STRUCTURE-ACTIVITY RELATIONSHIP OF s-TRIAZOLO-1,4-BENZODIAZEPINES IN CENTRAL NERVOUS DEPRESSANT ACTION

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A variety of the central depressant effects of 1, 4-benzodiazepines have been well documented (1-3). Shimamoto and Takaori (4), and Nakajima et al. (5) also have reported the potent sedative, taming, muscle relaxant and anti-convulsive properties of 1-alkylcarbamoyl-1, 4-benzodiazepin-2-ones synthesized by Usui et al. (6). Most of the chemical studies in this area have been devoted to syntheses of the derivatives of 1, 4-benzodiazepines with relatively simple substituents at 1, 2 or 3 position or in phenyl ring as exemplified by chlordiazepoxide, diazepam, oxazepam and nitrazepam. On the other hand, Meguro and Kuwada (7) in this Chemical Laboratories have synthesized a number of novel 6-phenyl-4H-s-triazolo-[4, 3a][1, 4] benzodiazepines with a tricyclic ring system.

The present report deals with the anticonvulsive, muscle relaxant, sedative and taming effects of these novel compounds in small experimental animals. In these respects, it was proved that several derivatives including 8-chloro-6-phenyl-4H-s-triazolo-[4, 3a][1, 4] benzodiazepine (D-40TA) and 1-methyl-8-chloro-6-phenyl-4H-s-triazolo [4, 3a][1, 4] benzodiazepine (D-65MT) were more effective in the central depression than diazepam and nitrazepam.

METHODS

Male ICR-JCL mice weighing 18-23 g and male SD-JCL rats weighing 250-350 g were used. All the test agents suspended in 5% arabic gum solution were administered orally or intraperitoneally. The ED50 of the test agents cited in most of the tests were calculated by the method of Litchfield-Wilcoxon (8) unless otherwise described. The other agents used were metrazol (Cardiazol®), methylhexabital sodium (Cyclopan natrium®) and morphine HCl.

Anticonvulsive effects in mice: Groups of mice consisting of 5 to 8 each were subjected to delivery of supramaximal electroshock (MES) of AC 400 V, 30 mA for 0.2 seconds on both eyeballs by the technique of Swinyard et al. (9) 45 minutes after oral administration of the test agents in various doses. The ED50 of the test agent preventing the tonic extension of the hind limbs in half the mice were determined.

The similarly pretreated groups of mice with the test agents were challenged with subcutaneous injection of 150 mg/kg of metrazol which caused the tonic convolution and
death within 10 minutes in all untreated animals. The absence of the tonic convulsion for more than 1 hour was taken as a measure of antimentrazol activity of the test agent.

**Muscle relaxant effects in mice:** The muscle relaxing effect was evaluated according to the traction and inclined screen tests at 10- or 30-minute intervals after oral administration of the test agents in groups of 5-8 mice. The muscle relaxant ED₅₀ was determined from the number of mice which slid down within 1 minute after being placed on a 60° inclined steel wire screen. The muscle relaxant and sedative ED₅₀ was also determined from the number of mice which lost the body traction or fell off within 30 seconds when the animals were suspended with forepaws on a horizontal wire of 2 mm in diameter.

**Antimorphine effect in mice:** Groups of 5 mice were treated with subcutaneous injection of 50 mg/kg of morphine HCl 30 minutes after oral administration of either the test agent or saline. The animals were placed individually in an 1 beaker, and the frequency of circle movements and the maximum tail response were determined for 30 seconds each 6 times at 10-minute intervals after morphine challenge. Tail response was rated by the method of Holton (10). The total value of 6 determinations for each response was calculated in each group, and the ED₅₀ producing 50% decrease of the value was estimated on semi-logarithmic paper.

**Calming effect in septal lesioned rats:** The electrolytic lesion in bilateral septal areas of the rats was produced by transmitting a direct current of less than 2 mA for 20 seconds through monopolar electrodes oriented according to the map of Krieg (11) under pentobarbital anesthesia. Several days after surgical operation, the behavioral hyperirritability of each rat was evaluated on a rating scale described by King and Meyer (12); startle or flight reaction to a rod presented before rat's eye, ii) startle response to a light tactile stimulation of the back with a rod, iii) struggle to hanging the body by clipping the tail with tongs, iv) muscular tone and resistance to handling, v) vocalization and vi) urination and defecation during the above testing. Each of these parameters was rated from 0 for no response to 4 for extremely violent response before and at 30-minute intervals after the intraperitoneal administration of the test agents at four dose levels in groups of 4 or 5 rats. The ED₅₀ producing 50% decrease of the score was graphically determined on semi-logarithmic paper.

**Potentiation of barbiturate hypnosis in mice:** Groups of 10 mice pretreated orally with various doses of the test agents received nonhypnotic dose of methylhexabital sodium (60 mg/kg) 30 minutes latter. Thereafter, the loss of righting reflex lasting for more than 30 seconds was considered as an indication of the potentiating effect of the test agents. The ED₅₀ of the test agent to induce the loss of righting reflex in half the mice given barbiturate was determined.

**RESULTS AND DISCUSSION**

The chemical structures and pharmacological activity of s-triazolobenzodiazepines tested are shown in Table 1. Chlordiazepoxide, diazepam, oxazepam, medazepam and nitrazepam were used as the reference compounds. Many of s-triazolobenzodiazepines were
TABLE 1. Pharmacological activity of s-triazolobenzodiazepines (ED<sub>50</sub>, mg kg).

![Chemical structure](image)

R<sub>1</sub>-C—N

N—C

N—CH<sub>2</sub>

C—N

R<sub>2</sub>

R<sup>+</sup>

| Compd. No. | Substituents | Anticonvulsive activity Mice, p.o. | Muscle relaxant activity Mice, p.o. | Calming activity Mice, p.o. | Rats, i.p. | Sedative activity Mice, p.o. |
|------------|-------------|-----------------------------------|-----------------------------------|-----------------------------|-------------|-----------------------------|
|            | R<sub>1</sub> R<sub>2</sub> R<sub>3</sub> | Antimetrazol | Antimax. shock | Inclined screen | Traction | Antimorphine Circle | Tail | Antiseptal irrit. | Potentiation of MHB hypnosis |
| 1          | H H H       | 3.0 | 14.8 | >200 | >50 | >50 | >50 | >50 | >50 | 15.0 |
| 2          | H CH<sub>3</sub> H | 3.9 | 50.0 | >200 | >50 | - | - | >50 | 1.0 |
| 3          | CH<sub>3</sub> H H | 24.0 | >50 | 50.0 | 14.2 | >50 | >50 | 26.0 | 19.1 |
| 4          | CH<sub>3</sub> CH<sub>3</sub> H | 25.5 | 26.0 | 36.0 | 10.0 | >50 | 30.0 | 10.2 | 6.1 |
| 5          | CH<sub>3</sub>O H H | >50 | >50 | 44.0 | 35.0 | 50.0 | 45.0 | 25.0 | 70.0 |
| 6          | CH<sub>3</sub>O CH<sub>3</sub> H | >50 | >50 | 57.0 | 43.0 | 50.0 | 40.0 | 19.8 | 18.0 |
| 7<sup>+</sup> | Cl H H | 2.2 | 4.6 | 6.1 | 0.72 | 12.4 | 2.77 | 4.1 | 0.35 |
| 8<sup>a</sup> | Cl CH<sub>3</sub> H | 0.25 | 1.6 | 3.2 | 0.28 | 3.1 | 0.95 | 2.9 | 0.13 |
| 9          | Cl C<sub>2</sub>H<sub>5</sub> H | 0.47 | 4.3 | 8.8 | 0.92 | 4.1 | 1.65 | 9.1 | 0.47 |
| 10         | Cl CH<sub>3</sub>(CH<sub>2</sub>)<sub>5</sub> H | >50 | >50 | >200 | >50 | >50 | >50 | >50 | - |

<sup>*<sup> Compounds 7 and 8 were also referred to as D-40TA and D65MT, respectively.
| No. | Substituent  | R  | R' | R'' | R''' | R'''' | R''''' | R'''''' | R'''''''' |
|-----|--------------|----|----|-----|------|-------|--------|----------|-----------|
| 11  | Cl           | C<sub>6</sub>H<sub>5</sub> | H  | 15.0 | >50  | >200  | 41.0   | >50      | >50       | >50       | 7.8       |
| 12  | Cl           | C<sub>4</sub>H<sub>9</sub>CH<sub>3</sub> | H  | >50  | >50  | >200  | >50    | >50      | >50       | >50       | >30       |
| 13  | NO<sub>2</sub>| H  | H  | 0.78 | 15.5 | 12.5  | 2.2    | 3.0      | 2.8       | 66.7      | 0.58      |
| 14  | NO<sub>2</sub>| CH<sub>3</sub> | H  | 0.34 | 1.8  | 2.7   | 0.63   | 2.3      | 1.5       | 3.3       | 0.13      |
| 15  | CF<sub>3</sub>| H  | H  | 1.2  | 10.5 | 3.0   | 2.7    | 7.0      | 4.9       | 8.1       | 1.0       |
| 16  | CF<sub>3</sub>| CH<sub>3</sub> | H  | 1.3  | 2.6  | 1.2   | 1.3    | 2.5      | 1.9       | 3.3       | 0.38      |
| 17  | Cl           | H  | CH<sub>2</sub>O | >30  | >50  | >200  | >50    | >50      | >50       | >50       | –         |
| 18  | Cl           | CH<sub>3</sub> | CH<sub>2</sub>O | >30  | >50  | >200  | >50    | >50      | >50       | >50       | –         |
|     | Chlordiazepoxide |      |     | 7.5  | 28.6 | 220.0 | 41.0   | 39.3     | 22.5      | 17.2      | 7.2       |
|     | Diazepam     |      |     | 3.0  | 14.0 | 44.0  | 3.8    | 1.6      | 0.8       | 8.1       | 1.7       |
|     | Oxazepam     |      |     | 0.52 | 1.5  | 61.0  | 2.5    | 3.0      | 4.5       | 16.6      | 2.8       |
|     | Medazepam    |      |     | 7.5  | 13.0 | 132.0 | 27.5   | 11.0     | 6.0       | 9.6       | 3.7       |
|     | Nitrazepam   |      |     | 0.8  | 8.4  | 5.8   | 0.8    | 3.8      | 1.3       | 9.5       | 1.5       |
shown to possess many aspects of the pharmacological properties qualitatively similar
to those of the reference compounds in calming effect, muscle relaxation, anticonvulsion
and sedation in mice and rats. Pharmacological activities of these compounds in each
test will be best compared by the present data obtained by the same investigator, since
most of biological assays show generally the considerable variation depending on the kind
of test, the species and strain of the animal used, and the individual differences of the ob-
servation technique. A top screening dose of the test agent was arbitrarily selected in
each test.

Anticonvulsant effects: As a rule, active compounds prevented metrazol-induced sei-
zure more remarkably than maximal electroshock seizure in mice, though there is one
exception of the compound 4 with similar potency in both seizure tests. Much differences
of the potency between antimetrazol and anti-electroshock effects were encountered in the
compounds 2 and 13. The most active group in antimetrazol effect included the com-
ounds 8, 9, 13 and 14, which were roughly equipotent to oxazepam and nitrazepam. The
second active group consisting of the compounds 1, 2, 7, 15 and 16 were roughly equivalent
to diazepam, while other compounds were either less active than chlordiazepoxide or in-
effective in antimetrazol test. The compounds 7, 8, 9, 14 and 16 included in the most or
second active antimetrazol groups were also classified to the most active group of anti-
electroshock effect, all of these compounds being more effective than diazepam, meda-
ezepam, nitrazepam and chlordiazepoxide. The compounds 1 and 15 were approximately
equipotent to diazepam and medazepam.

Muscle relaxant effect: As regards the compounds 7, 8, 9 and 14, their muscle re-
laxant activities were 4 to 11 times as potent as in the traction test than in the inclined screen
test, and moreover were more or equivalent to nitrazepam, the most active of the reference
compounds, in both tests. The compounds 13, 15 and 16 were also very effective in both
tests.

Calming effects: Antimorphine effect in mice and anti-hyperirritability in septal
lesioned rats were referred to tentatively as calming effect in order to differentiate from
sedative activity in the potentiation of barbiturate hypnosis in mice. In general, the tail
reaction to morphine was depressed by these benzodiazepines more remarkably than was
the circle movement. The most active group of s-triazolobenzodiazepines in preventing
both responses to morphine comprised the compounds 8, 9, 13, 14 and 16, which were
followed by the compounds 15 and 7 in the potency. These most active compounds ap-
peared to be a little more or equipotent to oxazepam or nitrazepam. Furthermore, most
of s-triazolo compounds in the most active antimorphine group, and the compounds 7
and 15 were more effective in calming the hyperirritable rats with septal lesion than dia-
ezepam which was most effective in the reference compounds. In contrast to potent effi-
cacy of the compound 13 in antimorphine effect, it was considerably weak in calming the
septal lesioned rats. On the contrary, both of the compounds 5 and 6 were active in the
latter effect despite almost lacking in antimorphine activity in the dose level of 50 mg/kg.

Sedative effects: All s-triazolobenzodiazepines exhibiting the potent activity in other
test showed the powerful potentiating effects of barbiturate hypnosis in mice, as indicated by the \( ED_{50} \) values of 1 mg/kg or below. Compared with nitrazepam, the most active of the reference compounds, the activity of the compounds 7, 8, 9, 13, 14 and 16 was 2.5-12 times. The compounds 15, 4, 2 and 11 were also considerably effective in the potentiation of barbiturate hypnosis, the effects of the latter two being contrast to their weak activities in other tests.

**Structure and activity relationship:** The modification of \( R_1 \) in phenyl ring was exclusively done in the present study, since this position was most accessible to be substituted in the first step of synthesis. As seen in a series of the compounds with hydrogen or methyl group in \( R_1 \) of s-triazol ring, introduction of such electron-withdrawing substituents as chlor, nitro or trifluoromethyl group in \( R_1 \) produced a pronounced enhancement of the over-all biological activity. However, the enhancing effect was not necessarily parallel to the electron-withdrawing power of substituent, and the compounds with chlor group appeared to be most potent in most of tests as seen in the compounds 7 and 8. On the other hand, the effect of such electron-releasing substituent as methyl or methoxy group in \( R_1 \) was not so remarkable as that of electron-withdrawing groups, but was effective to some extent in enhancing the muscle relaxant and calming activities.

Whatever substituent was introduced in \( R_1 \), substitution with methyl group in \( R_2 \) of s-triazolo ring was always effective in enhancing all parameters of the biological activities. Exploration of the effects of substitution with various kinds of groups in \( R_2 \) was made on the compound 7 with chlor group in \( R_1 \) of phenyl ring. The over-all biological activity was attained to maximum by replacement with methyl group and was decreased slightly by ethyl group. Substitution of alkyl group with long chain such as n-hexyl group, or of bulky phenyl or benzyl group in \( R_2 \) lowered the biological activity remarkably, as can be seen in the compounds 10, 11 and 12.

The effects of substituent in \( R_3 \) of phenyl ring of 6 position were studied only in the case of methoxy group. The presence of methoxy group in \( R_3 \) resulted in almost complete loss of activity, as shown in the compounds 17 and 18.

**SUMMARY**

Central depressant activity of 18 new type of 6-phenyl-4H-s-triazolo [4, 3a] [1, 4] benzodiazepines was studied by 7 standard biological tests in small experimental animals. Most of s-triazolobenzodiazepines with substituents of chlor, nitro or trifluoromethyl group in position 8 as well as hydrogen or methyl group in position 1 were equi- or more potent than the already known 1, 4-benzodiazepines in anticonvulsive, muscle relaxant, calming and sedative effects.

Structure-activity relationship of s-triazolobenzodiazepines can be summarized as follows:

1. Electron-withdrawing substituents in position 8 enhanced the activity, but electron-releasing substituents did not affect the activity remarkably.
2. Introduction of methyl group into position 1 was always most effective in increas-
ing the activity, and substituents larger than ethyl in the same position showed an unfavorable effect to the activity.

3. Substitution of methoxy group in para-position of 6-phenyl ring caused a pronounced decrease in activity.

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