ANTI-INFLAMMATORY PROPERTIES OF A NEWLY SYNTHETIZED COMPOUND, 6-CHLORO-4-OXYIMINO-1-PHENYL-1,2,3,4-TETRAHYDROQUINOLINE (M-7074)

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Abstract—Anti-inflammatory properties of a newly synthetized compound, 6-chloro-4-oxyimino-1-phenyl-1,2,3,4-tetrahydroquinoline (M-7074), have been investigated. Anti-edema activities of M-7074 were more potent than those of phenylbutazone in carrageenin, bradykinin and mustard edema tests in rats. M-7074 showed an inhibitory effect on adjuvant arthritis, especially on the secondary inflammatory lesions in rats. Inhibitory effect of M-7074 on cotton-pellet granuloma formation was all but equal to that of phenylbutazone in rats. M-7074 also showed inhibitory effects on ultraviolet erythema in guinea-pigs and increased vascular permeability in mice, moderate analgesic activity in rats and mice, and antipyretic activity in rats. Furthermore, inhibitory effects of M-7074 on prostaglandin biosynthesis in guinea-pig lung homogenate and arachidonic acid-induced aggregation of rabbit platelets were fairly equal to those of indomethacin. However, M-7074 showed no effect on humoral nor cellular immunity in mice. M-7074 possessed no ulcerogenic activity in rats and mice, and LD50 value of M-7074 was 8.01 g/kg, p.o. in mice. These data indicate that M-7074 is a novel anti-inflammatory agent with large margin of safety.

6-chloro-4-oxyimino-1-phenyl-1,2,3,4-tetrahydroquinoline (M-7074) shown in Fig. 1 is a newly synthetized compound. The results of experiments in which anti-inflammatory properties of M-7074 were compared with those of phenylbutazone and other known anti-inflammatory drugs are described herein. Acute toxicity of M-7074 in male mice was also studied.

MATERIALS AND METHODS

Drugs: M-7074 is a yellowish-white needle crystalline powder readily soluble in methanol and chloroform, and not so soluble in water. The melting point is 136–137°C. M-7074 and other drugs suspended or solubilized in 5% arabic gum solution were given orally, in in vivo experiments. All tested drugs used in in vitro experiments were
solubilized in 0.1N-NaOH solution and each alkaline solution was adjusted to pH 7 with 1N-HCl solution.

Animals: The experiments were conducted using male ddY mice and male Wistar rats. These animals were used after an acclimatization period, of at least 5 days to the laboratory environment. Illumination was automatically controlled with 12 hr of dark and the temperature was kept at 23±2°C and the relative humidity at 55±5%. The animals were housed in standard metal cages and provided food and water ad libitum.

Effect on carrageenin and bradykinin edema in rats: One hour after p.o. administration of various doses of test compounds to groups of 3 or 5 rats each, weighing 90–110 g, 0.1 ml of 1% carrageenin suspended in 0.9% sterile saline or 0.1 ml of bradykinin 5 mg in 0.9% sterile saline solution was given s.c. into the sole of the right hind paw. Volume of the injected foot to the level of the lateral malleolus was measured by water displacement immediately before drug administration and 3 hr after carrageenin injection or 1 hr after bradykinin injection. The intensity of edema expressed as percent increase in the foot volume was calculated by the following equation: (post-injection volume—pre-injection volume) x 100/pre-injection volume. Percent inhibition of edema was calculated by following equation: (mean edema intensity in control group—mean edema intensity in treated group) x 100/mean edema intensity in control group. ED50 values were then calculated according to the probit method.

Effect on mustard edema in rats: Rats, weighing 90–110 g, were given 0.1 ml of 5% mustard suspended in 0.9% sterile saline s.c. the sole of the right hind paw. Drugs were given p.o. twice a day for the first 3 days and the volume of the hind paw was estimated daily for 7 days by water displacement, as described in the edema test.

Effect on ultraviolet erythema in guinea-pigs: Guinea-pigs, weighing 300–400 g, were separated at random into groups of 5. Based on the method of Winder et al. (1), the guinea-pigs were put in a rubber glove with three closely spaced 5 mm holes at the point corresponding to the middle of the animals torso. One hour after p.o. administration of drugs, the animals were exposed at their torso to the ultraviolet lamp (H-400 type, Toshiba Co., Ltd.) 15 cm away for 60 seconds. The intensity of erythema was quantitated according to the method of Winder et al. (1). ED50 value were calculated according to Litchfield-Wilcoxon’s method.

Effect on increased vascular permeability in mice: Based on the method of Whittle (2), 0.1 ml of a 4% Pontamine sky blue solution was given i.v. to groups of 6 mice, weighing 18–22 g. 30 min after p.o. administration. Fifteen min later, 10 ml of a 0.6% acetic acid solution was given i.p. Thirty min after the injection of acetic acid solution, the mice were killed and the viscera were irrigated with distilled water. The combined washings made up to 10 ml and the absorption was read at 510 nm. The effects in terms of permeability were expressed as percentage inhibitions of control value. ED50 value was calculated according to the probit method.

Effect on weight of cotton pellet granuloma in rats: Based on the method of Meier et al. (3), a sterilized cotton pellet, weighing 30 mg, was implanted s.c. into the scapular region of rats anesthetized with ether. Drugs were given p.o. once daily ×7 from the day of implantation to groups of 6 rats each, weighing 160–200 g. The animals were killed on the eighth day and each granuloma together with the pellet was removed to assess the dry weight.

Effect on adjuvant arthritis in rats: Eight individually caged rats, weighing 130–170 g, were used in each test group. An adjuvant mixture of a volume of 0.05 ml containing
0.6 mg of Mycobacterium (Difco Laboratories) suspended in liquid paraffin was given i.d. into the volar surface of the right hind paw of rats. Drugs were given p.o. daily ×21. Volume of the injected and non-injected feet were measured by water displacement, as for the edema test.

Effect on the number of writhings induced by acetic acid in mice: Based on the method of Koster et al. (4), 10 ml/kg of 0.6% acetic acid was given i.p. to groups of 5 mice each weighing 17–20 g, 30 min after p.o. drug administration. The number of writhing per mouse was counted for a 20 min period immediately after injection of acetic acid. Percent inhibition was calculated from the mean value of each treated group and that of the control group. ED50 value was calculated according to the probit method.

Effect on pain threshold in rats tested by Randall-Sellito’s method: Based on the method of Randall and Sellito (5), 0.1 ml of 2% Brewer’s yeast suspension was given s.c. into the sole of the right hind paw 30 min after p.o. administration of drugs to groups of 6 rats, each weighing 110–130 g. The pain thresholds of the injected and non-injected feet were measured at 1, 2, 3 and 4 hr later. Total pain threshold for 4 hr was expressed as analgesic index: Total pain threshold for 4 hr in treated group/total pain threshold for 4 hr in control group.

Antipyretic activity in rats: Rats, weighing 180–210 g, were given 100 μg/kg i.v. of lipopolysaccharide B from E. coli, as a pyrogen, following food deprivation for 18 hr. Drugs were given p.o. to groups of 6 rats each with a rectal temperature of over 0.8°C. The rectal temperature were then measured at 1 hr intervals using a thermometer and thermister probe inserted to a constant depth of 3.5 cm.

Effect on delayed-type hypersensitivity in mice: Based on the method of Turk (7), drugs were given p.o. to groups of 5 mice each weighing 18–21 g, 1 hr after applying 7% of picryl chloride in ethanol to the surface of the abdominal skin. Seven days after the treatment, the ear of the animal was painted with 1% picryl chloride in olive oil. The thickness of the applied ears was measured.

Effect on arachidonic acid-induced aggregation of rabbit platelets: Platelet rich plasma (PRP) was prepared from rabbit blood containing 3.8% sodium citrate. Based on the method of Born (8) and O’Brien (9), 0.1 ml of test compound was added to 0.9 ml of PRP containing 5×10^8 platelets/ml. One minute later, 0.1 ml of arachidonic acid (1 mg/ml) was added and maximal aggregation was estimated by the change in the light transmission. IC50 values were calculated according to the probit method.

Effect on prostaglandin biosynthesis using guinea-pig lung: The lungs of guinea-pigs, weighing 300–500 g were homogenized in four vol. of 0.25 M sucrose solution, and centrifuged at 9,000×g for 60 min. The pellet was suspended in 0.4 M phosphate buffer (pH 7.4) and used as biosynthetic system of prostaglandins.

One ml of reaction solution (pH 7.4) containing biosynthetic system of prostaglandins (including 2 mg of protein), 50 μg of reduced glutathione, 20 mg of arachidonic acid and test compound was incubated for
30 min at 37°C. The reaction was stopped by immersion in boiling water for 1 min. Based on the method of Vane (10), the reaction solution was bioassayed for PGF2α-like activity using a rat fundus strip with oxygenated Tyrode's solution containing a mixture of antagonists to make the assay more specific.

**Ulcerogenic activity in rats:** Three and a half hours after p.o. drug administration to groups of 5 rats weighing 150–170 g and deprived of food for 18 hr, the stomach was removed, sectioned along its curvature and examined for the presence of gastric ulcer.

**Ulcerogenic activity in mice:** Based on the method of Fukawa et al. (11), four hr after p.o. administration to groups of 5 mice weighing 18–22 g and deprived of food for 18 hr, the stomach was removed, sectioned along its curvature and examined for the presence of gastric ulcer.

**Acute toxicity in mice:** Mice, weighing 23–27 g, were divided into groups of 6 and given 5.0, 6.0, 7.2, 8.6 and 10.3 g/kg of the drug orally. Mortality data were recorded for 7 days after drug administration. LD50 values were calculated according to Litchfield-Wilcoxon's method.

### RESULTS

**Effects on carrageenin and bradykinin edema in rats:** M-7074 showed a marked inhibition of carrageenin and bradykinin edema in rats. ED30 values of M-7074, phenylbutazone, aspirin and indomethacin in carrageenin edema were 28.0, 53.0, 160 and 6.84 mg/kg, respectively (Table 1).

ED30 values of M-7074 and phenylbutazone in bradykinin edema were 136 and >200 mg/kg, respectively (Table 1).

**Effect on mustard edema in rats:** M-7074 showed a significant inhibition of mustard edema in rats. The inhibitory effect of M-7074 was more potent than that of phenylbutazone (Fig. 2).

**Effect on ultraviolet erythema in guinea-pigs:** M-7074 showed a dose-dependent inhibition of ultraviolet erythema. ED50

| Table 1. Effects of M-7074 on carrageenin and bradykinin edema in rats |
|---------------------------------|------------------|------------------|------------------|------------------|
| **Compound**                   | **mg/kg p.o.**   | **Carrageenin**  | **Bradykinin**   |
|                                |                  | **Inhibition (%)** | **ED30 mg/kg** | **Inhibition (%)** | **ED30 mg/kg** |
| M-7074                         | 20               | 19.6             | 28.0            |
|                                | 40               | 41.8             |                  |
|                                | 80               | 58.9             |                  |
|                                | 100              |                  | 27.8            |
|                                | 200              |                  | 33.9            |
| Phenylbutazone                 | 25               | 18.8             | 136             |
|                                | 50               | 31.9             | >200            |
|                                | 100              | 37.0             |                  |
|                                | 200              |                  |                  |
| Aspirin                        | 50               | 8.8              |                  |
|                                | 100              | 18.4             | 160             |
|                                | 200              | 36.5             |                  |
| Indomethacin                   | 2.5              | 20.6             | 6.84            |
|                                | 5                | 25.5             |                  |
|                                | 10               | 34.5             |                  |

Each value of percent inhibition indicates the mean value from 3 rats in carrageenin edema test or 5 rats in bradykinin edema test.
values of M-7074 and phenylbutazone were 11.7 and 5.83 mg/kg, respectively (Table 2).

Effect on increased vascular permeability in mice: M-7074 showed a dose-dependent inhibition of increased vascular permeability. ED50 values of M-7074 and phenylbutazone were 125 and 68.1 mg/kg, respectively (Table 3).

Effect on weight of cotton-pellet granuloma in rats: M-7074 caused 14.1 and 14.4% inhibition of the granuloma formation at daily doses of 100 and 200 mg/kg, respectively. On the other hand, daily doses of 100 and 200 mg/kg of phenylbutazone caused 10.2 and 19.2% inhibition, respectively (Table 4).

Effect on adjuvant arthritis in rats: M-7074 showed a dose-dependent preventive effect on the development of adjuvant arthritis, especially on the secondary inflammatory lesions. This preventive effect of M-7074 was more potent than that of phenylbutazone, and more marked in the non-injected foot. The inhibitory action of M-7074 on the primary stage of inflammation was equal to or slightly less potent than that of phenylbutazone (Fig. 3).

Effect on the number of writhings induced by acetic acid in mice: M-7074 showed a dose-dependent preventive effect on the

| Table 2. Effect of M-7074 on ultraviolet erythema in guinea-pigs |
|-------------------|-----------------|------------------|-----------|
| Compound         | mg/kg p.o.     | Effectiveness    | ED50 mg/kg |
|                   |                 |                  |           |
| Phenylbutazone   | 50              | 1/5              |           |
|                   | 100             | 3/5              | 11.7      |
| M-7074           | 50              | 4/5              |           |
|                   | 105             | 4/5              |           |

Each value of percent inhibition indicates the mean value from 6 mice.

| Table 3. Effect of M-7074 on increased vascular permeability in mice |
|-------------------|-----------------|-------------------|-----------|
| Compound         | mg/kg p.o.     | Inhibition (%)    | ED50 mg/kg |
|                   |                 |                   |           |
| Phenylbutazone   | 50              | 42.7              | 125       |
|                   | 100             | 46.7              |           |
|                   | 200             | 54.8              |           |
| M-7074           | 10              | 44.4              | 68.1      |
|                   | 200             | 61.7              |           |

| Table 4. Effect of M-7074 on weight of cotton pellet granuloma in rats |
|-----------------------|-----------------|----------------|-----------|
| Compound              | mg/kg p.o.     | Granuloma dry weight (mg) | Inhibition (%) |
| Control               | 33.4±1.7        |                  |           |
| M-7074                | 28.7±1.7        | 14.1             |           |
|                       | 28.6±1.9        | 14.4             |           |
| Phenylbutazone        | 200             | 30.0±1.7         | 10.2      |
|                       | 26.9±2.3*       | 18.2             |           |

Each value indicates mean±s.e. of 6 rats. *: Significant difference from control group at p<0.05.
wring syndrome. ED50 values of M-7074 and aminopyrine were 84.0 and 24.8 mg/kg, respectively (Table 5).

**Effect on pain threshold in rats determined by Randall-Sellito’s test:** M-7074 showed a moderate effect on inflamed foot, but no effect on the normal foot (Table 6).

**Antipyretic activity in rats:** M-7074 showed a moderate antipyretic activity, at a dose of 200 mg/kg. The activity of M-7074 was less potent than that of phenylbutazone (Fig. 4).

**Effect on the number of plaque forming cells collected from mice:** M-7074 showed no effect on the number of plaque forming cells at oral doses of 1, 10 and 100 mg/kg.

**Effect on the delayed-type hypersensitivity in mice:** M-7074 showed no effect on the delayed-type hypersensitivity in mice at oral doses of 1, 10 and 100 mg/kg.

**Effect on arachidonic acid-induced aggregation of rabbit platelets:** M-7074 showed a marked inhibition of aggregation of rabbit

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**Table 5. Effect of M-7074 on number of writhings induced by acetic acid in mice**

| Compound    | mg/kg p.o. | Inhibition (%) | ED50 mg/kg |
|-------------|------------|----------------|------------|
| M-7074      | 50         | 27.8           | 84.0       |
|             | 100        | 57.8           |            |
| Aminopyrine | 25         | 41.2           | 24.8       |
|             | 50         | 61.2           |            |

Each value of percent inhibition indicates the mean value from 5 mice.

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**Table 6. Effect of M-7074 on pain threshold of rats in Randall-Sellito’s tests (5)**

| Compound    | mg/kg p.o. | Analgesic index |
|-------------|------------|-----------------|
|             |            | Inflamed foot   |
|             |            | Normal foot     |
| Control     | 1.00±0.03  | 1.00±0.03       |
| M-7074      | 1.14±0.06  | 0.99±0.02       |
| Aminopyrine | 1.27±0.04**| 1.09±0.02*      |
| Phenylbutazone | 1.26±0.04** | 1.06±0.02      |

Each value indicates mean±s.e. of 6 rats. * and **: Significant difference from control group at p<0.05 and p<0.01, respectively.
platelets. IC50 values of M-7074 and indomethacin were 4.82 and 7.84 µg/ml, respectively (Table 7).

Effect on prostaglandin biosynthesis: M-7074 showed a marked inhibition on prostaglandin biosynthesis. IC50 values of M-7074 and indomethacin were 4.01 and 2.23 µg/ml, respectively (Table 8).

Ulcerogenic activity in rats: M-7074 showed no ulcerogenic activity at a dose of 400 mg/kg in rats. Both phenylbutazone and aspirin induced multiple gastric ulcers at doses of 100 and 200 mg/kg, respectively (Table 9).

Ulcerogenic activity in mice: M-7074 showed no ulcerogenic activity at a dose of 4,000 mg/kg in mice. On the other hand, ulcer index tended to increase after administration of 200 mg/kg of phenylbutazone and significantly increased after administration of 100 mg/kg of aspirin (Table 10).

Acute toxicity in mice: In most cases, death occurred within the first 24 hr following administration of drug. Body weight decreases were observed in all survived mice. LD50 value of M-7074 was 8.01 g/kg (Table 11).

Table 7. Effect of M-7074 on arachidonic acid-induced aggregation of rabbit platelets

| Compound    | µg/ml | Inhibition (%) | IC50 (µg/ml) |
|-------------|-------|----------------|--------------|
|             | 1.25  | 20±3.1         |              |
| M-7074      | 2.5   | 37±4.0         | 4.82         |
|             | 5.0   | 72±3.5         |              |
| Indomethacin| 5.0   | 10±2.0         |              |
|             | 7.5   | 33±3.1         | 7.84         |
|             | 10.0  | 92±2.5         |              |

Each value indicates mean±s.e. of 3 experiments.

Table 8. Effect of M-7074 on prostaglandin biosynthesis

| Compound    | µg/ml | Inhibition (%) | IC50 (µg/ml) |
|-------------|-------|----------------|--------------|
|             | 3.0   | 22±2.0         |              |
| M-7074      | 5.0   | 74±2.1         | 4.01         |
|             | 10.0  | 94±3.1         |              |
| Indomethacin| 2.0   | 29±3.6         | 2.23         |
|             | 3.0   | 75±2.5         |              |
|             | 5.0   | 100±0.3        |              |

Each value indicates mean±s.e. of 3 experiments.

Table 9. Ulcerogenic activity of M-7074 in rats

| Compound    | mg/kg p.o. | No. of rats Ulcerogenic/Tested |
|-------------|-------------|--------------------------------|
| Control     |             | 0/5                            |
| M-7074      | 400         | 0/5                            |
| Aspirin     | 100         | 3/5                            |
| Phenylbutazone | 200     | 5/5                            |

Table 10. Ulcerogenic activity of M-7074 in mice

| Compound    | mg/kg p.o. | Ulcer index(n) (mm) |
|-------------|-------------|---------------------|
| Control     |             | 0.7±0.3             |
| M-7074      | 4.000       | 0.6±0.5             |
| Aspirin     | 200         | 2.7±0.7*            |
| 400         | 4.6±2.1     | 9.2±1.9**           |
| Phenylbutazone | 100     | 0.9±0.2             |
| 200         | 2.0±0.7     |                     |

a) Total length of gastric erosive lesions.
Each value indicates mean±s.e. of 5 mice.
* and **: Significant difference from control group at p<0.05 and p<0.01, respectively.
Non-steroidal anti-inflammatory drugs, in general have been classified as acidic and non-acidic agents according their chemical structure. It is well known that acidic non-steroidal anti-inflammatory drugs are effective on adjuvant arthritis and granuloma formation but that they do possess potent ulcerforming activity in experimental animals. On the other hand, non-acidic agents show a potent analgesic activity and an inhibitory effect on increased vascular permeability but are less effective on adjuvant and granuloma formation (12-14).

Therefore, the anti-inflammatory properties of M-7074 were compared with those of phenylbutazone and other non-steroidal anti-inflammatory agents.

M-7074 showed anti-edema activities. The anti-carrageenin edema activity of M-7074 was twice as potent as that of phenylbutazone and fivefold that of aspirin, in rats. Anti-bradykinin and mustard edema activities of M-7074 were more potent than those of phenylbutazone. In the adjuvant arthritis test, the effect of M-7074 was all but equal that of phenylbutazone in the primary stage of inflammation. The effect of M-7074 was more potent than that of phenylbutazone on the secondary inflammatory lesions in the adjuvant arthritis and is assumed to parallel human rheumatism (15-17). M-7074 also showed inhibited considerably the edema in the non-injected foot in the adjuvant arthritis test.

The inhibitory effect of M-7074 on granuloma formation appears to be much the same as that of phenylbutazone.

However, the inhibitory effects of M-7074 on ultraviolet erythema and increased vascular permeability were less than those seen with phenylbutazone. The analgesic and antipyretic activities of M-7074 were also less potent than those of aminopyrine or phenylbutazone.

On the other hand, M-7074 had a potent inhibitory effect on arachidonic acid-induced aggregation of platelets and prostaglandin biosynthesis, suggesting that anti-thrombotic effect of M-7074 should be further investigated. In fact, anti-inflammatory agents which showed inhibitory effects on aggregation of platelets have been assessed as possible anti-thrombotic agents, at the clinical stage (20, 21).

The ulcerogenic activity of acidic non-steroidal anti-inflammatory drugs is also attributed to inhibitory effects on prostaglandin biosynthesis (22). This hypothesis, however, was inconsistent with the results that M-7074 showed no ulcerogenic activity in rats and mice despite potent inhibitory effects on prostaglandin biosynthesis and aggregation of platelets. Recently, Fahrenholtz et al. (23) reported findings in which a certain agent had no ulcerogenic activity but did have inhibitory effects on prostaglandin biosynthesis. Therefore, the role of prostaglandins in ulcer formation requires further consideration.

M-7074 had a large LD50 value. When tested at a high dose of 10.3 g/kg of M-7074,
only a moderate reduction in locomotor activity was seen. These results indicate that M-7074 does not depress the central nervous system to any great extent, and that it does have a large margin of safety.

Considering the chemical structure of M-7074, this compound is apparently not acidic. Therefore, it is reasonable that the pharmacological profile of M-7074 in these laboratory tests is not always parallel to that of acidic non-steroidal anti-inflammatory agents such as phenylbutazone. In the present work, M-7074 showed strong anti-edema activity and significant inhibitory effects on secondary inflammatory lesions in induced adjuvant arthritis, prostaglandin biosynthesis and aggregation of platelets. These actions of M-7074 are similar to those of acidic agents. M-7074, however, showed moderate inhibitory effects on ultraviolet erythema and no ulcerogenic activity, in contrast to findings with acidic agents. M-7074 had a weak analgesic effect and a mild inhibitory effect on increased vascular permeability, in contrast to observations of non-acidic agents.

Thus, M-7074 appears to be a novel anti-inflammatory agent.

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