

**Zeitschrift:** Acta Tropica  
**Herausgeber:** Schweizerisches Tropeninstitut (Basel)  
**Band:** 41 (1984)  
**Heft:** 3

**Artikel:** Methyl 5(6)-4-2-pyridyl piperazino carbamoyl benzimidazole-2-carbamate : a new broad spectrum anthelmintic  
**Autor:** Katiyar, J.C. / Visen, P.K.S. / Misra, A.  
**DOI:** <https://doi.org/10.5169/seals-313303>

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Divisions of Parasitology and Medicinal Chemistry, Central Drug Research Institute, Lucknow, India

## Methyl 5(6)-4-2-pyridyl piperazino carbamoyl benzimidazole-2-carbamate – a new broad spectrum anthelmintic

J. C. KATIYAR, P. K. S. VISEN, A. MISRA, SUMAN GUPTA, A. P. BHADURI

### Summary

Methyl 5(6)-4-2-pyridyl piperazino carbamoyl benzimidazole-2-carbamate (CDRI Comp. 81-470) was tested against various nematode and cestode infections in different experimental and domestic animals. The compound showed 100% effectivity against adult of *Ancylostoma ceylanicum* (hookworm) in hamsters (6.25 mg/kg p.o.  $\times$  1), *Nippostrongylus brasiliensis* (trichostrongylid) in rats (100 mg/kg p.o.  $\times$  3), *Hymenolepis nana* (cestode) in rats (25 mg/kg p.o.  $\times$  1) and *Syphacia obvelata* (oxyurid) in mice (12.5 mg/kg p.o.  $\times$  3). It was also found highly effective against artificial and natural helminth parasites of higher animals. The compound removed all, *A. caninum* and *A. ceylanicum* (hookworms) and *Toxocara* sp. (ascarid) from dogs at a dose of 10 mg/kg p.o.  $\times$  3; *A. tubaeformis* (hookworm) and *Toxocara* sp. at 25 mg/kg p.o.  $\times$  3 and 2.5 mg/kg p.o.  $\times$  3 respectively from cats and *Ascaridia galli* (ascarid) at 10 mg/kg p.o.  $\times$  3 from fowl. The compound in doses of 1500 mg/kg by oral route and 1000 mg/kg by i.p. route did not cause any mortality or produce adverse effect in mice. The expanded anthelmintic action and large therapeutic index indicate compound's great anthelmintic potentiality.

**Key words:** anthelmintic; efficacy; intestinal parasites; rodents; dog; cat; fowl; toxicity.

### Introduction

Infestation with helminth parasites is exceedingly common in tropical world. In the absence of suitable biological control methods, chemotherapy remains the only tool to combat these infections. Because of frequent occur-

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Correspondence: Dr. J. C. Katiyar, Central Drug Research Institute, Division of Parasitology, P.O. Box 173, Lucknow-226001, India

rence of concurrent infections by more than one type of helminth parasites, to have a broad spectrum anthelmintic is obviously the need of the day. Of the various anthelmintics in use, mebendazole is the most effective drug. However, this too has limitations because of its teratogenic and embryotoxic effects (Annon, 1975). As such mebendazole is not indicated during pregnancy and in children below 2 years of age (Keystone and Murdoch, 1979). Hence, there is urgency to develop a new anthelmintic with wide spectrum of activity and a high cure rate, minimum side effects and suitable for population based chemotherapy.

In an endeavour to develop a highly effective and nontoxic broad spectrum anthelmintic, a number of variously substituted benzimidazoles were synthesized and tested against a variety of experimental helminth parasites. In this communication, we wish to report the efficacy of the compound methyl 5(6)-4-2-pyridyl piperazino carbamoyl benzimidazole-2-carbamate (CDRI Comp. 81-470) against different helminth parasites of experimental and domestic animals. The chemistry and synthesis of the compound has already been communicated elsewhere (Kumar et al., 1983).

## Materials and Methods

### Drug testing

The test Comp. 81-470 and the reference drug mebendazole (Mebex, CIPLA, India) being insoluble in water, were made to fine suspension with little of Tween 80. Three to five animals were used for each dose level. The experimental animals were sacrificed under deep chloroform anaesthesia.

1. *Ancylostoma ceylanicum* (hookworm): The drug testing was carried out by the technique of Ray et al. (1978) as modified by Misra et al. (1981). Golden hamsters of either sex weighing 40–60 g served as the experimental host. These were orally infected with  $60 \pm 5$  infective larvae ( $L_3$ ) of *A. ceylanicum*. The hamsters found positive by oviscopic examination on day 17 p.i. were used for therapeutic trials. The positive animals of one infected batch were randomly allocated for control and for each dose group of the test compound and the reference drug. The administration of test compound and the reference drug to hamsters was simultaneously done on any day between day 17 and 20 p.i. The efficacy was expressed in terms of absolute clearance of the host and percent worm reduction. The percent worm reduction in the treated group compared to untreated control group was obtained by the formula

$$\frac{N - n}{N} \times 100$$

(where 'N' and 'n' respectively stand for average numbers of worms in untreated control group and treated group). Based on percent worm reduction the  $ED_{50}$  was calculated by Maximum Likelihood estimation (Iterative method).

2. *Nippostrongylus brasiliensis* (trichostrongylid): The freshly weaned (35–40 g) male rats (UF strain) were infected with 500  $L_3$  subcutaneously. The therapeutic trials were initiated on day 9 p.i. and continued for 3 consecutive days. The efficacy assessment was made on percent worm reduction as described for *A. ceylanicum*.
3. *Nematospiroides dubius* (trichostrongylid): Male swiss mice (15–18 g) were orally infected with 200  $L_3$ . Administration of drug was initiated on any day between day 17 and 18 of infection and continued for three consecutive days. Drug efficacy was determined as described for *N. brasiliensis*.

4. *Syphacia obvelata* (oxyurid): The drug trials were carried out against natural infection of *S. obvelata* in mice of either sex weighing 18–20 g (Katiyar et al., 1982). The criterion of efficacy was the absolute clearance of parasites from the treated mice.
5. *Hymenolepis nana* (cestode): The testing method of Gupta et al. (1979) was followed. The efficacy was assessed against ova (200) induced infection of *H. nana* in male rats (25–40 g). The basis of drug efficacy was the absence of worms in treated animals on autopsy.

#### *Clinical evaluation in domestic animals*

The animals were treated with 3 doses of the test compound given on consecutive days. After instituting first dose, the stool voided during the preceding 24 h was examined for expelled worms. This exercise continued till the day of autopsy. When the worm repulsion ceased, animals were sacrificed, whole of the intestine was slit open and carefully observed for worms to verify drug's efficacy. Untreated control animals were similarly examined for the presence of worms in their stool and in the intestine on autopsy.

- *Dog*: The therapeutic trials were undertaken against artificially induced infection of *A. ceylanicum* ( $3000 \pm 100 L_3$ ) and the natural infection of *A. caninum* and *Toxocara canis*. The drug trials were carried out at doses 25, 10 and 5 mg/kg p.o.  $\times 3$ .
- *Cat*: The cats carrying single or mixed infections of *Toxocara* sp., *Toxascaris* sp., *Ancylostoma* sp. and *Taenia* sp. were dosed with Comp. 81-470 in doses 25, 10, 5, 2.5 and 1 mg/kg p.o.  $\times 3$ .
- *Fowl*: The adult fowls positive for round worms, were obtained from the local market. These were treated with the compound in doses 10 and 5 mg/kg for 3 successive days.
- *Acute toxicity test*: This was carried out in healthy adult mice. Different doses of the compound were administered orally or intraperitoneally to groups of 4–5 mice of similar weight. Control and untreated animals received only the medium by respective routes. The animals were kept under close observation for 24 h. The mortality or adverse effects noticed, if any, were recorded.

## Results

The antihookworm activity of Comp. 81-470 against *A. ceylanicum* in hamsters has been presented in Table 1. Repeated trials have confirmed that single dose of 250 mg/kg p.o. and down to 6.25 mg/kg b.w. cured all the treated animals. Based on worm reduction, the  $ED_{50}$  was found to be 3.64 mg/kg  $\times 1$  with fiducial limits 3.09 to 4.15 mg/kg. In simultaneous trials with mebendazole the cure of animals was obtained with a dose of 1 mg/kg  $\times 1$ .

Table 2 depicts the data of efficacy on other helminth parasites. Against trichostrongylid nematode *N. brasiliensis* in rats, a complete worm reduction was achieved at the dose of 100 mg/kg  $\times 3$ . The lesser amounts showed dose dependent efficacy. However, the compound was feebly effective in the other trichostrongylid worm, *N. dubius*, in mice.

The oxyurid worm, *S. obvelata*, was highly susceptible to the drug's action. All the 24 mice treated with doses from 50 mg/kg  $\times 3$  and down to 12.5 mg/kg  $\times 3$  expelled their parasites. A reasonable effectivity was still witnessed at 6.25 mg/kg  $\times 3$  dose level.

The rats infected with cestode *H. nana* were cured at 25 mg/kg  $\times 1$  and above.

Encouraged with the primary screening results, the drug was evaluated in higher animals (Table 3).

Table 1. Efficacy of Compound 81-470 and mebendazole against *Ancylostoma ceylanicum* (adult) in hamsters

| Dose<br>(mg/kg)<br>oral          | Group     | Animals<br>cured/<br>treated | Per-<br>cent<br>cure | Worms<br>recovered<br>(mean with<br>range) | Percent<br>worm<br>reduc-<br>tion | ED <sub>50</sub><br>(mg/kg) |
|----------------------------------|-----------|------------------------------|----------------------|--|-----------------------------------|-----------------------------|
| 6.25 and<br>above $\times 1^*$   | Exp. (12) | 30/30                        | 100                  | 0  | 100                               |                             |
|                                  | Control   | 0/34                         | —                    | 26<br>(11–44)                              | —                                 |                             |
| 4.41 $\times 1$                  | Exp. (2)  | 3/6                          | 50                   | 1<br>(0–2)                                 | 96.8                              |                             |
|                                  | Control   | 0/7                          | —                    | 32<br>(23–38)                              | —                                 |                             |
| 3.12 $\times 1$                  | Exp. (4)  | 4/10                         | 40                   | 5<br>(0–11)                                | 82.1                              | 3.64 $\times 1$             |
|                                  | Control   | 0/13                         | —                    | 26<br>(16–44)                              | —                                 | Fiducial limit<br>3.09–4.15 |
| 2.21 $\times 1$                  | Exp. (2)  | 0/7                          | 0                    | 8<br>(2–24)                                | 75.0                              |                             |
|                                  | Control   | 0/7                          | —                    | 32<br>(23–38)                              | —                                 |                             |
| 1.56 $\times 1$                  | Exp. (2)  | 0/6                          | 0                    | 14<br>(2–19)                               | 56.2                              |                             |
|                                  | Control   | 0/7                          | —                    | 32<br>(23–38)                              | —                                 |                             |
| 1.0 and<br>above $\times 1^{**}$ | Exp. (7)  | 19/19                        | 100                  | 0  | 100                               |                             |
|                                  | Control   | 0/24                         | —                    | 19<br>(5–38)                               | —                                 |                             |
| 0.5 $\times 1$                   | Exp. (1)  | 1/4                          | 25                   | 6<br>(0–10)                                | 85                                |                             |
|                                  | Control   | 0/3                          | —                    | 40<br>(36–43)                              | —                                 |                             |

\* Pooled efficacy data of doses from 250 mg/kg  $\times 1$  and down to 6.25 mg/kg  $\times 1$

\*\* Pooled efficacy data of doses from 5 mg/kg  $\times 1$  and down to 1 mg/kg  $\times 1$

In dogs, the Comp. 81-470 was fully effective in removing all the 382 *A. ceylanicum* at as low a dose as 5 mg/kg  $\times 3$ . Dogs harbouring dual infection of *A. caninum* and *T. canis* were also freed from parasites at 10 mg/kg  $\times 3$ .

The cats infected with *Toxocara* sp. were fully cleared of their parasites upto as low a dose as 2.5 mg/kg  $\times 3$ . A further low dose of 1 mg/kg  $\times 3$  administered similarly could also remove majority of the worms. Three cats infected with hookworms and treated at the dose of 25 mg/kg  $\times 3$  were also cured. But 10 mg/kg dose had only slight effect on hookworms. Tapeworms (*Taenis* spp.), however, remained unaffected. Mebendazole at 1 mg/kg  $\times 3$  resulted in a 100% cure in 2 cats harbouring 7 and 17 *Toxocara* sp. (not shown in the table).

Three fowls naturally infected with *Ascaridia galli* were cured when the compound was administered at 10 mg/kg  $\times$  3 dose level. In the third fowl, however, 3 doses each of 5 mg/kg failed to clear ascarids.

*Controls:* In infected and untreated animals stool did not contain any worms except for few gravid segments of tapeworms. The mice tolerated very high amounts of the compound both by oral and intraperitoneal routes. A dose of 1500 mg/kg given orally or 1000 mg/kg introduced intraperitoneally did not cause any death or adverse effect (Table 4).

## Discussion

For intestinal helminths, mebendazole is considered to be a wonder drug. But its toxic effects limit the use in various conditions (Annon, 1975). Gupta et al. (1983) observed that mebendazole had no action on *H. nana* in rats. It is also feebly effective against *N. brasiliensis* in rats and *N. dubius* in mice (Misra et al., 1981). Sharp and Westcott (1976) also did not get encouraging results with this drug against *S. obvelata* in mice.

To develop an effective anthelmintic a large scale screening of rationally synthesized compound was taken up. During the exercise, one compound (Comp. 81-470) possessing all qualities of an ideal broad spectrum anthelmintic with practically no toxicity was discovered. The Comp. 81-470 was found effective against a variety of natural and artificially adapted nematode and cestode parasites of laboratory animals. Besides, it was also found to exhibit efficacy against tissue dwelling filarial parasites in appreciable low doses (Fatima et al., 1983). Clinical evaluation in higher animals confirmed its therapeutic value.

The compound appears to be safe as a very high dose of 1500 mg/kg administered orally or 1000 mg/kg introduced intraperitoneally could not do any harm to the treated animals. Even at the dose of 4500 mg/kg p.o. the death of only one animal occurred, which could be due to other factors rather than drug as the survivors apparently did not show any adverse reaction.

The pharmacological and biochemical investigations of the compound are in progress. However, from the toxicity data one could surmise that even if the compound is absorbed freely, it will not produce toxic side effect in reasonably high doses as very high amounts by intraperitoneal route were harmless.

Thus, the compound possessing expanded anthelmintic activity coupled with large therapeutic index fulfills the requirements of an ideal broad spectrum anthelmintic. As such the compound appears to be a candidate broad spectrum anthelmintic and deserves detailed clinico-chemotherapeutic investigations.

Table 2. Activity of Compound 81-470 against other helminth parasites

| Parasite   | Host  | Dose<br>(mg/kg)<br>oral | No. of<br>experi-<br>ment | Animals<br>cured/<br>treated | %<br>animals<br>cured | Av. no. of<br>worms recovered<br>(mean with range) | % worm<br>reduction |
|--|-------|-------------------------|---------------------------|------------------------------|-----------------------|--|---------------------|
| 1. <i>Nippostrongylus<br/>brasiliensis</i><br>(trichostrongylid) | Rat   | 250 × 3                 | 2                         | 8/8                          | 100                   | 0  | 100                 |
|  |       | Control                 |                           | 0/9                          | –                     | 112 (86–129)                                       | –                   |
|  |       | 100 × 3                 | 4                         | 17/17                        | 100                   | 0  | 100                 |
|  |       | Control                 |                           | 0/14                         | –                     | 84.6 (58–120)                                      | –                   |
|  |       | 50 × 3                  | 6                         | 2/15                         | 13.3                  | 20.7 (0–50)  | 81.5                |
|  |       | Control                 |                           | 0/13                         | –                     | 111.7 (72–155)                                     | –                   |
|  |       | 25 × 3                  | 4                         | 1/12                         | 8.3                   | 63.6 (0–142)                                       | 23.6                |
|  |       | Control                 |                           | 0/12                         | –                     | 83.2 (57–147)                                      | –                   |
|  |       | 12.5 × 3                | 3                         | 0/10                         | 0                     | 99.8 (19–160)                                      | 4.0                 |
|  |       | Control                 |                           | 0/8                          | –                     | 104.0 (57–147)                                     | –                   |
| 2. <i>Nematospiroides<br/>dubius</i><br>(trichostrongylid)       | Mouse | 250 × 3                 | 2                         | 2/6                          | 33.3                  | 8.6 (0–16)   | 65.3                |
|  |       | Control                 |                           | 0/6                          | –                     | 23.1 (16–34)                                       | –                   |
|  |       | 100 × 3                 | 2                         | 0/7                          | 0                     | 17.4 (7–32)  | 24.6                |
|  |       | Control                 |                           | 0/6                          | –                     | 23.1 (16–34)                                       | –                   |
|  |       | 50 × 3                  | 1                         | 4/4                          | 100                   | 0  | –                   |
| 3. <i>Syphacia obvelata</i> *                                    | Mouse | Control                 |                           | 0/3                          | –                     | 7.0 (4–13)   | –                   |
|  |       | 25 × 3                  | 2                         | 8/8                          | 100                   | 0  | –                   |
|  |       | Control                 |                           | 0/10                         | –                     | 6.0 (1–16)   | –                   |
|  |       | 12.5 × 3                | 3                         | 12/12                        | 100                   | –  | –                   |
|  |       | Control                 |                           | 0/19                         | –                     | 13.24 (3–45)                                       | –                   |
|  |       | 6.25 × 3                | 3                         | 5/12                         | 42                    | 2.5 (0–15)   | –                   |
|  |       | Control                 |                           | 0/10                         | –                     | 6.0 (1–16)   | –                   |
|  |       | 3.125 × 3               | 0/4                       | 4/4                          | 0                     | 5.0 (2–8)  | –                   |
|  |       | Control                 |                           | 0/3                          | –                     | 7.3 (4–12)   | –                   |
|  |       | 50 × 1                  | 2                         | 6/6                          | 100                   | 0  | –                   |
| 4. <i>Hymenolepis nana</i> *<br>(cestode)                        | Rat   | Control                 |                           | 0/6                          | –                     | 18 (4–49)  | –                   |
|  |       | 25 × 1                  | 3                         | 9/9                          | 100                   | 0  | –                   |
|  |       | Control                 |                           | 0/9                          | –                     | 11 (5–19)  | –                   |
|  |       | 12.5 × 1                | 2                         | 0/6                          | 0                     | 9 (2–14)   | –                   |
|  |       | Control                 |                           | 0/6                          | –                     | 10 (3–21)  | –                   |
|  |       |                         |                           |                              |                       |  |                     |
|  |       |                         |                           |                              |                       |  |                     |

\* Efficacy based on complete worm clearance



Table 3. Efficacy of Compound 81-470 against different helminth parasites of cat, dog and fowl

| Animal      | Dose<br>(mg/kg)<br>oral | Parasites<br>present           | No. expelled<br>following<br>medication | No.<br>recovered<br>on autopsy |
|-------------|-------------------------|--------------------------------|---|--------------------------------|
| <i>Cat</i>  |                         |                                |   |                                |
| 1.          | 25 × 3                  | <i>Toxocara</i> sp.            | 3                                       | Nil (10)*                      |
|             |                         | <i>Ancylostoma tubaeformis</i> | 11                                      | Nil (10)                       |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 4 (10)                         |
| 2.          |                         | <i>Toxocara</i> sp.            | 3                                       | Nil (10)                       |
|             |                         | <i>A. tubaeformis</i>          | 3                                       | Nil (10)                       |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 11 (10)                        |
| 3.          |                         | <i>Toxocara</i> sp.            | 9                                       | Nil (10)                       |
|             |                         | <i>A. tubaeformis</i>          | 2                                       | Nil (10)                       |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 7 (10)                         |
| 4.          |                         | <i>Toxocara</i> sp.            | 2                                       | Nil (14)                       |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 4 (14)                         |
| 1.          | 10 × 3                  | <i>Toxocara</i> sp.            | 11                                      | Nil (5)                        |
|             |                         | <i>A. tubaeformis</i>          | 10                                      | 22 (5)                         |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 5 (5)                          |
| 1.          | 5 × 3                   | <i>Toxocara</i> sp.            | 12                                      | Nil (7)                        |
|             |                         | <i>A. tubaeformis</i>          | 4                                       | 11 (7)                         |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 4 (7)                          |
| 2.          |                         | <i>Toxocara</i> sp.            | 17                                      | Nil (5)                        |
|             |                         | <i>Taenia</i> sp.              | Many gravid segments                    | 5 (5)                          |
| 1.          | 2.5 × 3                 | <i>Toxocara</i> sp.            | 34                                      | Nil (6)                        |
| 1.          | 1 × 3                   | <i>Toxocara</i> sp.            | 10                                      | 3 (7)                          |
| 2.          |                         | <i>Toxocara</i> sp.            | 121                                     | 3 (7)                          |
| <i>Dog</i>  |                         |                                |   |                                |
| 1.          | 25 × 3                  | <i>Toxocara canis</i>          | 10                                      | Nil (6)                        |
|             |                         | <i>A. caninum</i>              | 10                                      | Nil (6)                        |
| 1.          | 10 × 3                  | <i>T. canis</i>                | 6                                       | Nil (7)                        |
|             |                         | <i>A. caninum</i>              | 7                                       | Nil (7)                        |
| 2.          |                         | <i>A. ceylanicum</i>           | 276                                     | Nil (5)                        |
| 1.          | 5 × 3                   | <i>A. ceylanicum</i>           | 382                                     | Nil (9)                        |
| 2.          |                         | <i>A. caninum</i>              | 3                                       | 23 (7)                         |
| <i>Fowl</i> |                         |                                |   |                                |
| 1.          | 10 × 3                  | <i>Ascaridia galli</i>         | 17                                      | Nil (7)                        |
| 2.          |                         | <i>A. galli</i>                | 14                                      | Nil (6)                        |
| 1.          | 5 × 3                   | <i>A. galli</i>                | Nil                                     | 8 (4)                          |

\* Day of autopsy after administration of the last dose



Table 4. Acute toxicity studies with Compound 81-470 in mice (observation period: 24 h)

|             | Dose<br>mg/kg $\times$ 1 | No. of<br>experiments | No. of mice<br>treated | No. of mice<br>died after 24 h |
|-------------|--------------------------|-----------------------|------------------------|--------------------------------|
| <i>Oral</i> | 4500                     | 2                     | 9                      | 1                              |
|             | 1500                     | 2                     | 8                      | 0                              |
|             | 1000                     | 2                     | 10                     | 0                              |
| <i>I.P.</i> | 1000                     | 2                     | 10                     | 0                              |
|             | 500                      | 2                     | 10                     | 0                              |

Oral LD<sub>50</sub>: 4500 mg/kg

I.P. LD<sub>50</sub>: 1000 mg/kg

Note: None of the 8 untreated control mice, kept simultaneously, died.

### Acknowledgments

We express sincere thanks to Dr. Nitya Nand, FNA, Director, CDRI, Lucknow, for his keen interest in the work and to Dr. A. B. Sen, Deputy Director and Head, Division of Parasitology for his valuable suggestions and going through the manuscripts. We are grateful to Messrs. S. C. Bhar and Shiv Ram for their able technical assistance. The award of Junior Research Fellowship by the ICMR to Sri P. K. S. Visen and Research Associateship by the University Grants Commission to Miss A. Misra is gratefully acknowledged. – This is CDRI Communication No. 3385.

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