 | 0.48 |
| 3 | 4-$COCH_3$ | 420 | 210 | *d* | 53 | *e* | *d* | 0.02 |
| 4 | H | 676 | 64 | *d* | 64 | 0.40 | *d* | 0.81 |
| 5 | 3,4-$Cl_2$ | 377 | 5 | 19 | 2 | 4.1 | 2.7 | 13.0 |
| 6 | 4-F | 250 | 34 | *d* | 69 | 3.3 | *d* | 1.7 |
| 7 | 4-$CH_3$ | 476 | 60 | 30 | 60 | 0.43 | 1.7 | 0.43 |

[a] Lowest concentration at which compound-induced cytotoxicity was apparent. [b] Minimum inhibitory concentration: the lowest concentration of compound to reduce the viral cytopathic effect by 50% or more compared to control. [c] The ratio of the $MIC_{50}$ of the positive control compound 1 to the test compound obtained in the same test. [d] Not tested or not determined. [e] Compound cytotoxicity in relation to activity was too great to permit a meaningful representation of antiviral activity.

**Table II.** Antipicornavirus Activity of 2-Phenoxy-5-nitrobenzonitriles



| compd | X | Tox μM | $MIC_{50}$, μM | | | rel act. | | |
|---|---|---|---|---|---|---|---|---|
| | | | RV-1A | RV-2 | coxsackie A21 | RV-1A | RV-2 | coxsackie A21 |
| 8 | 3,4-$Cl_2$ | >324 | 0.26 | 0.26 | 0.52 | 319 | 99.6 | 99.6 |
| 9 | 2,4-$Cl_2$ | >324 | 0.97 | 0.52 | 3.9 | 85.6 | 49.8 | 13.3 |
| 10 | 3,5-$Cl_2$ | >65 | 16.2 | 8.1 | 16.2 | 1.6 | 1.6 | 0.80 |
| 11 | 2,6-$Cl_2$ | >65 | 3.9 | 0.52 | 16.2 | 5.3 | 25.0 | 0.80 |
| 12 | 2,3-$Cl_2$ | >65 | 0.52 | 0.26 | 8.1 | 25.0 | 50.0 | 3.2 |
| 13 | 2,5-$Cl_2$ | >162 | 0.26 | 0.52 | 2.0 | 50.0 | 25.0 | 13.0 |
| 14 | 4-Cl | >364 | 1.4 | 2.8 | 3.6 | 18.5 | 9.3 | 7.2 |
| 15 | 3-Cl | >91 | 1.1 | 0.58 | 1.1 | 23.6 | 44.7 | 11.8 |
| 16 | 2-Cl | 45 | 4.5 | 4.5 | 5.7 | 4.6 | 4.6 | 2.3 |
| 17 | 4-Br | >313 | 3.8 | 3.8 | 7.8 | 6.8 | 6.8 | 3.3 |
| 18 | 2-Br | >31 | 1.6 | 0.94 | 0.41 | 12.9 | 11.1 | 3.3 |
| 19 | 4-$OCH_3$ | 93 | 46.3 | 11.6 | 5.7 | 0.56 | 2.2 | 2.2 |
| 20 | 3-$OCH_3$ | >185 | 23.0 | 9.3 | 4.6 | 1.1 | 1.4 | 2.8 |
| 21 | 2-$OCH_3$ | 18.5 | 9.2 | 9.2 | 18.5 | *a* | *a* | *a* |
| 22 | H | >42 | 5.4 | 5.4 | 2.7 | 3.8 | 3.8 | 0.96 |
| 23 | 4-$CH_3$ | 39 | 5.1 | 5.1 | 2.4 | 4.1 | 2.0 | 2.2 |
| 24 | 4-CN | >38 | 9.4 | 9.4 | 18.9 | 2.2 | 1.1 | 0.33 |
| 25 | 4-$CF_3$ | 162 | 10.0 | 1.0 | 2.0 | 1.3 | 13.0 | 13.0 |
| 26 | 3-$CF_3$ | 41 | 8.1 | 2.0 | 3.9 | 1.6 | 25.9 | 5.3 |
| 27 | 2-$CF_3$ | 32 | >324 | 3.9 | 32 | *a* | 3.3 | *a* |
| 28 | 3,4-$(OCH_3)_2$ | >167 | >167 | 20.7 | 10.3 | *b* | 0.63 | 1.3 |
| 29 | 4-COPh | >291 | >291 | >291 | 36.3 | *b* | *b* | 1.4 |
| 30 | 4-$COCH_3$ | >177 | 22.2 | 11.1 | 3.5 | 0.59 | 1.2 | 0.68 |
| 31 | 4-$SCH_3$ | 350 | >350 | 21.8 | 0.87 | *b* | 2.4 | 6.0 |
| 32 | 4-$SO_2CH_3$ | >314 | >314 | 39.3 | 2.0 | *b* | 1.3 | 2.6 |
| 33 | 2,3-$(CH)_4$ | >172 | 4.3 | 1.1 | 10.8 | 6.0 | 11.8 | 1.2 |

[a] See footnote *e*, Table I. [b] Not determined.

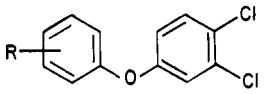
types 1 and 2 and coxsackievirus A21 were chosen for the initial tests. Since it was not possible to evaluate all compounds at the same time using the same stock of HeLa cells and the same virus challenge, we found it useful to obtain a measure of the relative activity of the compounds through the use of a positive control. 5-Cyano-2-(4-nitrophenoxy)pyridine (1), synthesized early in our program, was chosen for this purpose. Because the $MIC_{50}$ values for 1 showed significant variation from test to test, this positive control was included in a parallel experiment in each test of each compound in the Tables I–V. Relative activity was determined by taking the ratio of the $MIC_{50}$ for 1 found in that test to the $MIC_{50}$ for the test compound. Compounds that exhibited significant activity in the initial cell culture assays were chosen for further study against a broader spectrum of picornaviruses. Compounds selected on the basis of potency and spectrum were evaluated for potential oral activity in weanling mice infected with coxsackievirus A21, on the basis of a model first described by Delong and co-workers.[6] Mice were fed a diet containing 0.06% test compound or placebo for 24 h (approximately 75 mg/kg per day of drug[7]) before receiving a normally lethal intraperitoneal inoculation of coxsackievirus A21. Animals continued on treated or placebo feed for the duration of the test. Animals were observed daily and deaths recorded.[8]

## Results and Discussion

When antipicornavirus activity was discovered in a series of 2-phenoxycyanopyridines (Table I), we began a synthesis program to optimize activity by preparing and testing other

(6) Delong, D. C.; Doran, W. J.; Baker, L. A.; Nelson, J. D. *Ann. N.Y. Acad. Sci.* **1970**, *173*, 516.
(7) This concentration in feed was selected based on prior experience in screening compounds for oral activity in mice. It represents a level at which most compounds were palatable and caused no obvious signs of toxicity.
(8) The pharmacokinetic behavior of compounds significantly protective was studied as a part of a separate investigation.

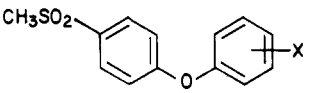
**Table III.** Antipicornavirus Activity of 1-Phenoxy-3,4-dichlorobenzenes



| compd | R | Tox, μM | MIC$_{50}$, μM | | | rel act. | | |
|---|---|---|---|---|---|---|---|---|
| | | | RV-1A | RV-2 | coxsackie A21 | RV-1A | RV-2 | coxsackie A21 |
| **34** | 4-$NO_2$ | 352 | 4.6 | 22.0 | 0.88 | 5.6 | 2.4 | 5.9 |
| **35** | 4-CN | 24 | 9.5 | 9.5 | 2.3 | 2.7 | 2.7 | 11.3 |
| **36** | 4-$CH_3SO_2$ | 158 | 0.79 | 0.79 | 4.1 | 32.8 | 32.8 | 3.2 |
| **37** | 4-$PhSO_2$ | 3.3 | 1.6 | 1.6 | 1.6 | *a* | *a* | *a* |
| **38** | 4-$CF_3SO_2$ | >135 | 33.7 | 16.8 | *b* | 0.39 | 1.5 | *b* |
| **39** | 4-Cl | 91 | 22.8 | 45.6 | 9.1 | 2.3 | *a* | 1.4 |
| **40** | 4-$CH_3CO$ | 49 | 2.2 | 2.2 | *b* | 11.8 | 11.8 | *b* |
| **41** | 2-CN | 379 | >379 | >379 | 23.7 | *b* | *b* | 1.1 |
| **42** | 2,4-$(NO_2)_2$ | 19 | >304 | >304 | 19.0 | *b* | *b* | *a* |
| **43** | 2,4-$(CN)_2$ | >173 | >173 | 10.7 | 10.7 | *b* | 2.4 | 3.1 |
| **44** | 2-$CO_2H$-4-$NO_2$ | 305 | 19.0 | 38.1 | 76.2 | 2.7 | 1.4 | 0.34 |
| **45** | 4-$CH_3SO_2$-2-$NO_2$ | >267 | >267 | 16.7 | >267 | *b* | 3.1 | *b* |
| **46** | 3-CN-4-$NO_2$ | 162 | *b* | 16.2 | 16.2 | *b* | 1.2 | 1.2 |
| **47** | 4-$CH_3SO_2$-2-CN | 292 | >292 | 18.3 | >292 | *b* | 2.8 | *b* |
| **48** | 4-$NH_2$-2-CN | 9.0 | 4.5 | 9.0 | 2.2 | *a* | *a* | 11.8 |
| **49** | 4-$CH_3CONH$-2-CN | 62 | 3.7 | 3.7 | 15.6 | 3.5 | 3.5 | 1.7 |
| **50** | 4-Cl-2-CN | >334 | >334 | >334 | 1.0 | *b* | *b* | 5.2 |
| **51** | 2-$NH_2$-4-$CH_3SO_2$ | 19 | 15.0 | 18.7 | 18.7 | *a* | *a* | *a* |
| **52** | 3-$CH_3$-4-$CH_3SO_2$ | >151 | 15.7 | 3-$CH_3$-4-$CH_3SO_2$ | >151 | 1.7 | 1.4 | *b* |

[a] See footnote *e*, Table I. [b] Not tested or not determined.

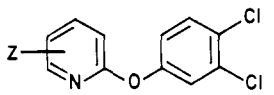
**Table IV.** Antipicornavirus Activity of 1-Phenoxy-4-(methylsulfonyl)benzenes



| compd | X | Tox, μM | MIC$_{50}$, μM | | | rel act. | | |
|---|---|---|---|---|---|---|---|---|
| | | | RV-1A | RV-2 | coxsackie A21 | RV-1A | RV-2 | coxsackie A21 |
| **53** | 4-$NO_2$ | 171 | 42.7 | 21.3 | 0.85 | 1.2 | 2.4 | 6.1 |
| **54** | 4-Cl | 44 | 4.4 | 4.4 | 2.2 | 11.8 | 5.9 | 11.8 |
| **55** | 4-CN | 183 | *a* | 45.8 | 11.4 | *a* | 0.56 | 2.3 |
| **56** | 4-Br | 38 | 1.9 | 1.0 | 7.6 | 9.8 | 19.7 | 2.5 |
| **57** | 4-PhCO | >284 | >284 | 142 | 0.88 | *a* | *b* | 5.9 |
| **58** | 4-$CH_3CO$ | 172 | 86.2 | 8.6 | 17.2 | *b* | 3.0 | 0.76 |
| **59** | 4-$CH_3$ | 203 | 50.8 | 25.4 | 25.4 | 0.51 | 1.0 | 1.0 |
| **60** | 4-$OCH_3$ | >180 | 22.5 | 45.0 | 90.0 | 0.58 | 0.58 | 0.29 |
| **61** | 4-OH | 95 | 94.7 | 94.7 | >189 | *b* | *b* | *a* |
| **62** | 3-Cl | 88 | *a* | 22.1 | 22.1 | *a* | 1.2 | 0.59 |
| **63** | 2-Cl | 88 | 88 | 44.2 | 11.0 | *b* | *b* | 1.2 |

[a] Not tested or not determined. [b] See footnote *e*, Table I.

**Table V.** Antipicornavirus Activity of 2-(3,4-Dichlorophenoxy)pyridines



| compd | Z | Tox, μM | MIC$_{50}$, μM | | | rel act. | | |
|---|---|---|---|---|---|---|---|---|
| | | | RV-1A | RV-2 | coxsackie A21 | RV-1A | RV-2 | coxsackie A21 |
| **64** | 3-CN | >189 | 47 | 24 | 12 | 0.55 | 1.1 | 1.1 |
| **65** | 4-CN | >189 | 24 | 12 | 12 | 1.1 | 2.2 | 1.1 |
| **66** | 6-CN | 378 | 94 | >378 | 23.5 | 0.28 | *a* | 0.91 |
| **67** | 5-$NH_2$ | 88 | 5 | *a* | 98 | 4.1 | *a* | *b* |
| **68** | 5-Br | 78 | 4 | 16 | 4 | 6.5 | 1.6 | 6.5 |
| **69** | 5-$SCH_3$ | 87 | 4 | 9 | 9 | 6.5 | 5.8 | 4.6 |
| **70** | 5-$SOCH_3$ | 83 | 2 | 4 | 83 | 6.5 | 3.3 | *b* |
| **71** | 5-$SO_2CH_3$ | 157 | 1 | 2 | 20 | 10.4 | 10.4 | 0.65 |
| **72** | 5-$SO_3H$ | 312 | 20 | >312 | 312 | 1.3 | *a* | *b* |
| **73** | 3-$SO_2CH_3$ | 157 | 79 | 157 | 157 | *b* | *b* | *b* |
| **74** | 4-$SO_2CH_3$ | 157 | 79 | 79 | 5 | *b* | *b* | 10.4 |
| **75** | 6-$SO_2CH_3$ | 157 | 10 | 39 | 157 | 2.6 | 0.66 | *b* |

[a] Not tested or not determined. [b] See footnote *e*, Table I.

phenoxy aromatic compounds. More promising activity was uncovered in a series of 2-phenoxy-5-nitrobenzonitriles (Table II).

Several observations from Table II are worth pointing out. In general, individual compounds tended to show similar activities against all three viruses, but there were exceptions. For example, naphthoxy compound **33** had fairly good rhinovirus activity, but only marginal activity

against coxsackievirus A21. The trifluoromethyl derivatives **25** and **26** were weak against RV-1A but much better against RV-2 and coxsackie A21. Halogens were favored substituents, with dichloro-substituted rings showing the best activity. There appeared to be no clear-cut preference for ortho, meta, or para substituents over the entire series. Neither strongly electron-withdrawing groups such as $SO_2CH_3$ (**32**) and CN (**24**) nor electron-releasing groups such as $OCH_3$ (**19–21**) were desirable.

Because the 3,4-dichloro-substituted derivative **8** appeared to be the most promising, a series of 1-(aryloxy)-3,4-dichlorobenzenes was prepared and likewise tested (Table III). All modifications in this series resulted in diminished activity compared with **8**. It is surprising that compounds with similarly electron-deficient rings such as **42** (2,4-$(NO_2)_2$), **43** (2,4-$(CN)_2$), **45** (4-$CH_3SO_2$-2-$NO_2$), and **47** (4-$CH_3SO_2$-2-CN) had little activity against any of the viruses. The 3-cyano isomer of **8** (**46**) was similarly only weakly active. The 4-methylsulfonyl derivative **36**, interestingly, had better activity than the closer 2-cyano-4-methylsulfonyl analogue of **8**, compound **47**. The presence of any electron-releasing substituents, as in compounds **48** (4-$NH_2$-2-CN), **51** (2-$NH_2$-4-$CH_3SO_2$), or **52** (3-$CH_3$-4-$CH_3SO_2$), was unfavorable. We conclude from Tables I–III that in vitro antiviral activity requires electron-poor aromatic rings and that the 2-CN-4-$NO_2$ substitution pattern is most desirable.

Because the 4-methylsulfonyl derivative **36** was the most promising member of Table III, further attempts to improve on its activity were made by altering substituents in the other ring (Table IV). A pattern of activity similar to that of Table II emerged, with halogen substituents affording the best activity. Again, electron-releasing substituents such as 4-$OCH_3$ (**60**), 4-OH (**61**), or 4-$CH_3$ (**59**) were not favorable. In the case of chloro-substituted compounds (**54**, **62**, **63**), para substitution was preferred.

Because of activity in the phenoxypyridine series (Table I), and because the electron-deficient nature of the pyridine ring may permit a molecule of similar characteristics to **8**, a series of (3,4-dichlorophenoxy)pyridines was synthesized (Table V). As expected, electron-withdrawing substituents provided the best activity. In addition, the position of the substituent was important, with 5-$SO_2CH_3$ (**71**) and 5-CN (**5**, Table I) being clearly favored over positional isomers.

There was not an apparent simple quantitative relationship between Hansch substituent parameters (e.g., $\sigma$, $\pi$, MR) and relative activity ratios. This may be due to a combination of factors including the observation that the $MIC_{50}$ values for many of the moderately to weakly active compounds were at the limit of solubility in the aqueous culture medium. In addition, multiple mechanisms of action may be operative (vide infra).

In summary, many diaryl ethers with one electron-deficient ring show in vitro antipicornavirus activity. Compounds with 3,4-dichloro substitution or with 2-CN-4-$NO_2$ substitution are favored. Also favored, but to a lesser degree, are phenoxybenzenes or phenoxypyridines with a methylsulfonyl group para to the ether linkage.

Several of the more active compounds selected from the initial tests were evaluated against a collection of rhinovirus serotypes. In the first series of experiments, HeLa cells were treated with compounds at doses (usually 25 $\mu$g/mL) exceeding their aqueous solubility to ensure a saturated solution and then were infected with rhinoviruses. A total of 20 rhinoviruses were selected, and the percentage of the 20 whose cytopathic effect was inhibited by <50%, 50–75%, and >75% is reported in Table VI.[9] Next, $MIC_{50}$ values were determined for compounds **8** and **71** against a series of 29 picornaviruses (Table VII). Compound **8** proved to be the superior of the two, in terms of both potency and spectrum of activity. Whereas **71** has little activity outside of the class of rhinoviruses, **8** appears to be a truly broad-spectrum antipicornavirus agent. This compound has been further evaluated against a larger number of viruses and in a variety of cell cultures.[10]

**Table VI.** Antirhinovirus Spectrum[a]

| compd | % rhinoviruses inhib by <50% | by 50–75% | by >75% |
|---|---|---|---|
| 1[b] | 8 | 7 | 85 |
| 8[c] | 0 | 0 | 100 |
| 34[c] | 30 | 5 | 65 |
| 36[c] | 10 | 5 | 85 |
| 71[c] | 25 | 10 | 65 |

[a] The rhinoviruses tested: RV-Hanks, 4,-6,-8,-10,-13,-17,-19,-21,-29,-39,-56,-58,-59,-60,-64,-68,-74,-75, and -81. [b] Tested at a dose of 50 $\mu$g/mL. [c] Tested at a dose of 25 $\mu$g/mL.

**Table VII.** Antiviral Spectrum of Selected Phenoxy Aromatics[a,b]

| virus | 8 | 71 | virus | 8 | 71 |
|---|---|---|---|---|---|
| RV-Hanks | 0.42 | 31 | RV-1A | 0.26 | 0.94 |
| RV-1B | 0.81 | <0.94 | RV-2 | 0.26 | 1.9 |
| RV-4 | 0.97 | 79 | RV-5 | 2.0 | >39 |
| RV-8 | 0.52 | >39 | RV-9 | 0.42 | 31 |
| RV-10 | <1.0 | <4.1 | RV-13 | 0.81 | >16 |
| RV-14 | 3.2 | >31 | RV-21 | 1.9 | 31 |
| RV-29 | 0.81 | 4.1 | RV-32 | 0.97 | 4.1 |
| RV-33 | 0.81 | 2.0 | RV-39 | 0.42 | 20 |
| RV-44 | 0.81 | 16 | RV-49 | 0.81 | <2.0 |
| RV-55 | 0.81 | <2.0 | RV-64 | 0.52 | 0.94 |
| RV-68 | ≤1.9 | >31 | RV-74 | <0.97 | 7.9 |
| RV-89 | <0.97 | 4.1 | Mengo | >1.6 | >16 |
| Coxsackie A21 | 0.52 | 20 | Coxsackie B3 | 4.9 | >7.9 |
| Coxsackie B4 | 4.9 | 39 | Polio 2 | 1.9 | >39 |
| Echo 12 | 0.97 | <4.1 | | | |

[a] Data expressed as $MIC_{50}$ ($\mu$M). [b] Compounds 8 and 71 were compared directly in the same test against each virus; therefore, it was not necessary to relate their activity to compound 1.

**Table VIII.** Oral Activity of Selected Phenoxy Aromatics in Coxsackievirus A21 Infected Mice

| compd[a] | S/T[b] | control S/T[c] | compd[a] | S/T[b] | control S/T[c] |
|---|---|---|---|---|---|
| 1 | 7/34 | 1/34 | 36 | 25/30 | 4/29 |
| 8 | 27/28 | 2/30 | 71 | 15/33 | 1/34 |
| 34 | 15/15 | 2/15 | | | |

[a] Compound administered in feed. Dose estimated at 75 mg/kg per day. [b] Survivors/Total. [c] Mice receiving no compound.

The results of oral activity testing of several of the promising compounds are presented in Table VIII. The compounds were able to increase the survival rate of mice, compared to controls, which had been infected with an otherwise lethal challenge of coxsackievirus A21. These data suggested that, pending pharmacokinetic and toxicological evaluation, diaryl ethers have potential as orally active antipicornavirus agents.

The biochemical basis of activity of diaryl ethers is not presently understood. Torney and co-workers have reported that **8** does not directly inactivate virus particles, but rather inhibits virus-directed RNA synthesis.[5] With three picornaviruses (Echo 12, Polio-2, RV-1A) made photosensitive with neutral red, **8** did not cause a significant reduction in their loss of photosensitivity during the

(9) We thank Prof. Jack Gwaltney, University of Virgina School of Medicine, for data used in Table VI.

(10) Powers, R. D.; Gwaltney, J. M., Jr.; Hyden, F. G. *Antimicrob. Agents Chemother.* **1982**, *22*, 639.

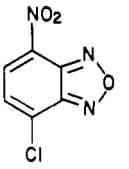
**Table IX.** Physical Data for Compounds of Tables I–V

| compd | mp, °C | ref | formula | anal.[a] | compd | mp, °C | ref | formula | anal.[a] |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 146–147 | *c* | $C_{12}H_7N_3O_3$ | C, H, N | 39 | 27–30 | *o* | $C_{12}H_7Cl_3O$ | C, H |
| 2 | 131–133 | *c* | $C_{12}H_7ClN_2O$ | C, H, N | 40 | 50–52 | *i* | $C_{14}H_{10}Cl_2O_2$ | C, H |
| 3 | 94–96 | *c* | $C_{14}H_{10}N_2O_2$ | C,[b] H, N | 41 | 97.5–98.5 | *p* | $C_{13}H_7Cl_2NO$ | C, H, N |
| 4 | 81–83 | *c* | $C_{12}H_8N_2O$ | C, H, N | 42 | 123–124 | *g* | $C_{12}H_6Cl_2N_2O_5$ | C, H, N |
| 5 | 138–141 | *c* | $C_{12}H_6Cl_2N_2O$ | C,[b] H, N | 43 | 174–175.5 | *i* | $C_{14}H_6Cl_2N_2O$ | C, H, N |
| 6 | 121–122 | *c* | $C_{12}H_7FNO$ | C, H, N | 44 | 188–190 | *j* | $C_{13}H_7Cl_2NO_5$ | C, H, N |
| 7 | 107–109 | *c* | $C_{13}H_{10}N_2O$ | C, H, N | 45 | 173–174 | *g* | $C_{13}H_9Cl_2NO_5S$ | C, H, N |
| 8 | 155–156 | *d* | $C_{13}H_5Cl_2N_2O_3$ | C, H, N | 46 | 110–111 | *h* | $C_{13}H_6Cl_2N_2O_3$ | C, H, N |
| 9 | 153.5–154.5 | *d* | $C_{13}H_5Cl_2N_2O_3$ | C, H, N | 47 | 162–163 | *g* | $C_{14}H_9Cl_2NO_3S$ | C, H, N |
| 10 | 196–198 | *e* | $C_{13}H_5Cl_2N_2O_3$ | C, H, N | 48 | 104–105.5 | *i* | $C_{13}H_8Cl_2N_2O$ | C, H, N |
| 11 | 157–159 | *e* | $C_{13}H_5Cl_2N_2O_3$ | C, H, N | 49 | 153.5–154.5 | *i* | $C_{15}H_{10}Cl_2N_2O_2$ | C, H, N |
| 12 | 146–147 | *e* | $C_{13}H_5Cl_2N_2O_3$ | C, H, N | 50 | 100–100.5 | *p* | $C_{13}H_6Cl_3NO$ | C, H, N |
| 13 | 167–168.5 | *e* | $C_{13}H_5Cl_2N_2O_3$ | C, H, N | 51 | 119–121 | *g* | $C_{13}H_{11}Cl_2NO_3S$ | C, H, N |
| 14 | 163–164 | *d* | $C_{13}H_7ClN_2O_3$ | C, H, N | 52 | 154–156 | *g* | $C_{14}H_{12}Cl_2O_3S$ | C, H |
| 15 | 130–131 | *f* | $C_{13}H_7ClN_2O_3$ | C, H, N | 53 | 132.5–133.5 | *g* | $C_{13}H_{11}NO_5S$ | C, H, N |
| 16 | 117–118.5 | *f* | $C_{13}H_7ClN_2O_3$ | C, H, N | 54 | 111–112 | *g* | $C_{13}H_{11}ClO_3S$ | C, H |
| 17 | 171–172 | *d* | $C_{13}H_7BrN_2O_3$ | C, H, N | 55 | 127–128.5 | *g* | $C_{14}H_{11}NO_3S$ | C, H, N |
| 18 | 108–109 | *f* | $C_{13}H_7BrN_2O_3$ | C, H, N | 56 | 127.5–129 | *g* | $C_{13}H_{11}BrO_3S$ | C, H |
| 19 | 111–112 | *f* | $C_{14}H_{10}N_2O_4$ | C, H, N | 57 | 164–165 | *g* | $C_{20}H_{16}O_4S$ | C, H |
| 20 | 102–103.5 | *e* | $C_{14}H_{10}N_2O_4$ | C, H, N | 58 | 131–132.5 | *g* | $C_{15}H_{14}O_4S$ | C, H |
| 21 | 118–120.5 | *f* | $C_{14}H_{10}N_2O_4$ | C, H, N | 59 | 129–132 | *k* | $C_{14}H_{14}O_2S$ | C, H |
| 22 | 121.5–123 | *f* | $C_{13}H_8N_2O_3$ | C, H, N | 60 | 151.5–153 | *g* | $C_{14}H_{14}O_4S$ | C, H |
| 23 | 124–126.5 | *f* | $C_{14}H_{10}N_2O_3$ | C, H, N | 61 | 129–130 | *g* | $C_{13}H_{12}O_4S$ | C, H |
| 24 | 187.5–189 | *f* | $C_{14}H_7N_3O_3$ | C, H, N | 62 | 84–87 | *k* | $C_{13}H_{11}ClO_3S$ | C, H |
| 25 | 154–155.5 | *e* | $C_{14}H_7F_3N_2O_3$ | C, H, N | 63 | 95.5–97.5 | *k* | $C_{13}H_{11}ClO_3S$ | C, H |
| 26 | 88–89.5 | *e* | $C_{14}H_7F_3N_2O_3$ | C, H, N | 64 | 114–115 | *c* | $C_{12}H_6Cl_2N_2O$ | C, H, N |
| 27 | 113–115 | *e* | $C_{14}H_7F_3N_2O_3$ | C, H, N | 65 | 94–95 | *c* | $C_{12}H_6Cl_2N_2O$ | C, H, N |
| 28 | 145–146.5 | *f* | $C_{15}H_{12}N_2O_5$ | C, H, N | 66 | 116–117 | *c* | $C_{12}H_6Cl_2N_2O$ | C, H, N |
| 29 | 179.5–180.5 | *f* | $C_{20}H_{12}N_2O_4$ | C, H, N | 67 | 78–79 | *i* | $C_{11}H_8Cl_2N_2O$ | C, H, N |
| 30 | 135–136.5 | *e* | $C_{15}H_{10}N_2O_4$ | C, H, N | 68 | 73–74 | *c* | $C_{11}H_6BrCl_2NO$ | C, H, N |
| 31 | 124–126 | *d* | $C_{14}H_{10}N_2O_3S$ | C, H, N | 69 | 145 (0.05)[l] | *m* | $C_{12}H_9Cl_2NOS$ | C, H, N |
| 32 | 206–207 | *d* | $C_{14}H_{10}N_2O_5S$ | C, H, N | 70 | 88–90 | *m* | $C_{12}H_9Cl_2NO_2S$ | C, H, N |
| 33 | 123–125 | *f* | $C_{17}H_{10}N_2O_3$ | C, H, N | 71 | 120–121 | *m* | $C_{12}H_9Cl_2NO_3S$ | C, H, N |
| 34 | 81–82 | *n* | $C_{12}H_7Cl_2NO_3$ | C, H, N | 72 | >250 | *i* | $C_{11}H_7Cl_2NO_4S$ | C, H, N |
| 35 | 77–78 | *p* | $C_{13}H_7Cl_2NO$ | C, H, N | 73 | 95–96 | *m* | $C_{12}H_9Cl_2NO_3S$ | C, H, N |
| 36 | 124.5–125.5 | *g* | $C_{13}H_{10}Cl_2O_3S$ | C, H | 74 | 95–96 | *m* | $C_{12}H_9Cl_2NO_3S$ | C, H, N |
| 37 | 145–146 | *g* | $C_{18}H_{12}Cl_2O_3S$ | C, H | 75 | 90–91 | *m* | $C_{12}H_9Cl_2NO_3S$ | C, H, N |
| 38 | 77–78 | *g* | $C_{13}H_7Cl_2F_3O_3S$ | C, H | | | | | |

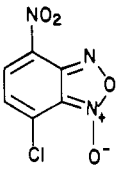
[a] Analysis was within 0.4% of the theoretical value unless otherwise noted. [b] **3.** C: calcd, 70.58; found, 71.00. **5.** C: calcd, 54.38; found, 53.97. [c] Prepared by method A. [d] U.S. Patent 4 254 144; *Chem. Abstr.* **1981**, *95*, 115090*h*. [e] U.S. Patent 4 332 820; *Chem. Abstr.* **1982**, 97, 91975*p*. [f] Prepared by method B. [g] U.S. Patent 4 349 568; *Chem. Abstr.* **1982**, *97*, 215735*g*. [h] Elsloger, E. F. et al. *J. Heterocycl. Chem.* **1972**, *9*, 759. [i] See the Experimental Section. [j] Deshpande, C. N. et al. *Chem. Abstr.* **1959**, 53, 14100*b*. [k] Prepared by method C. [l] Boiling point (torr). [m] U.S. Patent 4 371 537; *Chem. Abstr.* **1983**, *98*, 155211*s*. [n] Masson, G. W.; Brooker, E. G. *Proc. N.Z. Weed Pest Contr. Conf.* **1968**, *21*, 163; *Chem. Abstr.* **1967**, *70*, 77494*s*. [o] Norstrom, A.; Anderson, K. *Chemosphere* **1977**, 6, 237; *Chem. Abstr.* **1977**, *87*, 134231*f*. [p] Prepared by method D.

first 3 h of infection, thus ability of the viruses to uncoat was not affected. This result is in contrast to a similar study of the 4-methylsulfonyl derivative **71**, which clearly inhibited virus uncoating.[11] Thus, two apparently closely related structural analogues exhibit different mechanisms of action.

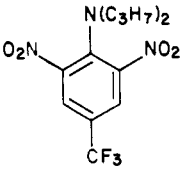
It is tempting to speculate that the activity of these electron-poor aromatic ethers may relate to their potential ability to form $\sigma$ complexes (Meisenheimer complexes)[12] with a nucleophilic region of a viral protein. A similar hypothesis has been advanced to account for the antileukemic activity of other electron-poor aromatic systems such as **76** and **77**[13] as well as the herbicidal activity of Treflan (**78**).[14] Benzofurazan **76** is a well-known fluorescent probe for cysteine.[15] Further developmental work in our laboratories has shown that **8** is cleaved to 3,4-dichlorophenol in rats and dogs, suggesting that nucleophilic aromatic substitution can occur under physiological conditions.




**76**




**77**




**78**

In summary, we have demonstrated that many diaryl ethers containing at least one electron-poor ring have in vitro antipicornavirus activity. Certain analogues are under development as potential chemotherapeutic agents because of their broad-spectrum activity, high therapeutic index in vitro, and most importantly their oral activity in our animal model.

## Experimental Section

Melting points were determined using a Thomas-Hoover capillary melting point apparatus and are uncorrected. All new compounds were characterized by proton NMR, infrared spectroscopy, and mass spectrometry and exhibited elemental analyses within ±0.4% of the theoretical value unless otherwise noted. Elemental analyses were determined by the Analytical Laboratory, The Dow Chemical Co., Midland, MI., the Analytical Chemistry

(11) Torney, H. L. unpublished results.
(12) For recent work see: Terrier, F. *Chem. Rev.* **1982**, *82*, 77. Sekiguchi, S.; Aizawa, T.; Aoki, M. *J. Org. Chem.* **1981**, *46*, 3657 and references therein. For a general discussion see: Feuer, H. "The Chemistry of the Nitro and Nitroso Groups", Part I; Wiley-Interscience: New York, 1969; p 520.
(13) Whitehouse, M. W.; Ghosh, P. B. *Biochem. Pharmacol.* **1968**, *17*, 158.
(14) Hall, R. C.; Giam, C. S. *J. Argric. Food. Chem.* **1974**, *22*, 461.
(15) Shipton, M.; Stuckbury, T.; Brocklehurst, K. *Biochem. J.* **1976**, *159*, 235.

Department of Merrell Dow, Cincinnati, OH, or the Applied Sciences and Technology Laboratories of Dow Chemical Co., Walnut Creek, CA.

**General Procedures. Method A.** To a solution of 1.0 equiv of the phenol in $Me_2SO$ (0.5 M) was added 1.05 equiv of 50% NaH, followed by 1.0 equiv of the appropriate chloropyridine. The mixture was warmed to 120 °C and when the reaction was judged complete, was poured onto crushed ice and extracted into 1,1,1-trichloroethane. The organic phase was separated, dried, and concentrated in vacuo to afford the crude product which was purified by recrystallization.

**Method B.** To a slurry of 7.5 equiv of solid anhydrous $K_2CO_3$ in $CH_3CN$ was added 1.5 equiv of the phenol, followed by 1.0 equiv of the appropriate aryl halide. Typically the mixtures were 0.25 M in aryl halide. The slurry was refluxed until TLC showed no remaining aryl halide and then was cooled, filtered, and concentrated at reduced pressure. The residue was taken up in ethyl acetate and extracted with 1 N NaOH and then with saturated NaCl, dried over $K_2CO_3$, and concentrated. The residue was purified by recrystallization.

**Method C.** To a solution of 1.6 equiv of potassium *tert*-butoxide and 1.5 equiv of the appropriate phenol in $Me_2SO$ was added a solution of 1.0 equiv of 4-fluorophenyl methyl sulfone in $Me_2SO$ to yield a mixture approximately 0.25 M in aryl halide. The mixture was heated to 120 °C until the starting aryl halide was consumed, and then the mixture was cooled and partitioned between $CH_2Cl_2$ and 1 N NaOH. The $CH_2Cl_2$ layer was washed with 1 N NaOH and then twice with saturated NaCl, dried over $K_2CO_3$, and concentrated. The residue was purified by recrystallization.

**Method D.** To a slurry of 1.1 equiv of solid NaOH in $Me_2SO$ (typically 0.2–0.4 M) was added 1.1 equiv of the phenol, followed by 1.0 equiv of the appropriate aryl halide. The reaction mixture was heated, if necessary, under $N_2$, until TLC showed no remaining starting material. The mixture was allowed to cool and poured into water, and the separated product was collected by filtration, washed free of $Me_2SO$, and vacuum dried. Recrystallization from appropriate solvent, typically ethanol, afforded the pure diaryl ether.

**Specific Procedures. 1-[4-(3,4-Dichlorophenoxy)phenyl]ethanone (40).** A mixture of 13.8 g (0.10 mol) of 4-fluoroacetophenone, 12.6 g (0.20 mol) of 3,4-dichlorophenol, 27.6 g (0.20 mol) of anhydrous $K_2CO_3$, and 150 mL of $Me_2SO$ was heated under $N_2$ at 170 °C for 1.5 h. The cooled reaction mixture was poured into dilute aqueous NaOH, and the crystalline precipitate was filtered off, washed with water, and dried. The crude product was purified by distillation (bp 174–178 °C (0.3 torr)) followed by recrystallization from hexane: yield 9.2 g (33%); mp 50–52 °C.

**4-(3,4-Dichlorophenoxy)-1,3-benzenedicarbonitrile (43).** To a slurry of 45.6 g (0.25 mol) of 2-chloro-5-nitrobenzonitrile in a mixture of 500 mL of 2-propanol and 250 mL of concentrated HCl was added 207.5 g (0.92 mol) of $SnCl_2 \cdot 2H_2O$ in small portions. The temperature rose to 72 °C. The mixture was refluxed for 1 h and then concentrated in vacuo. The resulting solid residue (amine HCl and tin salts) was washed with concentrated HCl and then water. The residue was suspended in water, and 2 N NaOH was added until the mixture was basic. The desired 2-chloro-5-aminobenzonitrile was extracted into $CH_2Cl_2$, washed with water, and dried over $Na_2SO_4$. The solvent was removed in vacuo, and the residue was recrystallized from aqueous ethanol to afford 26.1 g (68%) of pure product, mp 130–131.5 °C (lit.[16] mp 133 °C).

To a slurry of 24.3 g (0.159 mol) of 2-chloro-5-aminobenzonitrile in a mixture of 400 mL of acetic acid, 160 mL of water, and 40 mL of concentrated HCl at 0 °C was added dropwise 11.77 g (0.171 mol) of $NaNO_2$ dissolved in 80 mL of water. The yellow solution was stirred at 3–5 °C for 1 h and then added to a slurry consisting of 800 mL of $CHCl_3$, 71.5 g (0.80 mol) of CuCN, and 79.5 g (1.62 mol) of NaCN dissolved in 800 mL of water, maintained at 10 °C. The resulting mixture was stirred for 1 h at 5 °C, then 1 h at 22 °C, and then 1 h at reflux. After cooling, the organic layer was separated, filtered, washed with 1 N NaOH and water, and then dried over $Na_2SO_4$. Removal of solvent in vacuo afforded 22.7 g of 2-chloro-1,3-benzenedicarbonitrile. The crude product was purified by sublimation (130 ° (0.5 torr)) and recrystallization from ethanol to furnish 18.9 g (73% yield) of pure product, mp 158.5–160 °C (lit.[17] mp 157 °C).

The preparation of 4-(3,4-dichlorophenoxy)-1,3-benzenedicarbonitrile was completed using 4-chloro-1,3-benzenedicarbonitrile and 3,4-dichlorophenol according to method B. The yield in the final step was 69%.

**5-Amino-2-(3,4-dichlorophenoxy)benzonitrile (48).** To a slurry of 6.18 g (0.020 mol) of **8** in 60 mL of 2-propanol and 30 mL of concentrated HCl was added 16.54 g (0.0733 mol) of $SnCl_2 \cdot 2H_2O$. The mixture was refluxed for 1 h, cooled, and concentrated in vacuo. The amine hydrochloride residue was suspended in concentrated HCl, filtered, and washed with concentrated HCl and then ether. The crude product was then suspended in water and made basic with 2 N NaOH. The amine was taken up in $CH_2Cl_2$, washed with water, and dried over $Na_2SO_4$. Removal of solvent and recrystallization of the residue from aqueous ethanol yielded 4.9 g (88%) of pure **48**, mp 104–105.5 °C.

***N*-[3-Cyano-4-(3,4-dichlorophenoxy)phenyl]acetamide (49).** To a solution of 7.5 g (0.0269 mol) of **48** in 50 mL of pyridine was added dropwise 2.3 g (0.0293 mol) of acetyl chloride, and the mixture was warmed to 60 °C for 1 h. Upon cooling, the mixture was poured into dilute HCl. The crystalline precipitate was filtered off, washed with water, and dried. Recrystallization from a mixture of ethyl and isopropyl acetates gave 2.5 g (29%) of pure **49**, mp 153.5–154.5 °C.

**6-(3,4-Dichlorophenoxy)-3-pyridinamine (67).** 2-(3,4-Dichlorophenoxy)-5-nitropyridine (available from 2-chloro-5-nitropyridine and 3,4-dichlorophenol according to method A) was hydrogenated at ambient temperature in absolute ethanol in a Parr apparatus over 5% Pd/C at 50 psi. From 92.8 g of the nitropyridine was obtained 70.8 g (83%) of **67**.

**6-(3,4-Dichlorophenoxy)-5-pyridinesulfonic Acid (72).** A solution of 12.0 g (0.05 mol) of **67** in 56 mL of acetic acid was chilled to 10 °C. Concentrated HCl, 11.7 g, was added followed by 3.3 g (0.05 mol) of $NaNO_2$ in small portions, keeping the temperature at 10–15 °C. When the addition was complete, the resulting diazonium salt solution was added slowly to a solution of 37 mL of acetic acid, 17.2 g of $SO_2$, 1.85 g of $CuCl_2 \cdot 2H_2O$, and 2.2 mL of water at 5 °C. The mixture was allowed to warm to room temperature and stirred for 1.5 h until gas evolution ceased. It was then placed in a freezer overnight, and the resulting solid was collected and dried to yield 15.1 g of sulfonyl chloride.

A 5.0-g (0.015 mol) portion of the sulfonyl chloride was taken up in 30 mL of $CH_2Cl_2$ and 2 mL of pyridine was added. The solvent was removed by heating on a steam bath, and 20 g of ice was added to the residue. Concentrated $H_2SO_4$ (5 mL) was added to precipitate a solid, which was collected by filtration and washed with water. The aqueous washings were acidified to give a solid that was collected and recrystallized from ethanol to afford 2.2 g (46%) of sulfonic acid **72**.

**Cell Culture Experiments.** HeLa cells (GIBCO, Grand Island, NY) were grown and maintained at 36 °C in Corning 75-$cm^2$ tissue culture flasks (Scientific Products, McGaw Park, IL) using Eagles Minimum Essential Medium with Earles Salts (E-MEM) (GIBCO) supplemented with 1% antibiotic stock solution (PSN, GIBCO). Heat-inactivated (7–10%) fetal calf serum (HIFCS, MA Bioproducts, Walkersville, ME) was added to the medium for cell growth (growth medium) and the concentration reduced to 1–2% for cell maintenance (maintenance medium).

**Test Viruses.** Rhinovirus types 1A (RV-1A) and 2 (RV-2) were obtained from Dr. B. D. Korant (E. I. du Pont de Nemours and Co.) and propagated in HeLa cells for the preparation of stock virus. Weanling mouse-grown coxsackievirus A21, obtained from Dr. D. C. DeLong (Eli Lilly and Co.), was grown in weanling mice and then passed once in HeLa cells to prepare stock virus.

**In Vitro Assay.** HeLa cells were transferred to 24-well microtiter plates (Costar, Cambridge, MA) at a concentration of (1.0–1.3) × $10^5$ cells/well in 1.0 mL of growth medium. After 24-h growth at 36 °C in a humidified $CO_2$ (5% $CO_2$, 95% air) incubator, the cultures covered 60–75% of the dish surface. Two milligrams of each test compound or the control compound 1 were mixed

(16) Dey, B. B.; Doraiswami, Y. G. *J. Indian Chem. Soc.* **1933**, *10*, 309.

(17) Wallenfiels, K.; et al. *Tetrahedron* **1967**, *23*, 1353.

with 0.02 mL of $Me_2SO$ followed by addition of 10 mL of maintenance medium to give a stock concentration of 200 μg of compound/mL.[18] Dilutions were then prepared in the same medium to give the various test concentrations. HeLa cell cultures in the microtiter plates were drained of medium and refed with 1.0 mL of compound-containing or compound-free maintenance medium. Appropriate monolayers were then challenged with 0.05–0.1 mL (10–100 $TCID_{50}$) virus while cytotoxicity and control cultures remained free of virus. Cell cultures were incubated at 36 °C in the humidified $CO_2$ incubator and examined microscopically at 48 and 72 h after challenge for compound cytotoxicity and viral cytopathic effect (CPE). Viral CPE was graded as a percent of the cell monolayer destroyed by the virus (percent CPE). The lowest concentration of compound reducing viral CPE by 50% or more compared to virus control was considered the minimum inhibitory concentration ($MIC_{50}$) of that compound. Cell toxicity was graded according to the degree of host cell death and/or a reduction in the growth or change in morphology of treated nonchallenged cultures compared to control cultures.

**Mouse Feed Test for in Vivo Activity.** Mouse feed containing compound was prepared by dissolving 150 mg of test compound in 10–15 mL of acetone and then adsorbing the acetone solution onto silica gel (Hi-SiL-233, PPG Industries, Pittsburgh, PA). The resulting silica gel containing compound was then mixed with granular mouse food (Wayne Pet Food Division, Continental Grain Co., Chicago, IL) on an automatic roller for 1 h to give a final concentration of 150 mg of compound/250 g of feed. This resulted in a feeding dose of about 75 mg/kg per day.[7] Control feed contained quantities of acetone/silica gel comparable to the test feeds. Cox/Swiss albino male mice (9-12 g; 19-day old) (Harlen Sprague–Dawley, Inc., Indianapolis, IN) were used in all in vivo assays. Animals were supplied ad libitum with compound-containing or control feed starting 24 h before virus challenge and continuing for the duration of the test. Test animals were challenged with an intraperitoneal injection of phosphate-buffered saline, pH 7.1–7.3 (GIBCO) supplemented with 1% antibiotic stock solution (PSN, GIBCO) and containing sufficient infectious virus to cause 85–100% mortality mice within 10 days of challenge. Animals were observed daily and deaths recorded.

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**Registry No.** **2**, 99902-70-2; **3**, 99902-71-3; **4**, 99902-72-4; **5**, 99902-73-5; **6**, 99902-74-6; **7**, 99902-75-7; **8**, 78940-62-2; **9**, 78940-63-3; **10**, 82674-08-6; **11**, 82674-07-5; **12**, 82674-05-3; **13**, 82674-06-4; **14**, 78940-65-5; **15**, 99902-76-8; **16**, 99902-77-9; **17**, 78940-64-4; **18**, 99902-78-0; **19**, 99902-79-1; **20**, 82673-96-9; **21**, 99902-80-4; **22**, 63707-35-7; **23**, 99902-81-5; **24**, 99902-82-6; **25**, 82673-97-0; **26**, 82674-10-0; **27**, 82674-09-7; **28**, 99902-83-7; **29**, 78940-72-4; **30**, 82673-98-1; **31**, 78940-67-7; **32**, 78940-69-9; **33**, 99902-84-8; **34**, 22532-80-5; **35**, 99922-95-9; **36**, 83642-20-0; **37**, 83642-29-9; **38**, 83642-43-7; **39**, 63646-51-5; **40**, 99922-96-0; **41**, 99902-85-9; **42**, 22532-87-2; **43**, 99902-86-0; **44**, 99585-43-0; **45**, 83642-35-7; **46**, 38710-80-4; **47**, 83649-25-6; **48**, 99902-87-1; **49**, 99902-88-2; **50**, 99902-89-3; **51**, 83642-36-8; **52**, 83642-38-0; **53**, 36089-89-1; **54**, 83642-21-1; **55**, 83642-27-7; **56**, 83642-28-8; **57**, 83642-23-3; **58**, 83642-22-2; **59**, 99902-90-6; **60**, 83642-37-9; **61**, 80622-22-6; **62**, 99902-91-7; **63**, 99902-92-8; **64**, 99902-93-9; **65**, 99902-94-0; **66**, 99902-95-1; **67**, 25935-30-2; **68**, 99902-96-2; **69**, 85330-94-5; **70**, 85330-95-6; **71**, 99902-97-3; **72**, 99902-98-4; **73**, 85331-25-5; **74**, 85331-19-7; **75**, 85331-21-1; *p*-$ClC_6H_4NO_2$, 100-00-5; *p*-$ClC_6H_4Cl$, 623-03-0; *p*-$MeSO_2C_6H_4Cl$, 98-57-7; *p*-$PhSO_2C_6H_4Cl$, 80-00-2; *p*-$CF_3SO_2C_6H_4Cl$, 383-11-9; *p*-$Cl_2C_6H_4$, 106-46-7; *p*-$AcC_6H_4Cl$, 99-91-2; *o*-$ClC_6H_4CN$, 873-32-5; *p*-$ClC_6H_4OH$, 106-48-9; *p*-$AcC_6H_4OH$, 99-93-4; PhOH, 108-95-2; *p*-$FC_6H_4OH$, 371-41-5; *p*-$MeC_6H_4OH$, 106-44-5; *m*-$ClC_6H_4OH$, 108-43-0; *o*-$ClC_6H_4OH$, 95-57-8; *p*-$BrC_6H_4OH$, 106-41-2; *o*-$BrC_6H_4OH$, 95-56-7; *p*-$MeOC_6H_4OH$, 150-76-5; *m*-$MeOC_6H_4OH$, 150-19-6; *o*-$MeOC_6H_4OH$, 90-05-1; *p*-$HOC_6H_4CN$, 767-00-0; *p*-$HOC_6H_4CF_3$, 402-45-9; *m*-$F_3CC_6H_4OH$, 98-17-9; *o*-$F_3CC_6H_4OH$, 444-30-4; *p*-$HOC_6H_4COPh$, 1137-42-4; *p*-$ACC_6H_4OH$, 99-93-4; *p*-$HOC_6H_4SMe$, 1073-72-9; *p*-$HOC_6H_4SO_2Me$, 14763-60-1; *p*-$HOC_6H_4NO_2$, 100-02-7; *p*-$HOC_6H_4OH$, 123-31-9; *p*-$FC_6H_4SO_2Me$, 455-15-2; *p*-$AcC_6H_4F$, 403-42-9; 2-chloro-5-cyanopyridine, 33252-28-7; 2-chloro-5-nitrobenzonitrile, 16588-02-6; 1-chloro-2,4-dinitrobenzene, 97-00-7; 1-chloro-2,4-dicyanobenzene, 4387-30-8; 1-chloro-2-carboxy-4-nitrobenzene, 2516-96-3; 1-chloro-2-nitro-4-(methylsulfonyl)-benzene, 97-07-4; 1-chloro-3-cyano-4-nitrobenzene, 34662-31-2; 1-chloro-2-cyano-4-(methylsulfonyl)benzene, 99902-99-5; 1-chloro-2-cyano-4-aminobenzene, 35747-58-1; 1-chloro-2-cyano-4-acetamidobenzene, 53312-85-9; 2,5-dichlorobenzonitrile, 21663-61-6; 2-chloro-5-(methylsulfonyl)aniline, 16328-56-6; 5-chloro-2-(methylsulfonyl)toluene, 10200-36-9; 2-chloro-3-cyanopyridine, 6602-54-6; 2-chloro-4-cyanopyridine, 33252-30-1; 2-chloro-6-cyanopyridine, 33252-29-8; 2-chloro-5-aminopyridine, 5350-93-6; 2-chloro-5-bromopyridine, 53939-30-3; 2-chloro-5-(methylthio)-pyridine, 41288-94-2; 2-chloro-5-(methylsulfinyl)pyridine, 99903-00-1; 2-chloro-5-(methylsulfonyl)pyridine, 99903-01-2; 2-chloro-5-pyridinesulfinic acid, 99903-02-3; 2-chloro-3-(methylsulfonyl)pyridine, 70682-09-6; 2-chloro-4-(methylsulfonyl)pyridine, 99903-03-4; 2-chloro-6-(methylsulfonyl)pyridine, 87512-29-6; 3,4-dichlorophenol, 95-77-2; 2,4-dichlorophenol, 120-83-2; 3,5-dichlorophenol, 591-35-5; 2,6-dichlorophenol, 87-65-0; 2,3-dichlorophenol, 576-24-9; 2,5-dichlorophenol, 583-78-8; 3,4-dimethoxyphenol, 2033-89-8; α-naphthol, 90-15-3; 2-chloro-5-aminobenzonitrile, 35747-58-1; 2-chloro-1,3-benzenedicarbonitrile, 28442-78-6; 2-(3,4-dichlorophenoxy)-5-nitropyridine, 25935-29-9.

(18) While $Me_2SO$ was not included in control wells, final $Me_2SO$ concentrations in test wells never exceeded 0.1% of total volume. Earlier tests (data not shown) indicated that no deleterious effects on host cells or viruses would occur at this level.

# *Book Reviews*

**Multidimensional Pharmacochemistry—Design of Safer Drugs.** By Peter P. Mager. Academic Press, New York. 1984. xiv + 418 pp. $89.00.

Unlike the now familiar multiple regression approach to QSAR, multidimensional pharmacochemistry attempts to simultaneously correlate all of the diverse biological effects of a given series of drugs with their associated physical properties. It should thus be possible not only to allow for the interactions between biological properties (metabolism, transport, binding, etc.) but also to design compounds with an ideal combination of high activity, low toxicity, and optimum pharmacokinetic properties.

Mager has published over 100 papers in this field, particularly as it relates to biorhythmic and other time- and dose-dependent phenomena. In the course of that work he and his colleagues have developed a unified series of programs devoted to the multivariate analysis of drug action, which Mager refers to as the "MASCA model of pharmacochemistry". The main components of the MASCA model are a range of statistical programs for the analysis of multivariate bioassays, together with multivariate QSAR analysis of these data in terms of their associated physicochemical properties. The description of these components makes up the bulk of the book, with the remainder being devoted to two short chapters describing Mager's personal and sometimes unorthodox views on the subject of physicochemical parameters and biochemical/pharmacological design.

The main value of the book will be to provide Mager's fellow scientists in the west with an overview of the work going on in his laboratory. There is little discussion of the relative merits