

present may not be those seen in the solid state and complicated distributions of polyoxometalate species may be established on time scales that are short with respect to the time for the drug to be administered and to take effect.

### Experimental Section

**Materials.** The polyoxometalates were prepared by standard literature procedures.<sup>4a,5a,14a,18</sup> All polyoxometalates were purified by recrystallization, and their purity was assessed spectroscopically with <sup>183</sup>W and <sup>31</sup>P NMR, UV-visible, and infrared absorption spectroscopies.

**Spectroscopic Methods.** The <sup>31</sup>P NMR spectra were taken on an IBM WP-200SY instrument. Chemical shifts are referenced to a 1% TMP (trimethyl phosphate) solution in D<sub>2</sub>O, by running the reference in a 5-mm coaxial tube, inside the 10-mm sample tube. The D<sub>2</sub>O in the coaxial tube proved sufficient to provide a lock, so D<sub>2</sub>O was not used in the buffer solutions. Infrared spectra were run on a Perkin-Elmer 1430 ratio recording spectrophotometer. Samples were run as KBr pellets (2-4 wt % in KBr). Electronic absorption spectra were run on a Hewlett-Packard Model 8451A diode-array spectrophotometer.

**Cell Culture Assays.** The compounds were evaluated in human mitogen-stimulated PBM cells infected with HIV-1 (strain

LAV-1), as described previously.<sup>19</sup> Virus was harvested after 6 days. Stock solutions (2 mM) of the compounds were freshly prepared in water prior to testing. Compounds 7, 15, 16, and 20 were dissolved in DMSO and then diluted with medium so that the final concentration of DMSO was less than 0.05%. At that concentration, DMSO has no effect on the viral yield.

**Cytotoxicity Assays in PBM Cells.** The drugs were evaluated for their potential toxic effects on uninfected mitogen-stimulated human PBM cells. Flasks were seeded so that the final cell concentration was  $2 \times 10^5$  cells/mL. The cells were cultured with and without drug for 6 days at which time aliquots were counted for cell viability, as assessed by the trypan blue dye-exclusion method using a hemacytometer.<sup>19</sup> The EC<sub>50</sub> and IC<sub>50</sub> were calculated by using the median effect method.<sup>20</sup>

**Acknowledgment.** This work was supported by Public Health Service Grants AI 26055 (CLH and RFS) from the National Institutes of Health, and the Department of Veterans Affairs (RFS). The excellent technical assistance of D. Cannon, B. Oswald, and V. Saalman is gratefully acknowledged.

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## 2,4-Dihydro-3H-1,2,4-triazol-3-ones as Anticonvulsant Agents

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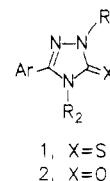
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A series of 5-aryl-2,4-dihydro-3H-1,2,4-triazol-3-ones was evaluated for anticonvulsant activity. In general the members of this series were prepared by the alkaline cyclization of 1-aryl-4-alkylsemicarbazides. The resulting 2-unsubstituted 3H-1,2,4-triazol-3-ones were then alkylated, yielding 2,4-dialkyl-3H-1,2,4-triazol-3-ones. Approximately one-third of the compounds examined exhibited activity against both maximal electroshock- and pentylenetetrazole-induced seizures in mice. Receptor-binding studies suggest that this activity was not a consequence of activity at either benzodiazepine or NMDA-type glutamate receptors. From this series, compound 45 was selected for further evaluation where it was also found to be active against 3-mercaptopropionic acid, bicuculline, and quinolinic acid induced seizures in mice. In addition, 45 also protected gerbils from hippocampal neuronal degeneration produced by either hypoxia or intrastriatal quinolinic acid injection.

We have recently been investigating a series of 2,4-dihydro-3H-1,2,4-triazole-3-thiones 1 as potential antidepressant agents.<sup>1</sup> Selected members of this series exhibited potent behavioral effects in test systems traditionally used to detect these agents. The mechanism of action of these compounds, however, remains uncertain. While attempting to establish some correlation between the structure and the activity of these triazoles, we prepared several 2,4-dihydro-3H-1,2,4-triazol-3-ones 2. Surprisingly, these compounds were completely devoid of antidepressant-like activity and instead exhibited anticonvulsant activity against a variety of convulsant stimuli. In order to more fully investigate these findings, we have prepared additional derivatives of 2 and we now report the anticonvulsant activities associated with members of this series.

### Chemistry

The triazol-3-ones (Table I) which were evaluated in this study were prepared via the three routes depicted in



Scheme I. The synthesis of 2,4-dihydro-5-phenyl-3H-1,2,4-triazol-3-one (3) was accomplished by the method of Lipkin.<sup>2</sup> Thus, condensation of benzoic acid hydrazide (4) and urea (5) gave 3 in a single, low-yielding step. The synthesis of 2,4-dihydro-2-methyl-5-phenyl-3H-1,2,4-triazol-3-one (6) was accomplished via a new route which involved S-methylation of triazole-3-thione 7.<sup>3</sup> The resultant thioether 8<sup>4</sup> was then oxidized with 2.5 equiv of *m*-chloroperoxybenzoic acid (MCPBA), affording sulfone 9. Alkaline hydrolysis of 9 cleanly afforded 6, which was

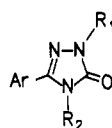
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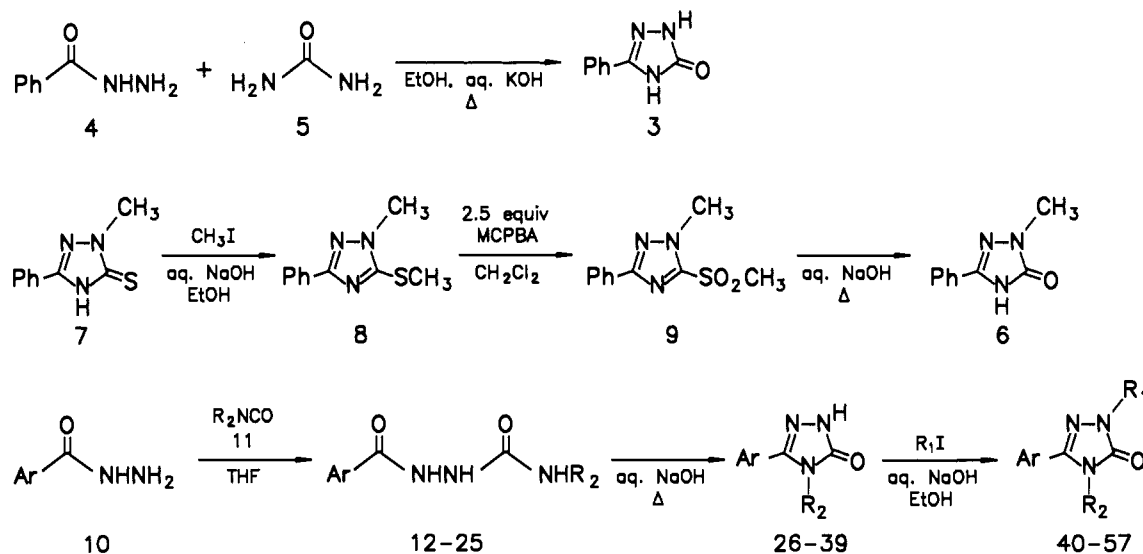
Table I. 2,4-Dihydro-3H-1,2,4-triazol-3-ones



| compd | Ar  | R <sub>1</sub>                          | R <sub>2</sub>                | mp, °C               | crystn solvent <sup>a</sup> | % yield | formula <sup>b</sup>  |
|-------|---|---|-------------------------------|----------------------|-----------------------------|---------|---|
| 3     | C <sub>6</sub> H <sub>5</sub>   | H                                       | H                             | >310 <sup>c</sup>    | A                           | 10      | C <sub>8</sub> H <sub>7</sub> N <sub>3</sub> O                  |
| 6     | C <sub>6</sub> H <sub>5</sub>   | CH <sub>3</sub>                         | H                             | 218–219 <sup>d</sup> | B                           | 90      | C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O                  |
| 26    | C <sub>6</sub> H <sub>5</sub>   | H                                       | CH <sub>3</sub>               | 177–178 <sup>e</sup> | A                           | 74      | C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O                  |
| 27    | C <sub>6</sub> H <sub>5</sub>   | H                                       | C <sub>2</sub> H <sub>5</sub> | 163–165 <sup>f</sup> | A                           | 69      | C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O                |
| 28    | 2-ClC <sub>6</sub> H <sub>4</sub>   | H                                       | CH <sub>3</sub>               | 168–170              | C                           | 71      | C <sub>9</sub> H <sub>8</sub> ClN <sub>3</sub> O                |
| 29    | 4-ClC <sub>6</sub> H <sub>4</sub>   | H                                       | CH <sub>3</sub>               | 213–215 <sup>g</sup> | D                           | 69      | C <sub>9</sub> H <sub>8</sub> ClN <sub>3</sub> O                |
| 30    | 4-ClC <sub>6</sub> H <sub>4</sub>   | H                                       | CH <sub>3</sub>               | 199–201 <sup>h</sup> | A                           | 83      | C <sub>10</sub> H <sub>10</sub> ClN <sub>3</sub> O              |
| 31    | 3,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>   | H                                       | CH <sub>3</sub>               | 170–172              | C                           | 72      | C <sub>9</sub> H <sub>7</sub> Cl <sub>2</sub> N <sub>3</sub> O  |
| 32    | 2-FC <sub>6</sub> H <sub>4</sub>  | H                                       | CH <sub>3</sub>               | 189–191              | E                           | 54      | C <sub>9</sub> H <sub>8</sub> FN <sub>3</sub> O                 |
| 33    | 4-FC <sub>6</sub> H <sub>4</sub>  | H                                       | CH <sub>3</sub>               | 216–218 <sup>i</sup> | D                           | 53      | C <sub>9</sub> H <sub>8</sub> FN <sub>3</sub> O                 |
| 34    | 3-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub>   | H                                       | C <sub>2</sub> H <sub>5</sub> | 130–131              | F                           | 52      | C <sub>11</sub> H <sub>10</sub> F <sub>3</sub> N <sub>3</sub> O |
| 35    | 4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>   | H                                       | CH <sub>3</sub>               | 206–208              | D                           | 78      | C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O                |
| 36    | 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>  | H                                       | CH <sub>3</sub>               | 174–176              | A                           | 86      | C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>   |
| 37    | 4-CH <sub>3</sub> O-3-( <i>n</i> -C <sub>4</sub> H <sub>9</sub> O)C <sub>6</sub> H <sub>3</sub> | H                                       | CH <sub>3</sub>               | 96–98                | C                           | 70      | C <sub>14</sub> H <sub>19</sub> N <sub>3</sub> O <sub>3</sub>   |
| 38    | 2-C <sub>4</sub> H <sub>3</sub> S   | H                                       | CH <sub>3</sub>               | 183–185 <sup>j</sup> | A                           | 82      | C <sub>7</sub> H <sub>7</sub> N <sub>3</sub> OS                 |
| 39    | 4-C <sub>6</sub> H <sub>4</sub> N   | H                                       | CH <sub>3</sub>               | 249–251 <sup>k</sup> | D                           | 71      | C <sub>8</sub> H <sub>8</sub> N <sub>4</sub> O                  |
| 40    | C <sub>6</sub> H <sub>5</sub>   | CH <sub>3</sub>                         | CH <sub>3</sub>               | 140–141 <sup>l</sup> | D                           | 64      | C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O                |
| 41    | C <sub>6</sub> H <sub>5</sub>   | CH <sub>3</sub>                         | C <sub>2</sub> H <sub>5</sub> | oil <sup>m</sup>     |                             | 64      | C <sub>11</sub> H <sub>13</sub> N <sub>3</sub> O                |
| 42    | C <sub>6</sub> H <sub>5</sub>   | C <sub>2</sub> H <sub>5</sub>           | CH <sub>3</sub>               | 87–89 <sup>n</sup>   | G                           | 61      | C <sub>11</sub> H <sub>13</sub> N <sub>3</sub> O                |
| 43    | 2-ClC <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>                         | CH <sub>3</sub>               | 61–63                | G                           | 51      | C <sub>10</sub> H <sub>10</sub> ClN <sub>3</sub> O              |
| 44    | 4-ClC <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>                         | CH <sub>3</sub>               | 126–128 <sup>o</sup> | C                           | 48      | C <sub>10</sub> H <sub>10</sub> ClN <sub>3</sub> O              |
| 45    | 4-ClC <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>                         | C <sub>2</sub> H <sub>5</sub> | 73–75 <sup>p</sup>   | G                           | 58      | C <sub>11</sub> H <sub>12</sub> ClN <sub>3</sub> O              |
| 46    | 4-ClC <sub>6</sub> H <sub>4</sub>   | C <sub>2</sub> H <sub>5</sub>           | CH <sub>3</sub>               | 79–81 <sup>q</sup>   | G                           | 53      | C <sub>11</sub> H <sub>12</sub> ClN <sub>3</sub> O              |
| 47    | 4-ClC <sub>6</sub> H <sub>4</sub>   | C <sub>2</sub> H <sub>5</sub>           | C <sub>2</sub> H <sub>5</sub> | 62–64 <sup>r</sup>   | H                           | 50      | C <sub>12</sub> H <sub>14</sub> ClN <sub>3</sub> O              |
| 48    | 4-ClC <sub>6</sub> H <sub>4</sub>   | <i>n</i> -C <sub>3</sub> H <sub>7</sub> | C <sub>2</sub> H <sub>5</sub> | oil                  |                             | 55      | C <sub>13</sub> H <sub>16</sub> ClN <sub>3</sub> O              |
| 49    | 3,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>   | CH <sub>3</sub>                         | CH <sub>3</sub>               | 107–109              | B                           | 65      | C <sub>10</sub> H <sub>9</sub> Cl <sub>2</sub> N <sub>3</sub> O |
| 50    | 2-FC <sub>6</sub> H <sub>4</sub>  | CH <sub>3</sub>                         | CH <sub>3</sub>               | 69–71                | G                           | 57      | C <sub>10</sub> H <sub>10</sub> FN <sub>3</sub> O               |
| 51    | 4-FC <sub>6</sub> H <sub>4</sub>  | CH <sub>3</sub>                         | CH <sub>3</sub>               | 104–106 <sup>s</sup> | G                           | 63      | C <sub>10</sub> H <sub>10</sub> FN <sub>3</sub> O               |
| 52    | 3-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>                         | C <sub>2</sub> H <sub>5</sub> | 49–51                | I                           | 41      | C <sub>12</sub> H <sub>12</sub> F <sub>3</sub> N <sub>3</sub> O |
| 53    | 4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>                         | CH <sub>3</sub>               | 92–94                | G                           | 56      | C <sub>11</sub> H <sub>13</sub> N <sub>3</sub> O                |
| 54    | 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>  | CH <sub>3</sub>                         | CH <sub>3</sub>               | 109–111              | G                           | 36      | C <sub>11</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>   |
| 55    | 4-CH <sub>3</sub> O-3-( <i>n</i> -C <sub>4</sub> H <sub>9</sub> O)C <sub>6</sub> H <sub>3</sub> | CH <sub>3</sub>                         | CH <sub>3</sub>               | 112–114              | C                           | 33      | C <sub>15</sub> H <sub>21</sub> N <sub>3</sub> O <sub>3</sub>   |
| 56    | 2-C <sub>4</sub> H <sub>3</sub> S   | CH <sub>3</sub>                         | CH <sub>3</sub>               | 108–110 <sup>t</sup> | C                           | 59      | C <sub>8</sub> H <sub>9</sub> N <sub>3</sub> OS                 |
| 57    | 2-C <sub>4</sub> H <sub>3</sub> S   | C <sub>2</sub> H <sub>5</sub>           | CH <sub>3</sub>               | 47–49                | J                           | 53      | C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> OS                |

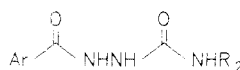
<sup>a</sup> A = 2-propanol, B = aqueous ethanol, C = ethyl acetate/hexane, D = ethanol, E = 2-butanone, F = aqueous 2-propanol, G = cyclohexane, H = hexane, I = Kugelrohr distilled 130–135 °C (0.5 mm), J = Kugelrohr distilled 210–225 °C (0.3 mm). <sup>b</sup> Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds. <sup>c</sup> Literature<sup>2</sup> mp 326–327 °C. <sup>d</sup> Literature<sup>21</sup> mp 218–219 °C. <sup>e</sup> Literature<sup>4</sup> mp 179–180 °C. <sup>f</sup> Literature<sup>26</sup> mp 168 °C. <sup>g</sup> Literature<sup>27</sup> mp 213–215 °C. <sup>h</sup> Literature<sup>28</sup> mp 199–200 °C. <sup>i</sup> Literature<sup>27</sup> mp 216–218 °C. <sup>j</sup> Literature<sup>29</sup> mp 183–185 °C. <sup>k</sup> Literature<sup>29</sup> mp 249–251 °C. <sup>l</sup> Literature<sup>4</sup> mp 144–145 °C. <sup>m</sup> Literature<sup>30</sup> bp 130 °C (0.2 mm). <sup>n</sup> Literature<sup>27</sup> mp 87–89 °C. <sup>o</sup> Literature<sup>27</sup> mp 126–128 °C. <sup>p</sup> Literature<sup>27</sup> mp 73–75 °C. <sup>q</sup> Literature<sup>27</sup> mp 79–81 °C. <sup>r</sup> Literature<sup>27</sup> mp 62–64 °C. <sup>s</sup> Literature<sup>27</sup> mp 104–106 °C. <sup>t</sup> Literature<sup>29</sup> mp 108–110 °C.

## Scheme I



isolated in 90% yield. It should be noted that, under the same conditions, attempted hydrolysis of thioether 8 re-

turned only starting material. With the above two exceptions, all the remaining triazol-3-ones were prepared

**Table II.** 1-Aroyl-4-alkylsemicarbazides

| compd | Ar  | R <sub>2</sub>                | mp, °C               | crystn solvent <sup>a</sup> | % yield | formula <sup>b</sup>   |
|-------|---|-------------------------------|----------------------|-----------------------------|---------|--|
| 12    | C <sub>6</sub> H <sub>5</sub>   | CH <sub>3</sub>               | 181–183 <sup>c</sup> | A                           | 77      | C <sub>9</sub> H <sub>11</sub> N <sub>3</sub> O <sub>2</sub>                 |
| 13    | C <sub>6</sub> H <sub>5</sub>   | C <sub>2</sub> H <sub>5</sub> | 175–177 <sup>d</sup> | B                           | 85      | C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>                |
| 14    | 2-ClC <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>               | 184–186              | C                           | 84      | C <sub>9</sub> H <sub>10</sub> ClN <sub>3</sub> O <sub>2</sub>               |
| 15    | 4-ClC <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>               | 234–236 <sup>e</sup> | C                           | 91      | C <sub>9</sub> H <sub>10</sub> ClN <sub>3</sub> O <sub>2</sub>               |
| 16    | 4-ClC <sub>6</sub> H <sub>4</sub>   | C <sub>2</sub> H <sub>5</sub> | 237–239 <sup>f</sup> | C                           | 89      | C <sub>10</sub> H <sub>12</sub> ClN <sub>3</sub> O <sub>2</sub>              |
| 17    | 3,4-Cl <sub>2</sub> C <sub>6</sub> H <sub>3</sub>   | CH <sub>3</sub>               | 245–247              | C                           | 56      | C <sub>9</sub> H <sub>8</sub> Cl <sub>2</sub> N <sub>3</sub> O <sub>2</sub>  |
| 18    | 2-FC <sub>6</sub> H <sub>4</sub>  | CH <sub>3</sub>               | 122–124              | B                           | 42      | C <sub>9</sub> H <sub>10</sub> FN <sub>3</sub> O <sub>2</sub>                |
| 19    | 4-FC <sub>6</sub> H <sub>4</sub>  | CH <sub>3</sub>               | 222–224 <sup>g</sup> | C                           | 75      | C <sub>9</sub> H <sub>10</sub> FN <sub>3</sub> O <sub>2</sub>                |
| 20    | 3-CF <sub>3</sub> C <sub>6</sub> H <sub>4</sub>   | C <sub>2</sub> H <sub>5</sub> | 188–189              | C                           | 29      | C <sub>11</sub> H <sub>12</sub> F <sub>3</sub> N <sub>3</sub> O <sub>2</sub> |
| 21    | 4-CH <sub>3</sub> C <sub>6</sub> H <sub>4</sub>   | CH <sub>3</sub>               | 194–196              | C                           | 86      | C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>2</sub>                |
| 22    | 4-CH <sub>3</sub> OC <sub>6</sub> H <sub>4</sub>  | CH <sub>3</sub>               | 180–181              | C                           | 52      | C <sub>10</sub> H <sub>13</sub> N <sub>3</sub> O <sub>3</sub>                |
| 23    | 4-CH <sub>3</sub> O-3-( <i>n</i> -C <sub>4</sub> H <sub>9</sub> O)C <sub>6</sub> H <sub>3</sub> | CH <sub>3</sub>               | 195–197              | C                           | 83      | C <sub>14</sub> H <sub>21</sub> N <sub>3</sub> O <sub>4</sub>                |
| 24    | 2-C <sub>4</sub> H <sub>9</sub> S   | CH <sub>3</sub>               | 165–167              | C                           | 84      | C <sub>7</sub> H <sub>9</sub> N <sub>3</sub> O <sub>2</sub> S                |
| 25    | 4-C <sub>6</sub> H <sub>4</sub> N   | CH <sub>3</sub>               | 224–225              | C                           | 80      | C <sub>8</sub> H <sub>10</sub> N <sub>4</sub> O <sub>2</sub>                 |

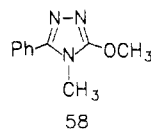
<sup>a</sup> A = water, B = 2-propanol, C = ethanol. <sup>b</sup> Satisfactory analyses (C, H, and N; ±0.4% of theoretical values) were obtained for all compounds. <sup>c</sup> Literature<sup>31</sup> mp 181 °C. <sup>d</sup> Literature<sup>31</sup> mp 175 °C. <sup>e</sup> Literature<sup>27</sup> mp 234–236 °C. <sup>f</sup> Literature<sup>27</sup> mp 237–239 °C. <sup>g</sup> Literature<sup>27</sup> mp 222–224 °C.

by the method of Kubota and Uda.<sup>4</sup> Thus, reaction of hydrazides 10 and isocyanates 11 gave aroylsemicarbazides 12–25 (Table II). Cyclization of these semicarbazides in refluxing aqueous sodium hydroxide afforded 2-unsubstituted triazol-3-ones 26–39, which were subsequently N-alkylated with alkyl iodides, yielding 2,4-dialkyltriazol-3-ones 40–57.

### Results and Discussion

The anticonvulsant activities of the triazoles listed in Table I were initially evaluated against maximal electroshock- and pentylenetetrazole-induced seizures in mice. The results are listed in Table III, where it can be seen that approximately one-third of the compounds exhibited activity against both convulsant stimuli. Also listed in Table III are LD<sub>50</sub> estimates which show that the acute toxicity associated with the use of these compounds is reasonably low, three-fourths of the compounds having an estimated LD<sub>50</sub> of greater than 400 mg/kg. In terms of structure it can be observed that all of the least toxic compounds (LD<sub>50</sub> > 800 mg/kg) are unsubstituted in the 2-position of the triazole ring.

The correlation between structure and activity in this series was not straightforward; however, some trends could be observed. As alluded to earlier, the most obvious structural feature necessary for anticonvulsant activity was the carbonyl moiety. Changing this group to either a thiocarbonyl group, e.g. 1 (Ar = C<sub>6</sub>H<sub>5</sub>, R<sub>1</sub> = R<sub>2</sub> = CH<sub>3</sub>, X = S),<sup>5</sup> or a methyl ether, e.g. 58,<sup>4</sup> resulted in a loss of



anticonvulsant activity. In general, the aryl groups of the active compounds were monohalogenated (compounds 28, 29, 33, 43, 44, 45, and 47) although phenyl derivative 26 and 3-(trifluoromethyl)phenyl derivative 52 also had activity. In contrast, aromatic rings containing either alkyl groups (compound 53) or alkoxy groups (compounds 36, 37, and 54) were generally inactive as were compounds containing heteroaryl groups (compounds 38, 39, 56, and 57). Substitution at the 2-position of the triazole nucleus

**Table III.** Biological Activities for 2,4-Dihydro-3H-1,2,4-triazol-3-ones

| compd                            | estimated LD <sub>50</sub> ,<br>mg/kg ip | activity <sup>a,b</sup> |                  | IC <sub>50</sub> , μM          |                                   |
|----------------------------------|--|-------------------------|------------------|--------------------------------|-----------------------------------|
|                                  |  | MES <sup>c</sup>        | PTZ <sup>d</sup> | [ <sup>3</sup> H]flunitrazepam | [ <sup>3</sup> H]CPP <sup>e</sup> |
| 1 <sup>f</sup>                   | 100–200                                  | –                       | –                |                                |                                   |
| 3                                | >800                                     | –                       | –                |                                |                                   |
| 6                                | 400–800                                  | –                       | ++               |                                |                                   |
| 26                               | >800                                     | ++                      | +++              | >100                           | >100                              |
| 27                               | >800                                     | –                       | ++               |                                |                                   |
| 28                               | 400–800                                  | ++                      | +++              | >100                           | >100                              |
| 29                               | >800                                     | +++                     | +++              | >100                           | >100                              |
| 30                               | 200–400                                  | –                       | +++              | >100                           | >100                              |
| 31                               | 200–400                                  | +                       | +                | >100                           | >100                              |
| 32                               | >800                                     | –                       | +++              |                                |                                   |
| 33                               | >800                                     | +++                     | +++              | >100                           | >100                              |
| 34                               | 200–400                                  | –                       | ++               |                                |                                   |
| 35                               | >800                                     | –                       | ++               |                                |                                   |
| 36                               | >800                                     | –                       | –                |                                |                                   |
| 37                               | 100–200                                  | –                       | –                |                                |                                   |
| 38                               | 200–400                                  | –                       | –                | >100                           | >100                              |
| 39                               | >800                                     | –                       | –                | >100                           | >100                              |
| 40                               | 400–800                                  | –                       | ++               |                                |                                   |
| 41                               | 400–800                                  | ++                      | +                |                                |                                   |
| 42                               | 400–800                                  | –                       | ++               |                                |                                   |
| 43                               | 400–800                                  | +                       | +++              |                                |                                   |
| 44                               | 400–800                                  | ++                      | +++              | >100                           | >100                              |
| 45                               | 200–400                                  | ++                      | +++              | >100                           | >100                              |
| 46                               | 400–800                                  | –                       | +++              |                                |                                   |
| 47                               | 200–400                                  | ++                      | ++               |                                |                                   |
| 48                               | 400–800                                  | –                       | –                |                                |                                   |
| 49                               | 100–200                                  | +                       | +                |                                |                                   |
| 50                               | 400–800                                  | –                       | ++               |                                |                                   |
| 51                               | 400–800                                  | +                       | +                |                                |                                   |
| 52                               | 400–800                                  | ++                      | +++              | >100                           | >100                              |
| 53                               | 400–800                                  | –                       | –                |                                |                                   |
| 54                               | 400–800                                  | –                       | –                |                                |                                   |
| 55                               | 400–800                                  | –                       | ++               | 33.2 ± 1.2                     | >100                              |
| 56                               | 400–800                                  | –                       | –                |                                |                                   |
| 57                               | 400–800                                  | –                       | –                |                                |                                   |
| 58                               | >800                                     | –                       | –                |                                |                                   |
| N-methyl-D-aspartate<br>diazepam |  |                         |                  | 0.012                          | 6                                 |

<sup>a</sup> Activity = protection of >50% of the treated mice. <sup>b</sup> + = activity at 1/4 LD<sub>50</sub> range, ++ = activity at 1/8 LD<sub>50</sub> range, +++ = activity at 1/16 LD<sub>50</sub> range. <sup>c</sup> MES = antagonism of maximal electroshock-induced seizures in mice. <sup>d</sup> Antagonism of pentylenetetrazole-induced seizures in mice. <sup>e</sup> 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid. <sup>f</sup> Ar = C<sub>6</sub>H<sub>5</sub>, R<sub>1</sub> = R<sub>2</sub> = CH<sub>3</sub>, X = S (ref 5).

did not appear to dramatically affect the anticonvulsant activity. For example, compounds which were either unsubstituted (compounds 26, 28, 29, and 33), methylated (compounds 43, 44, 45, and 52), or ethylated (compound

**Table IV.** Anticonvulsant Activities for 45 and Reference Compounds

| compound      | ED <sub>50</sub> , mg/kg ip |                  |                  |                  |                  |                 |
|---------------|-----------------------------|------------------|------------------|------------------|------------------|-----------------|
|               | MES <sup>a</sup>            | PTZ <sup>b</sup> | MPA <sup>c</sup> | BIC <sup>d</sup> | STR <sup>e</sup> | QA <sup>f</sup> |
| 45            | 48                          | 17               | 31               | 15               | ~101             | 26              |
| carbamazepine | 13-25                       | >200             | 86               |                  | 61               | 25              |
| phenobarbital | 20                          | 24               | 30               | 9                | 52               | 52              |
| phenytoin     | 26                          | >32              | 89               | >200             | >200             | g               |
| valproate     | >200                        | 100-200          | 183              | 28               | >200             | >256            |
| clonazepam    | 25-50                       | 0.06             | 0.20             | 0.07             | ~2 <sup>h</sup>  | <8 <sup>i</sup> |

<sup>a</sup> Antagonism of maximal electroshock-induced seizures in mice.

<sup>b</sup> Antagonism of pentylenetetrazole-induced seizures in mice.

<sup>c</sup> Antagonism of 3-mercaptopropionic acid induced seizures in mice.

<sup>d</sup> Antagonism of bicuculline-induced seizures in mice.

<sup>e</sup> Antagonism of strychnine-induced seizures in mice. <sup>f</sup> Antagonism

of quinolinic acid induced seizures in mice. <sup>g</sup> Maximum effect,

50% at 20 mg/kg. <sup>h</sup> Irregular dose-response function. <sup>i</sup> Irregular

dose-response function; 90% effect at 8 mg/kg.

47) at this position had activity. On the other hand, alkyl substitution at the 4-position of the triazole ring appeared to be more necessary, accepting either methyl (compounds 26, 28, 29, 33, 43, and 44) or ethyl (compounds 45, 47, and 52) substitution. Of the two examples which were unsubstituted at this position, one (compound 3) was inactive against both seizure stimuli while the other (compound 6) was active against only pentylenetetrazole-induced seizures.

From the examples listed in Table I, we selected compound 45 for further evaluation. Thus, 45 was examined in several additional seizure models, and these results are presented in Table IV. With the exception of strychnine-induced seizures, 45 was active against all of the seizure models within a relatively narrow dose range. The anticonvulsant profile observed for 45 appeared to be somewhat better than those observed for either carbamazepine, phenytoin, or sodium valproate and was similar to that observed for phenobarbital. On the other hand, 45 was considerably less potent than clonazepam in all of the seizure models with the exception of maximal electroshock-induced seizures, where their activities were comparable.

Among the possible mechanisms which are known to inhibit seizure activity are enhancement of GABA transmission<sup>6,7</sup> and antagonism of glutamate transmission.<sup>8,9</sup> Accordingly, a number of the triazoles listed in Table I were examined for affinity at the benzodiazepine binding site on the GABA<sub>A</sub> receptor complex and at the glutamate site on the NMDA subtype of the glutamate receptor. The results are presented in Table III, showing that none of the compounds tested had any appreciable affinity for either of these sites. In addition, compound 45 did not exhibit any appreciable affinity for either the kainate or quisqualate receptor subtypes (IC<sub>50</sub> values > 100 μM).

Recent studies have suggested that excessive glutamate receptor activation may be involved in various neurodegenerative disorders.<sup>10-13</sup> Since quinolinic acid is an agonist at glutamate receptors, the activity we observe

**Table V.** Activity against Quinolinic Acid Induced Seizures and Effect on Hypoxia-Induced Neuronal Damage of PCP and Compounds 28, 30, and 45

| compound         | QA <sup>b</sup> | damage scores <sup>a</sup><br>(mean ± SEM) |                          |
|------------------|-----------------|--|--------------------------|
|                  |                 | sham                                       | hypoxic                  |
| water            |                 | 0.33 ± 0.09                                | 2.98 ± 0.24              |
| PCP <sup>c</sup> |                 | 1.06 ± 0.48                                | 1.14 ± 0.38 <sup>d</sup> |
| 28               | ~65             | 0.22 ± 0.16                                | 2.95 ± 0.19              |
| 30               | ~51             | 0.33 ± 0.16                                | 2.64 ± 0.48              |
| 45               | 26              | 0.33 ± 0.24                                | 0.97 ± 0.52 <sup>d</sup> |

<sup>a</sup> Hippocampal (CA1 region) neuronal damage scores assessed in gerbils pretreated with drug and subjected to bilateral carotid occlusion for 5 min (or sham operation). Compounds 28, 30, and 45 were administered at 40 mg/kg ip 20 min prior to occlusion. <sup>b</sup> ED<sub>50</sub>, mg/kg ip in mice against quinolinic acid induced seizures. <sup>c</sup> Phencyclidine hydrochloride (15 mg/kg ip). <sup>d</sup> *p* < 0.05 vs water.

against quinolinic acid induced seizures might represent a functional antagonism at the glutamate receptor.<sup>14-17</sup> Accordingly, three of the triazoles which had exhibited activity against quinolinic acid induced seizures were examined for activity against the hippocampal neurodegeneration produced by hypoxia in gerbils.<sup>18,19</sup> As can be seen in Table V, compound 45 produced a significant protection, decreasing hypoxic damage to only 33% of that seen in controls, while 28 and 30 were essentially inactive. This protective activity was equal to that produced by phencyclidine, a known NMDA antagonist with neuroprotective activity.<sup>14</sup>

In summary, a series of 3H-1,2,4-triazol-3-ones has been examined for anticonvulsant activity. Approximately one-third of the compounds in this series exhibited activity against both maximal electroshock- and pentylenetetrazole-induced seizures in mice. Of these examples, triazole 45 was examined in several additional seizure models where it was found to be active. This generalized antiseizure activity suggests that compounds such as 45 might find utility against a variety of seizure disorders. Mechanistically, these compounds do not directly interact with either the benzodiazepine binding site on the GABA<sub>A</sub> receptor complex or the glutamate binding site on the NMDA subtype of the glutamate receptor. In addition, triazole 45 also protected against hypoxic neuronal degeneration in gerbils, suggesting that it also might find utility as a neuroprotective agent.

### Experimental Section

Melting points were determined in open capillaries on a Thomas-Hoover apparatus and are uncorrected. Elemental analyses were performed on site and were within ±0.4% of the theoretical values. All compounds were routinely examined by proton NMR (Varian FT80A, EM390, XL300, and Gemini 300), IR (Perkin-Elmer 180), and TLC (silica gel).

**1-Methyl-5-(methylsulfonyl)-3-phenyl-1H-1,2,4-triazole (9).** MCPBA (14.7 g, 68.1 mmol, commercial 80% mixture) was added portionwise to a stirred, 0 °C, solution of 1-methyl-5-(methylthio)-3-phenyl-1H-1,2,4-triazole<sup>4</sup> (5.6 g, 27 mmol) and

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CH<sub>2</sub>Cl<sub>2</sub> (180 mL). After being stirred overnight at room temperature, the reaction was diluted with CH<sub>2</sub>Cl<sub>2</sub> (300 mL). The reaction was transferred to a separatory funnel where it was washed two times with saturated aqueous NaHCO<sub>3</sub> and once with saturated aqueous NaCl. The CH<sub>2</sub>Cl<sub>2</sub> layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> before it was evaporated at reduced pressure. The resulting solid was purified by flash chromatography<sup>20</sup> (2% EtOAc/CH<sub>2</sub>Cl<sub>2</sub>). Subsequent crystallization from EtOAc/hexane afforded colorless needles: 4.7 g (73%); mp 112–114 °C; <sup>1</sup>H NMR (CDCl<sub>3</sub>) δ 3.49 (s, 3 H), 4.24 (s, 3 H), 7.42–7.51 (m, 3 H), 8.04–8.12 (m, 2 H); <sup>13</sup>C NMR (CDCl<sub>3</sub>) 37.91, 42.91, 126.39, 128.63, 129.36, 129.94, 151.72, 160.79 ppm. Anal. (C<sub>10</sub>H<sub>11</sub>N<sub>3</sub>O<sub>2</sub>S), C, H, N.

**2,4-Dihydro-2-methyl-5-phenyl-3H-1,2,4-triazol-3-one (6).** A stirred mixture of **9** (2.84 g, 12.0 mmol) and 1 M aqueous NaOH (30 mL) was heated to reflux. After refluxing 30 min, the reaction was allowed to cool to room temperature at which time it was acidified by the addition of 1 M aqueous HCl (32 mL). The resulting precipitate was collected by filtration. Crystallization from aqueous EtOH afforded colorless needles: 1.88 g (90%); mp 218–219 °C (lit.<sup>21</sup> mp 218–219 °C).

**General Procedure for the Preparation of 1-Aroyl-4-alkylsemicarbazides 12–25.** A stirred mixture of hydrazide **10** (30 mmol) and dry THF was warmed until a homogeneous solution was obtained. The alkyl isocyanate **11** (33 mmol) was then added dropwise. After 17 h the mixture was diluted with Et<sub>2</sub>O (80 mL) and the precipitate was collected by filtration. The collected solid was washed with Et<sub>2</sub>O and subsequently purified by crystallization from one of the solvents listed in Table II.

**General Procedure for the Preparation of 4-Alkyl-5-aryl-2,4-dihydro-3H-1,2,4-triazol-3-ones 26–39.** A stirred mixture of the 1-aryl-4-alkylsemicarbazide (98.1 mmol) and 1 M aqueous NaOH (118 mL) was heated to reflux. After 23 h the mixture was allowed to cool to room temperature when it was acidified by the addition of 1 M aqueous HCl (130 mL). The mixture was cooled in an ice bath and the precipitate was collected by filtration. With the exception of **31**, which was flash chromatographed (EtOAc) prior to crystallization, all of the products were purified by crystallization from one of the solvents listed in Table I.

**General Procedure for the Preparation of 5-Aryl-2,4-dialkyl-2,4-dihydro-3H-1,2,4-triazol-3-ones 40–57.** To a stirred solution of the 4-alkyl-5-aryl-2,4-dihydro-3H-1,2,4-triazol-3-one (26.8 mmol) and 1 M aqueous NaOH (30 mL) was added a solution of the alkyl iodide (40 mmol) in EtOH (10 mL). The reaction was routinely monitored by removal of a small aliquot, acidification, and TLC of an EtOAc extract. In general, methylations were complete in 24 h whereas ethylation and propylation took considerably longer (27 days in the case of **48**). The reaction was transferred to a separatory funnel where it was extracted several times with EtOAc. The extracts were combined, washed with saturated aqueous NaCl, and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. The drying agent was removed by filtration and the filtrate was evaporated at reduced pressure, leaving the crude product which was purified by a combination of flash chromatography (generally 50% EtOAc/CH<sub>2</sub>Cl<sub>2</sub>) and crystallization from one of the solvents listed in Table I.

**Anticonvulsant Activity.** Groups of five or ten CD-1 male mice were housed individually before being administered the test compound ip as a solution in distilled H<sub>2</sub>O or a suspension in distilled H<sub>2</sub>O/Tween 80. Reference compounds were prepared similarly. The H<sub>2</sub>O/Tween 80 vehicle had no effect in any of the test systems. Thirty minutes after administration of the test compound, mice were administered the convulsant stimulus: (a) maximal electroshock (MES, 50 mA, 0.2 s, corneal electrodes); (b) pentylenetetrazole (PTZ, 60 mg/kg iv); (c) 3-mercaptopropionic acid (MPA, 100 mg/kg iv); (d) bicuculline (BIC, 0.6 mg/kg iv); (e) strychnine sulfate (STR, 2.7 mg/kg ip); (f) quinalinic acid (QA, 7.7 μg icv). In each of these tests, mice were considered protected according to the following criteria: (a) MES, absence of tonic hind-limb extension; (b) PTZ, absence of clonic seizures for 2 min after PTZ administration; (c) MPA, absence of seizures for 5 min after MPA administration; (d) BIC, absence of the typical clon-

ic-tonic seizure syndrome for 5 min after BIC administration; (e) STR, absence of tonic extension for more than 15 min after STR administration; (f) QA, absence of clonic-tonic seizures for 15 min after QA administration. The ED<sub>50</sub> was defined as that dose causing significant protection from seizures in 50% of the mice and it was calculated, when appropriate, with a computer program for analysis of quantal data. For all calculated ED<sub>50</sub> values, 95% confidence limits are within the range 0.5–2.0 ED<sub>50</sub>. All ED<sub>50</sub> values were calculated from the results of at least four doses, each administered to at least one group of 10 mice.

**Intraperitoneal LD<sub>50</sub> Estimation.** Groups of three CD-1 male mice (18–26 g) were administered graded doses of the test compounds prepared as either solutions in distilled H<sub>2</sub>O or suspensions in distilled H<sub>2</sub>O/Tween 80. Standard compounds were prepared similarly. Deaths occurring during the next 7 days were recorded, and the dose range encompassing the 50% lethal effect was recorded as the estimated LD<sub>50</sub>.

**Receptor Binding.** Male, Sprague-Dawley rats (Charles River, 150–200 g) were used as the source of the cortical or hippocampal membrane preparations used in the in vitro receptor binding assays. 3-(2-Carboxypiperazin-4-yl)propyl-1-phosphonic acid ([<sup>3</sup>H]CPP, 30.7 Ci/mmol) binding was performed as previously described.<sup>22</sup> Frozen membranes were thawed at room temperature and washed with ice cold assay buffer (3 × 30 mL). Assays (1 mL) were conducted in scintillation minivials containing 10 nM [<sup>3</sup>H]CPP, 200 μg of membrane protein (Bio-Rad protein assay), 50 mM Tris-HCl buffer (pH 7.4 at 25 °C), various concentrations of test compounds (pH adjusted to 7.4) or 1 mM unlabeled glutamate (nonspecific binding). Following a 15-min incubation at 25 °C, vials were centrifuged at 46000g for 10 min. Supernatants were decanted and the pellets were solubilized overnight in Ready Protein<sup>+</sup> (4 mL, Beckman). Specific binding accounted for 76% of the total binding, which was 11 000 to 20 000 dpm/assay vial. The [<sup>3</sup>H]flunitrazepam (81.8 Ci/mmol) binding assay was performed as previously described.<sup>23</sup>

[<sup>3</sup>H]-α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid ([<sup>3</sup>H]AMPA) binding was performed as previously described.<sup>24</sup> Assay vials contained 0.5 mg of membrane protein, 5 nM [<sup>3</sup>H]-AMPA (Du Pont NEN, 27.6 Ci/mmol), 100 mM KSCN, various concentrations of test compounds, 2.5 mM CaCl<sub>2</sub>, 30 mM Tris-HCl (pH 7.4 at 4 °C) in a final volume of 0.5 mL. Nonspecific binding was defined using 1 mM L-glutamate. Specific binding accounted for >85% of total binding. Following incubation on ice for 60 min, bound ligand was collected by centrifugation.

[<sup>3</sup>H]Kainate binding was performed as previously described.<sup>25</sup> Assay vials contained 0.5 mg of membrane protein (prepared as above for [<sup>3</sup>H]AMPA binding), 5 nM [<sup>3</sup>H]kainate (Du Pont NEN, 58 Ci/mmol), various concentrations of test compounds, 50 mM Tris-HCl (pH 7.4 at 4 °C) in a final volume of 1 mL. Nonspecific binding was defined using 100 μM unlabeled kainic acid. Specific binding accounted for >80% of the total binding. Following incubation on ice for 60 min, bound ligand was collected by centrifugation.

**Neuroprotective Activity.** Male gerbils (Tumblebrook Farms, 60–80 g) were treated with either water (*n* = 24), phenylcyclidine hydrochloride (15 mg/kg ip, *n* = 12), **28** (40 mg/kg ip, *n* = 12), **30** (40 mg/kg ip, *n* = 11), or **45** (40 mg/kg ip, *n* = 9) 20 min prior to a 5-min hypoxic episode. The gerbils were anesthetized with 3–4% halothane, a midline incision was made, and both carotid arteries were exposed. Hypoxia was induced

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by occluding the carotid arteries with 7-mm Mayfield aneurysm clips for 5 min. Interruption of the carotid blood flow was confirmed by observation of the blanching of radial arteries of the retina using an ophthalmoscope. Following removal of the clips, the incision was closed and the animal was allowed to recover. In sham animals, the carotids were exposed, and each animal was maintained under anesthesia for 5 min. After complete recovery from surgery, animals were returned to home cages. Seven days

after surgery, the gerbils were killed and their brains were prepared for histological evaluation. Left and right hemispheres were assessed separately for hippocampal damage by three independent investigators who were blind to the treatments. Damage was quantified by using a scoring system which has previously been reported.<sup>14</sup> Damage scores for each group were averaged to obtain the reported values. Comparisons were made by using the Student's *t* test with the significance level set at  $p < 0.05$ .

## Binding of Indolylalkylamines at 5-HT<sub>2</sub> Serotonin Receptors: Examination of a Hydrophobic Binding Region

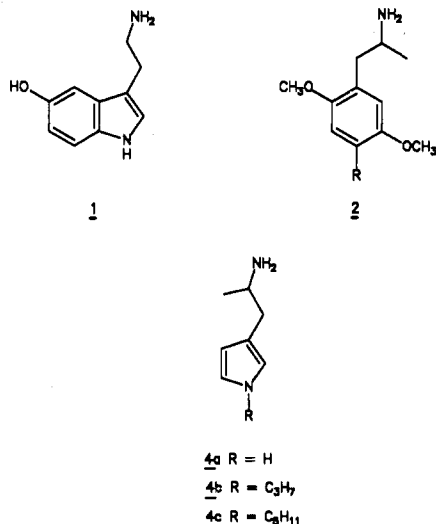
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Received March 16, 1990

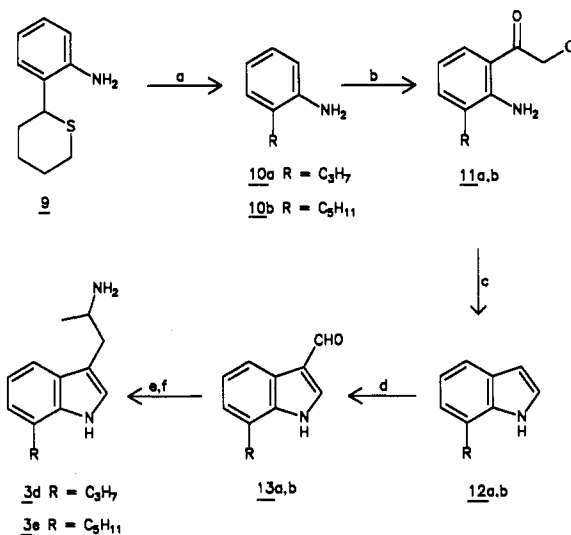
Taking advantage of a proposed hydrophobic region on 5-HT<sub>2</sub> receptors previously identified by radioligand-binding studies utilizing various phenylisopropylamine derivatives, we prepared and evaluated several *N*<sub>1</sub>- and/or *C*<sub>7</sub>-alkyl-substituted derivatives of  $\alpha$ -methyltryptamine in order to improve its affinity and selectivity. It was determined that substitution of an *n*-propyl or amyl group has similar effect on affinity regardless of location (i.e., *N*<sub>1</sub> or *C*<sub>7</sub>). The low affinity of several *N*<sub>1</sub>-alkylpyrroleethylamines suggests that the benzene portion of the  $\alpha$ -methyltryptamines is necessary for significant affinity. Whereas tryptamine derivatives generally display little selectivity for the various populations of 5-HT receptors, *N*<sub>1</sub>-*n*-propyl-5-methoxy- $\alpha$ -methyltryptamine (**3h**) binds with significant affinity ( $K_i = 12$  nM) and selectivity at 5-HT<sub>2</sub> receptors relative to 5-HT<sub>1A</sub> ( $K_i = 7100$  nM), 5-HT<sub>1B</sub> ( $K_i = 5000$  nM), 5-HT<sub>1C</sub> ( $K_i = 120$  nM), and 5-HT<sub>1D</sub> ( $K_i > 10000$  nM) receptors. As a consequence, this is the most 5-HT<sub>2</sub>-selective indolylalkylamine derivative reported to date.

The 5-HT<sub>2</sub> population of serotonin (5-hydroxytryptamine; 5-HT) receptors is currently of clinical interest because of its potential role in cardiovascular function and possible involvement in various mental disorders such as schizophrenia, depression, hallucinations, and anxiety (see ref 1 for a review). To date, there are two major classes of 5-HT<sub>2</sub> agonists: indolylalkylamines, such as 5-HT (1)



itself, and phenylisopropylamines, such as certain 4-substituted derivatives of 1-(2,5-dimethoxyphenyl)-2-amino-propane (2,5-DMA; **2**, R = H) (e.g. **2**, R = methyl, *n*-propyl, and bromo). In general, indolylalkylamines are nonselective agents that bind at multiple populations of 5-HT receptors.<sup>2,3</sup> The phenylisopropylamines, on the other hand, are considerably more selective and bind primarily at 5-HT<sub>2</sub> sites (with a significant, though lower, affinity

### Scheme I<sup>a</sup>



<sup>a</sup> (a) Raney Ni; (b) BCl<sub>3</sub>/AlCl<sub>3</sub>, ClCH<sub>2</sub>CN; (c) NaBH<sub>4</sub>; (d) POCl<sub>3</sub>/DMF; (e) EtNO<sub>2</sub>; (f) LiAlH<sub>4</sub>.

for 5-HT<sub>1C</sub> sites);<sup>4</sup> however, certain of these agents may only be partial agonists.<sup>5</sup> The selectivity and affinity of the phenylisopropylamines for 5-HT<sub>2</sub> sites is related to the nature of the 4-position substituent; specifically, we have found that high affinity is associated with increased lipo-

- (1) Glennon, R. A. *Neurosci. Biobehav. Rev.* 1990, 14, 35.
- (2)  $\alpha$ -Methyl-5-HT has been claimed by some to be a 5-HT<sub>2</sub>-selective agent. However, we have recently shown (Ismail, A. M.; Titeler, M.; Miller, K. M.; Smith, T. S.; Glennon, R. A. *J. Med. Chem.* 1990, 33, 755) that  $\alpha$ -methyl-5-HT is not nearly as selective as previously suspected.
- (3) Glennon, R. A. *J. Med. Chem.* 1987, 30, 1.
- (4) Titeler, M.; Lyon, R. A.; Glennon, R. A. *Psychopharmacology* 1988, 94, 213.
- (5) Glennon, R. A. *Neuropsychopharmacology*, in press.

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