

Antiviral Agents. 5*H*-as-Triazino[5,6-*b*]indoles

Jan M. Z. Gladych, Roy Hornby, John H. Hunt, David Jack,  
Research Division, Allen and Hanburys Ltd., Ware, Hertfordshire, England

John J. Boyle, Robert J. Ferlauto, Richard F. Haff,\* Charles G. Kormendy, Frederick J. Stanfield,  
and Richard C. Stewart

Research and Development Division, Smith Kline and French Laboratories, Philadelphia, Pennsylvania 19101.  
Received November 10, 1970

Sixty-four substituted 5*H*-as-triazino[5,6-*b*]indoles (II) have been synthesized by cyclization of isatin-3-thiosemicarbazones (I) followed by alkylation or displacement reactions. Many of these compds are active *in vitro* against a variety of viruses including several strains of rhinovirus. Attempts to establish structure-activity relationships are reported and the most promising compds against rhinovirus infections appear to be those with hydroxyalkylamino substituents in the C-3 position.

The reported antiviral activity of *as*-triazines<sup>1</sup> and well-documented clinical efficacy of methisazone<sup>2</sup> (I, R = Me) prompted us to prepare a series of 5*H*-as-triazino[5,6-*b*]indoles (II and III) and to evaluate these as antiviral agents.

Although the action of isatin-3-thiosemicarbazone (I, R = H) is limited mainly to poxviruses<sup>3</sup> methisazone shows a wider activity.<sup>3-5</sup> The antiviral activity of two triazinoindoles (23 and 61) has been reported.<sup>5</sup>

**Chemistry.** N-Substituted isatins were prepared by published methods<sup>6</sup> and their 3-thiosemicarbazones (I) were cyclized<sup>7</sup> to the 5*H*-as-triazino[5,6-*b*]indole-3-thiones (II) (Table I) in aqueous K<sub>2</sub>CO<sub>3</sub>. Steric factors markedly affected the rate of cyclization. Thus, when R = C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub> the reaction required 6 days at reflux, but when R = C<sub>6</sub>H<sub>5</sub>CH<sub>2</sub>CH<sub>2</sub> it was complete in 10 hr, and with R = Me in 7 hr.

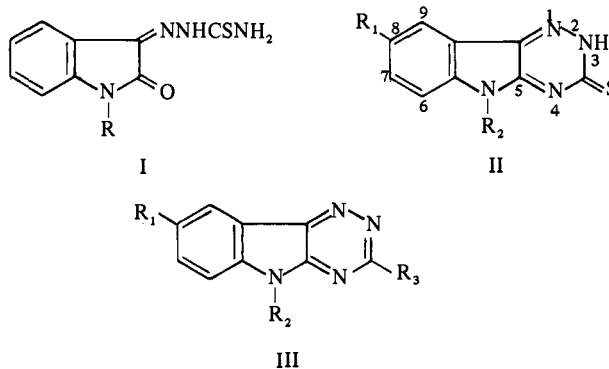
The thiones were alkylated on the S atom in aq alkali to give the alkylthio deriv (III, R<sub>3</sub> = S-alkyl) (Table II). Where R<sub>2</sub> = H further alkylation at N-5 could be effected with an alkyl halide and NaH in DMF. The latter procedure is particularly useful for the synthesis of compds of type III where a bulky R<sub>2</sub> group hinders cyclization of the thiosemicarbazone.

The thiones (II) or the thioethers (III, R = SMe) with amines gave the 3-amino compds listed in Table III.

**Virology. Plaque Inhibition Test.**<sup>9</sup> Compds were tested for their ability to inhibit focal areas of cell necrosis (plaques) produced by viral infection in monolayer cell cultures contained in petri dishes. The infected cultures, overlaid with a solid agar maintenance medium, were incubated with superimposed cellulose disks impregnated with 100 μg of test compd. The compd, diffusing into the medium, provided a concentration gradient so that antiviral activity could be expressed as a plaque-free zone in an area of healthy cells surrounding the disk or zone of compd toxicity.

The following viruses and cell substrates were employed in the primary screen: influenza A/WSN—primary cultures of chick embryo cells (CE); influenza A<sub>2</sub>/Jpt—CE; parainfluenza 1/Sendai—CE; respiratory syncytial/Long—WI-26 or WI-38 (human diploid); rhinovirus strains 1059 or HGP—WI-26 or WI-38; and vaccinia/WH—CE. The results are shown in Table IV.

In an extended screen, selected compds were tested against the following additional viruses: Coxsackie B-1/Conn. 5—HEp-2; Coxsackie B-3/Nancy—HEp-2; feline rhinotracheitis/C-27—primary feline kidney cells; herpes simplex/HF—CE; herpes zoster/EY—WI-26 or WI-38; influenza A/NWS—CE; Newcastle disease/Roakin—CE; Newcastle disease/Victoria



—CE; polio I/Brunhilde—HeLa; polio II/Lansing—HeLa; Polio III/Leon—HeLa; pseudorabies/Aujeszký—CE; Semliki Forest/original—CE; vesicular stomatitis/Indiana—CE. Results are shown in Table V.

**Tube Dilution Test.** Compds were tested for their ability to inhibit cell necrosis produced by rhinovirus infection in human diploid cells, strain WI-26 or WI-38, cultivated on the walls of culture tubes in liquid medium.<sup>5</sup> The following rhinovirus strains were used: 1059, HGP, 2060, and 33342.

The well-tolerated dose (WTD) is the highest concentration of test compd which produced no evidence of cytopathology in uninfected cultures. The minimal effective concn (MEC) is the lowest dose which provided 50% or less destruction than in control cultures. The ratio of the WTD to MEC is considered the therapeutic index (TI). All values are the geometric mean of at least two determinations. Concentrations refer to added amounts of test compds and are

Table I. 5*H*-as-Triazino[5,6-*b*]indole-3(2*H*)-thiones

Compd	R <sub>1</sub>	R <sub>2</sub>	Mp, °C	Formula	Anal.
1	H	H	>300	C <sub>9</sub> H <sub>6</sub> N <sub>4</sub> S	C, H, S; N <sup>a</sup>
2	H	Me	279–281	C <sub>10</sub> H <sub>9</sub> N <sub>4</sub> S	C, H, N, S
3	H	Et	294	C <sub>11</sub> H <sub>10</sub> N <sub>4</sub> S	C, S; H, N <sup>b</sup>
4	H	Pr	278	C <sub>12</sub> H <sub>12</sub> N <sub>4</sub> S	C, H, N, S
5	Cl	Me	297	C <sub>10</sub> H <sub>7</sub> ClN <sub>4</sub> S	C, H, N, S
6	Cl	Pr	270–275	C <sub>12</sub> H <sub>11</sub> ClN <sub>4</sub> S	C, H, Cl, N, S
7	NO <sub>2</sub>	Me	283–284	C <sub>10</sub> H <sub>7</sub> N <sub>5</sub> O <sub>2</sub> S	C, H, N, S
8	H	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	269–271	C <sub>16</sub> H <sub>12</sub> N <sub>4</sub> S	C, H, N, S
9	H	C <sub>6</sub> H <sub>5</sub> (CH <sub>2</sub> ) <sub>2</sub>	245–246	C <sub>17</sub> H <sub>14</sub> N <sub>4</sub> S	C, H, S; N <sup>c</sup>

<sup>a</sup>N: calcd, 27.70; found, 27.70. <sup>b</sup>H: calcd, 4.40; found, 4.35.  
N: calcd, 24.30; found, 24.15. <sup>c</sup>N: calcd, 18.30; found, 17.75.

Table II. 5-Alkylthio-5*H*-as-triazino[5,6-*b*]indoles

Compd	R <sub>1</sub>	R <sub>2</sub>	Mp, °C	Formula	Anal.
10	Me	Me	185	C <sub>11</sub> H <sub>10</sub> N <sub>4</sub> S	H, N; C, S <sup>a</sup>
11	Me	CH <sub>2</sub> =CHCH <sub>2</sub>	141-143	C <sub>13</sub> H <sub>12</sub> N <sub>4</sub> S	C, H, N, S
12	Me	<i>N</i> -Morpholinylethyl	145-146	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> OS	C, H, N, S
13	H	Et	303-304	C <sub>11</sub> H <sub>10</sub> N <sub>4</sub> S	C, H, N, S
14	Pr	Me	129-130	C <sub>13</sub> H <sub>14</sub> N <sub>4</sub> S	C, H, N, S
15	Me	Et <sub>2</sub> N(CH <sub>2</sub> ) <sub>2</sub>	101-103	C <sub>16</sub> H <sub>21</sub> N <sub>5</sub> S	C, H, N, S
16	Me	C <sub>6</sub> H <sub>3</sub> -2,4-(NO <sub>2</sub> ) <sub>2</sub>	209-211	C <sub>16</sub> H <sub>10</sub> N <sub>6</sub> O <sub>4</sub> S	C, H, N, S
17	Me	Me <sub>2</sub> N(CH <sub>2</sub> ) <sub>2</sub>	128-130	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> S	C, H, N, S
18	Me	<i>N</i> -Morpholinylpropyl	101-102	C <sub>17</sub> H <sub>21</sub> N <sub>5</sub> OS	C, H, N, S
19	Me	C <sub>5</sub> H <sub>10</sub> N(CH <sub>2</sub> ) <sub>2</sub>	144	C <sub>17</sub> H <sub>21</sub> N <sub>5</sub> S	C, N, S; H <sup>b</sup>

<sup>a</sup>C: calcd, 57.35; found, 57.80. S: calcd, 13.90; found, 13.30. <sup>b</sup>H: calcd, 6.42; found, 6.89.

Table III. 3-Amino-5*H*-as-triazino[5,6-*b*]indoles

Compd	R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	Mp, °C	Formula	Anal.
20	H	H	HO(CH <sub>2</sub> ) <sub>2</sub> NH	270-271	C <sub>11</sub> H <sub>11</sub> N <sub>5</sub> O	C, H; N <sup>a</sup>
21	H	H	HO(CH <sub>2</sub> ) <sub>3</sub> NH	248-249	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub> O	C, H, N
22	H	Me	HO(CH <sub>2</sub> ) <sub>2</sub> NH	235-236	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub> O	C, H, N
23	H	Me	HO(CH <sub>2</sub> ) <sub>3</sub> NH	164-165	C <sub>13</sub> H <sub>15</sub> N <sub>5</sub> O	C, H, N
24	H	H	NH <sub>2</sub> <sup>b</sup>	>360	C <sub>9</sub> H <sub>7</sub> N <sub>5</sub>	C, H, N
25	NO	H	NH <sub>2</sub> <sup>c</sup>	>350	C <sub>9</sub> H <sub>6</sub> N <sub>6</sub> O <sub>2</sub>	C, H, N
26	H	Me	NH <sub>2</sub> <sup>b</sup>	335	C <sub>10</sub> H <sub>9</sub> N <sub>5</sub>	C, H, N
27	NO	Me	NH <sub>2</sub> <sup>d</sup>	>300	C <sub>10</sub> H <sub>8</sub> N <sub>6</sub> O <sub>2</sub>	C, H, N
28	MeO	H	NH <sub>2</sub> <sup>e</sup>	330	C <sub>10</sub> H <sub>9</sub> N <sub>5</sub> O	C, H, N
29	H	Me	Me <sub>2</sub> N-	170-173	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub>	C, H, N
30	H	Me	EtNH-	214	C <sub>12</sub> H <sub>13</sub> N <sub>5</sub>	C, H, N
31	H	Me	Morpholinyl	169-170	C <sub>14</sub> H <sub>15</sub> N <sub>5</sub> O	C, H, N
32	H	Me	Me <sub>2</sub> CH(CH <sub>2</sub> ) <sub>2</sub> NH	187-189	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub>	C, H, N
33	H	Me	Me(CH <sub>2</sub> ) <sub>11</sub> NH	119-121	C <sub>22</sub> H <sub>33</sub> N <sub>5</sub>	C, H, N
34	H	Me	CH <sub>2</sub> (CH <sub>2</sub> ) <sub>1</sub> NH	104-105	C <sub>28</sub> H <sub>45</sub> N <sub>5</sub>	C, H, N
35	Cl	Me	HO(CH <sub>2</sub> ) <sub>3</sub> NH	203-204	C <sub>13</sub> H <sub>14</sub> ClN <sub>5</sub> O	C, H, N
36	H	Me	HOCH <sub>2</sub> CH(OH)CH <sub>2</sub> NH <sup>f</sup>	194-195	C <sub>13</sub> H <sub>15</sub> N <sub>5</sub> O <sub>2</sub>	H, N; C <sup>g</sup>
37	H	Me	HO(CH <sub>2</sub> ) <sub>4</sub> NH	146-147	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O	C, H, N
38	H	Me	MeO(CH <sub>2</sub> ) <sub>3</sub> NH	141	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O	C, H; N <sup>h</sup>
39	H	Me	HO(CH <sub>2</sub> ) <sub>2</sub> NH(CH <sub>2</sub> ) <sub>2</sub> NH	174-175	C <sub>14</sub> H <sub>18</sub> N <sub>6</sub> O	C, H, N
40	H	Me	Me <sub>2</sub> N(CH <sub>2</sub> ) <sub>1</sub> NH	154	C <sub>15</sub> H <sub>20</sub> N <sub>6</sub>	C, H; N <sup>i</sup>
41	H	Me	HO(CH <sub>2</sub> ) <sub>6</sub> NH	124-125	C <sub>16</sub> H <sub>21</sub> N <sub>5</sub> O	C, H, N
42	H	Me	2-Pyridyl-(CH <sub>2</sub> ) <sub>2</sub> NH	214	C <sub>17</sub> H <sub>16</sub> N <sub>6</sub>	C, H, N
43	H	Me	H <sub>2</sub> NNH	212-214	C <sub>10</sub> H <sub>10</sub> N <sub>6</sub>	C, H, N
44	H	Me	<i>N</i> -Morpholinyl-(CH <sub>2</sub> ) <sub>3</sub> NH	183-184	C <sub>17</sub> H <sub>22</sub> N <sub>6</sub> O	H, N; C <sup>j</sup>
45	H	Me	CH <sub>3</sub> N <sub>2</sub> C <sub>4</sub> H <sub>8</sub> <sup>k</sup>	174-175	C <sub>15</sub> H <sub>25</sub> N <sub>7</sub>	C, H, N
46	H	Me	MeCOO(CH <sub>2</sub> ) <sub>3</sub> NH	186	C <sub>15</sub> H <sub>17</sub> N <sub>5</sub> O <sub>2</sub>	C, H, N
47	H	Et	HO(CH <sub>2</sub> ) <sub>3</sub> NH	151-152	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O	N; C, H <sup>l</sup>
48	H	Me	HO(CH <sub>2</sub> ) <sub>2</sub> N <sub>2</sub> C <sub>4</sub> H <sub>9</sub> <sup>m</sup>	156-157	C <sub>16</sub> H <sub>20</sub> N <sub>6</sub> O	C, H, N
49	H	Me	MeCH <sub>2</sub> CH(CH <sub>2</sub> OH)NH	176	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O	C, H, N
50	H	Pr	HO(CH <sub>2</sub> ) <sub>3</sub> NH	142-143	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O	C, H, N
51	H	Me	EtCOO(CH <sub>2</sub> ) <sub>3</sub> NH	158-160	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O <sub>2</sub>	C, H, N
52	H	Me	MeS(CH <sub>2</sub> ) <sub>3</sub> NH	150-151	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> S	C, H, N, S
53	H	Me	HO(CH <sub>2</sub> ) <sub>5</sub> NH	158	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O	C, H, N
54	H	Me	HO <sub>2</sub> C(CH <sub>2</sub> ) <sub>2</sub> COO(CH <sub>2</sub> ) <sub>3</sub> NH	186-187	C <sub>17</sub> H <sub>19</sub> N <sub>5</sub> O <sub>4</sub>	C, H, N
55	H	C <sub>6</sub> H <sub>5</sub> (CH <sub>2</sub> ) <sub>2</sub>	HO(CH <sub>2</sub> ) <sub>3</sub> NH	154-155	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O	C, H, N
56	H	Me	HOCH <sub>2</sub> C(C <sub>6</sub> H <sub>5</sub> )(Me)CH <sub>2</sub> NH	187-188	C <sub>20</sub> H <sub>21</sub> N <sub>5</sub> O	C, H, N
57	H	Me	HOCH <sub>2</sub> C(Me <sub>2</sub> )CH <sub>2</sub> NH	228	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O	H, N; C <sup>n</sup>
58	H	Me	H <sub>2</sub> N(CH <sub>2</sub> ) <sub>3</sub> NH	155-156	C <sub>13</sub> H <sub>16</sub> N <sub>6</sub>	C, H, N
59	H	Me	MeCONH(CH <sub>2</sub> ) <sub>3</sub> NH	192-193	C <sub>15</sub> H <sub>18</sub> N <sub>6</sub> O	C, H, N
60	H	Me	HOCH(Me)(CH <sub>2</sub> ) <sub>2</sub> NH	169	C <sub>14</sub> H <sub>17</sub> N <sub>5</sub> O	H, N; C <sup>o</sup>
61	H	Me	HOC(Me <sub>2</sub> )(CH <sub>2</sub> ) <sub>2</sub> NH	194	C <sub>15</sub> H <sub>19</sub> N <sub>5</sub> O	C, H, N
62	H	Me	HOC <sub>6</sub> H <sub>4</sub> NH- <i>p</i>	184-185 <sup>p</sup>	C <sub>16</sub> H <sub>19</sub> N <sub>5</sub> O	C, H, N
63	H	C <sub>6</sub> H <sub>5</sub> CH <sub>2</sub>	HO(CH <sub>2</sub> ) <sub>3</sub> NH	186-187	C <sub>19</sub> H <sub>19</sub> N <sub>5</sub> O	C, H, N

<sup>a</sup>N: calcd, 30.55; found, 30.65. <sup>b</sup>Reference 8. <sup>c</sup>By nitration of 24. <sup>d</sup>By nitration of 26. <sup>e</sup>By cyclization of 5-methoxyisatin, guanil-hydrazone by the method in ref 8. <sup>f</sup>Anal. calcd to include 0.5 mole of EtOH. <sup>g</sup>C: calcd, 56.75; found, 56.20. <sup>h</sup>N: calcd, 26.25; found, 25.80. <sup>i</sup>N: calcd, 29.55; found, 30.10. <sup>j</sup>C: calcd, 62.55; found, 62.00. <sup>k</sup>CH<sub>3</sub>N<sub>2</sub>C<sub>4</sub>H<sub>8</sub> is 4-methyl-1,4-diazacyclohexyl. <sup>l</sup>C: calcd, 61.95; found, 62.55. H: calcd, 6.30; found, 6.75. <sup>m</sup>N<sub>2</sub>C<sub>4</sub>H<sub>9</sub> is 1,4-diazacyclohexane-1,4-diyl. <sup>n</sup>C: calcd, 63.15; found, 63.60. <sup>o</sup>C: calcd, 61.95; found, 61.35. <sup>p</sup>Hydrogen maleate.

Table IV. Antiviral Activity (Plaque Inhibition Tests)<sup>c</sup>

Compd <sup>a</sup>	Viruses						
	Rhinoviruses		Vaccinia	Influenza A	Influenza A <sub>2</sub>	Parainfluenza 1 <sup>b</sup>	Respiratory syncytial
1059	HGP						
2	+	+	+	ND	-	ND	-
12	+	+	+	+	ND	+	-
14	+	ND	-	-	ND	ND	ND
15	+	ND	-	-	-	-	-
17	-	ND	+	-	ND	ND	ND
18	-	ND	ND	-	+	+	-
23	+	+	+	+	-	-	-
26	-	ND	+	-	+	ND	-
35	-	ND	-	+	ND	-	-
36	-	ND	+	-	ND	ND	-
37	+	+	+	+	-	ND	-
38	+	+	+	-	+	-	-
41	+	+	+	+	-	+	-
47	+	+	+	+	+	-	-
48	+	ND	+	+	+	-	-
49	+	+	+	+	-	-	-
50	ND	ND	+	-	-	-	-
53	+	ND	+	-	+	-	-
54	-	+	+	-	+	-	-
55	+	+	+	+	-	ND	-
56	+	ND	ND	-	+	-	-
57	+	ND	ND	-	ND	ND	-
59	+	-	-	-	ND	ND	-
61	+	ND	ND	ND	ND	ND	-
62	-	-	+	+	ND	-	-
63	+	ND	+	-	-	ND	-

<sup>a</sup>Compds not shown were inactive against rhinovirus 1059, vaccinia, and influenza A. Except where indicated in footnote *b* they were not tested against other viruses. <sup>b</sup>Compds 1, 3, 4, 5, 9, and 10 were inactive. <sup>c</sup>+ = Active, - = inactive, ND = not done.

Table V. Spectrum of Activity (Plaque Inhibition Tests)<sup>a</sup>

Virus challenge	Compd			
	2	37	41	55
Coxsackie B-1	-	+	+	-
Coxsackie B-3	+	ND	+	-
Feline Rhinotracheitis	ND	ND	-	-
Herpes Simplex	+	+	+	ND
Herpes Zoster	ND	-	ND	ND
Newcastle Disease/Roakin	-	+	+	ND
Newcastle Disease/Victoria	-	-	-	ND
Poliovirus I	-	+	-	-
Poliovirus II	-	ND	ND	-
Poliovirus III	-	ND	-	-
Pseudorabies	+	+	+	ND
Semliki Forest	-	+	-	ND
Vesicular Stomatitis	-	-	-	ND

<sup>a</sup>- = Inactive, + = active, ND = not done.

not necessarily amounts in soln. At the higher concentrations employed many compds were only partially soluble. Results are shown in Table VI.

**Structure-Activity Correlations.** The structural requisites for maximum activity against rhinoviruses are difficult to define, particularly since results of the plaque inhibition (PI) and tube-dilution (TD) assays did not always coincide. Thus, it is difficult to explain, for example, why, considering the thiones (II) (1-9), the *N*-Me (2) and *N*-Pr (4) compds are active in the TD test when the *N*-Et compd (3) is not, and why of these only 2 is active in the PI test. Again, in the 3-alkylthio compds (10-19) the *N*-Me compd (10) is inactive whereas the *N*-Pr deriv (14) is positive in both PI and TD tests.

The most potent of these triazino[5,6-*b*]indoles with an S-containing substituent at C-3 is the morpholinoethyl compd (12) but the corresponding diethylaminoethyl derivative (15) is active only in the PI test.

In the face of such inconsistencies in which presumably physical factors such as diffusability, solubility, and particle

size play a key role it appears fruitless to attempt to rationalize these results on purely chemical considerations. Similar problems appear to apply to compounds with substituents containing N atoms attached directly to the 3 position of the triazino[5,6-*b*]indole nucleus and only fairly tentative generalizations can be made. Thus, on the whole, simple primary, secondary, and tertiary alkylamines are relatively poorly active (33 is an exception but only in the TD test). Potency is enhanced when an OH group is present in the alkyl chain of a secondary amine at least 3 C atoms away from the N atom (*e.g.*, 23, 36, 37, 41, 55, 60, 61).

A substituent other than H on N-5 is essential but its size is not critical (47, 50, 55, 53). A Cl substituent at C-8 seems undesirable (35). The hydroxyalkylamine chain at C-3 may be branched (56, 57, 60, 61, 62) and the alcohol etherified (38) without loss of activity, although Ac (46), propionyl (51), and succinyl (54) esters were virtually inactive.

Replacement of the OH group by methylthio (52) abolished activity in the PI test but had relatively little effect on the TD result. The corresponding primary amine (58) was active only near toxic dose levels but the acetamido compd (59) was just as active but possessed a better therapeutic index.

## Conclusions

*In vitro* testing of the 5*H*-triazino[5,6-*b*]indoles listed above shows a broad spectrum of antiviral activity against both DNA and RNA viruses. A notable feature of the series is the widespread action against several strains of rhinovirus which is most consistently shown by compds containing hydroxyalkylamino substituents at C-3. Some of these compds including 23, 55, and 61 have been evaluated against other human rhinovirus serotypes *in vitro* and against rhinovirus infections in animals and in man. These results with 23 and 61 in part have been reported.<sup>5b,c</sup>

Table VI. Antirhinovirus Activity (Tube Dilution Tests)

Compd	Rhinoviruses								
	1059			HGP		2060		33342	
	WTD, <sup>a</sup> μg/ml	MEC, <sup>b</sup> μg/ml	TI <sup>c</sup>	MEC, μg/ml	TI	MEC, μg/ml	TI	MEC, μg/ml	TI
Methisazone	40	11	3.6	22	1.8	— <sup>d</sup>	—	21	1.9
1	100	—	—	—	—	ND <sup>d</sup>	—	ND	—
2	100	20	5	45	2.2	—	—	0.16	625
3	100	—	—	—	—	ND	—	ND	—
4	100	20	5	—	—	ND	—	ND	—
5	500	20	25	—	—	ND	—	—	—
6	ND	—	—	ND	—	ND	—	ND	—
7	ND	—	—	ND	—	ND	—	ND	—
8	100	—	—	—	—	ND	—	ND	—
9	100	10	10	—	—	ND	—	ND	—
10	100	—	—	—	—	ND	—	ND	—
11	100	—	—	—	—	ND	—	ND	—
12	100	4	25	—	—	20	5	0.16	625
13	100	—	—	—	—	ND	—	—	—
14	100	20	5	20	5	—	—	4	25
16	100	—	—	—	—	ND	—	ND	—
19	20	—	—	—	—	—	—	—	—
20	100	—	—	—	—	ND	—	ND	—
22	20	—	—	—	—	ND	—	ND	—
23	159	50	3.2	63	2.5	50	3.2	13	12.2
24	20	—	—	—	—	ND	—	ND	—
25	380	220	1.7	380	1.0	380	1.0	—	—
27	100	—	—	—	—	ND	—	ND	—
28	500	100	5.0	—	—	—	—	—	—
29	500	100	5.0	45	11.1	—	—	45	11.1
30	100	—	—	—	—	ND	—	ND	—
31	300	100	3.0	220	1.4	—	—	100	3.0
32	20	—	—	—	—	ND	—	ND	—
33	500	4	125	—	—	—	—	ND	—
34	100	—	—	—	—	ND	—	ND	—
35	380	220	1.7	100	3.8	—	—	45	8.4
36	170	20	8.5	100	1.7	—	—	170	1.0
37	220	20	11.0	10	22	45	4.9	20	11.0
38	500	20	25.0	220	2.3	—	—	100	5.0
39	100	—	—	—	—	—	—	—	—
40	20	20	1.0	—	—	—	—	4	5.0
41	150	20	7.5	20	7.5	100	1.5	6	25
42	500	220	2.3	—	—	—	—	—	—
43	100	—	—	—	—	ND	—	ND	—
44	100	—	—	—	—	ND	—	ND	—
45	100	—	—	—	—	ND	—	ND	—
46	380	220	1.7	220	1.7	—	—	20	19.0
47	100	45	2.2	100	1.0	—	—	20	5
48	20	—	—	—	—	ND	—	ND	—
49	390	220	1.8	100	3.9	—	—	4	98
50	45	20	2.2	—	—	ND	—	ND	—
51	330	220	1.5	330	1.0	—	—	ND	—
52	500	45	11.1	—	—	ND	—	ND	—
53	330	45	7.3	45	7.3	ND	—	ND	—
54	500	—	—	100	5.0	—	—	ND	—
55	180	4	45	45	4.0	180	1.0	4	45
56	220	45	4.9	220	1.0	ND	—	ND	—
57	500	20	25	4	125	220	2.3	ND	—
58	20	10	2.0	10	2.0	—	—	ND	—
59	150	10	15.0	100	1.5	—	—	—	—
60	500	45	11.1	45	11.1	—	—	—	—
61	252	40	6.3	50	5.0	50	5.0	20	12.6
62	220	20	11	10	22	—	—	—	—
63	600	4	125	—	—	—	—	30	20

<sup>a</sup>Well-tolerated dose. <sup>b</sup>Minimum effective concn. <sup>c</sup>Therapeutic index (WTD/MEC). <sup>d</sup>— = negative, ND = not done.

### Experimental Section†

**General Procedure for Thiones (II).** The appropriate isatin (0.1 mole), H<sub>2</sub>NNHCSNH<sub>2</sub> (0.11 mole), K<sub>2</sub>CO<sub>3</sub> (0.15 mole), and H<sub>2</sub>O (500 ml) were stirred and refluxed for 7–10 hr. On cooling, the

†Melting points were determined on a Townson-Mercer app corrected for the exposed thermometer stem. Microanalyses were performed by Alfred Hernhardt, 5251 Elbach uber Engelskirchen, West Germany, and by Drs. Weiler and Strauss, Oxford, England. Where analyses are indicated by the symbols of the elements, values for those elements were within ±0.4% of the calculated values.

mixt was filtered, and the filtrate acidified with AcOH. The solid was filtered off, washed with H<sub>2</sub>O, and dried. For analysis, a sample was recrystallized from EtOH or DMF.

**General Procedure for 3-Alkylthiotriazinoindoles.** Alkyl halide (0.11 mole) was added during 2–5 min to a stirred soln of the appropriate thione (0.1 mole) in NaOH soln (1 N; 0.115 mole). The mixt was stirred for a further 30 min and the solid was filtered off, washed with H<sub>2</sub>O, and dried. With the more soluble tertiary aminoalkylthio compds, the reaction mixt was poured into an excess of H<sub>2</sub>O and the product salted out. For analysis, a sample was recrystallized from MeOH or EtOH.

**General Procedure for N-5-Alkylation of 3-Alkylthiothiazinoindoles.** NaH (4.8 g, 50% dispersion in oil; 0.104 mole) was added to a stirred suspension of the appropriate 3-alkylthio compd (0.1 mole) in anhyd HCONMe<sub>2</sub> (200 ml) and the appropriate alkyl halide (0.106 mole) in anhyd C<sub>6</sub>H<sub>6</sub> was then added and the mixt refluxed for 24 hr, cooled, and filtered. After the removal of solvent, the residue was extd with hot cyclohexane or EtOH. On cooling the product crystd. For analysis, a sample was recrystd from MeOH or EtOH.

**General Procedure for 3-Aminotriazinoindoles.** A soln of the appropriate thione or 3-alkylthio compd and the amine (3 ml/g of S compd) was heated at 160–180° until the evolution of H<sub>2</sub>S or alkanethiol was complete (5–6 hr). For low boiling amines, an EtOH soln in a pressure vessel was used. On cooling, the mixt was stirred with an excess of H<sub>2</sub>O, and the product was filtered off, washed with H<sub>2</sub>O, and dried. For analysis, a sample was recrystallized from EtOH.

**Acknowledgments.** We wish to thank the following for experimental assistance: Miss P. R. Schaay, Mr. I. J. Gibson, and Mrs. A. G. Hartley of Allen and Hanburys Laboratories; Mrs. B. D. Margolis, Miss C. Zapiec, and Mr. E. Flannery of Smith Kline & French Laboratories.

## References

- (1) T. Ueda and I. Nakata, *Yakugaku Zasshi*, 80, 1068 (1960); *Chem. Abstr.*, 55, 562 (1961).
- (2) D. J. Bauer and F. W. Sheffield, *Nature (London)*, 184, 1496 (1959).
- (3) D. J. Bauer and P. W. Sadler, *Brit. J. Pharmacol.*, 15, 101 (1960).
- (4) (a) D. J. Bauer, L. St. Vincent, C. H. Kempe, and A. W. Downie, *Lancet*, ii, 494 (1963); (b) D. J. Bauer and K. Apostolov, *Science*, 154, 796 (1966); (c) D. J. Bauer, K. Apostolov, and J. W. T. Selway, *Ann. N. Y. Acad. Sci.*, 173, 314 (1970).
- (5) (a) R. F. Haff, J. J. Boyle, R. C. Stewart, R. J. Ferlauto, J. M. Z. Gladych, J. H. Hunt, and D. Jack, *Nature (London)*, 221, 286 (1969); (b) J. J. Boyle, W. G. Raupp, F. J. Stanfield, R. F. Haff, E. C. Dick, D. D'Alessio, and C. R. Dick, *Ann. N. Y. Acad. Sci.*, 173, 477 (1970); (c) R. F. Haff, *Int. Congr. Chemother., Proc.*, 6th, 1969, 855 (1970).
- (6) W. C. Sumpter and F. M. Miller, *Chem. Heterocycl. Compounds*, 8, 110 (1954).
- (7) Allen and Hanburys Limited, Netherlands Patent 6410823 (1965); *Chem. Abstr.*, 63, 13295 (1965).
- (8) H. King and J. Wright, *J. Chem. Soc.*, 2314 (1948).
- (9) E. C. Herrmann, Jr., *Proc. Soc. Exp. Biol. Med.*, 107, 142 (1961).

## Anthelmintic Activity in Sheep of Some Compounds Related to Pyrantel and Morantel

W. C. Austin,\* R. L. Cornwell, R. M. Jones,

Research Division, Pfizer Limited, Sandwich, Kent, United Kingdom

and M. Robinson

Pfizer Pty, West Ryde, Sydney, Australia. Received April 19, 1971

The anthelmintic activity in sheep of pyrantel, morantel, and other cyclic amidines, of a series of thiazoline and dihydrothiazine analogs of pyrantel, and of a related series of 1-(2-arylvinyl)pyridinium compounds correlated with those previously reported for the *Nematospiroides dubius* rodent screen. The rodent screen was shown to be a good early indicator for activity against the gastrointestinal nematodes of sheep for these classes of compounds, although it became clear subsequently that for some species, e.g., *Trichostrongylus colubriformis*, the screen was not always valid.

The discovery of a new series of highly active anthelmintic compounds exhibiting broad spectrum activity in domestic animals has been reported.<sup>1</sup> Details of the structure-activity relationship of a large number of compounds in this and

Table I. Pyrantel, Morantel and Other Thiophene-Substituted Cyclic Amidines

Compound number <sup>a</sup>	X	n	R <sub>1</sub>	R <sub>2</sub>
1	CH <sub>2</sub> S	2	H	H
8	CH <sub>2</sub> CH <sub>2</sub>	2	H	H
10	CH <sub>2</sub> CH <sub>2</sub>	3	H	H
21/22	CH <sub>2</sub> CH <sub>2</sub>	2	H	CH <sub>3</sub>
37/38	CH <sub>2</sub> CH <sub>2</sub>	3	H	CH <sub>3</sub>
66	CH=CH	2	H	CH <sub>3</sub>
70	CH=CH	3	H	H
71 (pyrantel)	CH=CH	3	H	CH <sub>3</sub>
74 (morantel)	CH=CH	3	CH <sub>3</sub>	CH <sub>3</sub>
78	CH=CH	3	C <sub>2</sub> H <sub>5</sub>	CH <sub>3</sub>
79	CH=CH	3	Br	CH <sub>3</sub>

<sup>a</sup>Number corresponding with identification used in McFarland, *et al.*<sup>2</sup>

†Pyrantel tartrate, Banminth, Strongid.

‡Morantel tartrate, Banminth II.

Table II. Substituted Styryl Tetrahydropyrimidines

Compound number <sup>a</sup>	R <sub>1</sub>	R <sub>2</sub>
82	H	H
84	CH <sub>3</sub>	H
93	Cl	H
97	Br	H
102	H	OCH <sub>3</sub>
103	OC <sub>2</sub> H <sub>5</sub>	H
106	NO <sub>2</sub>	H

<sup>a</sup>Corresponding to number used for identification in McFarland, *et al.*<sup>2</sup>

closely related series using the mouse nematode *Nematospiroides dubius* have been published.<sup>2-5</sup> Two compounds, *trans*-1,4,5,6-tetrahydro-1-methyl-2-[2-(2-thienyl)vinyl]pyrimidine (pyrantel)† and its 3-methyl-2-thienyl analog (morantel),‡ have been extensively evaluated in sheep,<sup>6-9</sup> cattle,<sup>10</sup> pigs,<sup>11,12</sup> horses,<sup>13-14</sup> dogs,<sup>15-18</sup> and man.<sup>19,20</sup>

Evaluation of these series in sheep has involved primarily the testing of compounds showing sufficient promise in the primary mouse screen. Some compounds showing little activity in mice have, however, also been tested in sheep because of the need to establish a correlation between the