**The involvement of flumazenil, amphetamine and chlorpromazine in the anticonvulsant effect of methanol root extract of *Securidaca longepedunculata* (Fresen) in mice model**

**ABSTRACT**

**Objective**

This study aimed to assess the potential effects of methanol root extract of *Securidaca longepedunculata* on seizures induced experimentally in mice and to evaluate the roles of flumazenil, amphetamine and chlorpromazine in mice.

**Materials and Methods:**

Animal behaviours were evaluated using hexobarbitone-induced sleep model, and anticonvulsant activity using picrotoxin- and pentylenetetrazole (PTZ)-induced convulsions. Attempt to understand the mode of action of the anticonvulsant activity of the plant; flumazenil (3 mg/kg), chlorpromazine, and amphetamine (3 mg/kg) were used.

**Results**

The methanol root extract of *Securidaca longepedunculata* (MRESL) potentiates the sleeping time of hexobarbitone-induced hypnosis in a dose-related manner. The root extracts significantly (p<0.05) prolonged the onset and attenuated the duration of seizure in PTZ-induced at 600 mg/kg body weight and a dose-dependent manner in picrotoxin-induced seizure.

However, the anticonvulsant activity of the methanol root extract of *Securidaca longepedunculata* was significantly reversed following intraperitoneal pre-treatment with flumazenil (GABA receptor antagonist) in picrotoxin-induced convulsion but not in PTZ-induced seizure. Amphetamine significant potentiated anti-seizure property of the extract (p < 0.05), while co-administration of the extract (600 mg/kg) with chlorpromazine (5 mg/kg) did not protect the mice against this seizure in either model.

**Conclusion:**

The findings suggest that methanol root extract of *Securidaca longepedunculata* is effective in reducing the severity of seizures. This positive effect may be mediated through signaling pathways that involve benzodiazepine-activated γ-aminobutyric acid receptors and anti-seizure property of the extract was potentiated by amphetamine.

**Key Words:** *Securidaca longepedunculata*, Seizure, Amphatamine, Pentylenetetrazole, picrotocin.

**INTRODUCTION**

Epilepsy is a serious disorder of the central nervous system that affects millions of people at some point in their lives. It is characterized by recurrent seizures caused by abnormal electrical discharges in the brain[1]. These synchronized discharges among brain cells result in changes in sensory, motor, and other functions. When the electrical activity occurs in a specific area of the brain, it is known as a partial seizure. In contrast, if the activity spreads and involves both sides of the brain, it is classified as a generalized seizure. Various factors can lead to epilepsy, such as brain damage from trauma, infections, strokes, or developmental abnormalities[2]. The condition significantly impacts patients physically, socially, and psychologically, highlighting the need for effective treatments to alleviate their suffering. The search for anticonvulsant drugs has produced numerous synthetic options. Among the antiepileptic drugs (AEDs) widely used in clinical settings are phenytoin, carbamazepine, ethosuximide, phenobarbital, and clonazepam[3]. These medications work by interacting with neurotransmitters that are involved in both inhibitory and excitatory signals in the brain. However, many of these drugs are associated with a high incidence of adverse effects. Additionally, the cost of treatment can be prohibitive for some, especially those living in rural areas or in countries with weaker economies. This has increased scientists' interest in finding alternative methods for treating seizures[4].

The use of medicinal plants has gained popularity worldwide, as many have been traditionally reported to help manage seizures[5]. One such plant is Securidaca longepedunculata Fresen. (Polygalaceae), a tree or shrub commonly found in parts of Africa, especially in Western, Northern, and Eastern Nigeria, where it is widely used for medicinal purposes. The roots and bark of this plant are consumed orally, either in powdered form or as infusions, to treat various ailments, including chest problems, headaches, inflammation, abortion, ritual suicide, tuberculosis, infertility, venereal diseases, and constipation[6]. Chewing the roots can also relieve toothache[7]. Mixed roots of the violet tree and dwarf custard apple are additionally used to treat gonorrhea. Ethnobotanical research has demonstrated that the root of S. longepedunculata is particularly valuable for managing inflammatory diseases. Consequently, this study aimed to investigate the anticonvulsant activity of the root extract of *S. longepedunculata* in albino mice.

**MATERIALS AND METHODS**

**Identification and Preparation of Plant Material**

The plant material used for this study was collected from Ajibode village, Ibadan, Nigeria. It was identified at the University of Ibadan Herbarium. The root bark was washed, dried, and powdered. The powdered sample was kept in an air-tight bottle at 4°C for further experimental use.

**Preparation of Extract**

The powdered sample 600 g was extracted in 2L of absolute methanol for 72 h using the cold maceration method, with occasional stirring. The liquid extract was filtered with Whatman filter paper No. 1, and the filtrate was concentrated using a rotary evaporator. The extract was stored at 4°C for further experimental use.

**Animal material**

Healthy male Swiss mice (20-30 g) obtained from the Animal House of the Ladoke Akintola University of Technology, Ogbomosho, Oyo state, Nigeria, were used for this study. Animals were housed in standard cages of six animals per cage. General housing was in temperature-controlled (22.5°C ± 2.5°C) quarters with the light on/off routine at 7 o’clock. Mice were given free access to food and water except during behavioral tests. All animals were fed with commercial standard rodent chow (calories: 29% protein, 13% fat, 58% carbohydrate) all through the experimental period. All rules about animal safety and care were observed.

**Hexobarbitone-induced Sleep in Mice**

The method utilized in this study was based on the approach described by Leite et al. (1982)[8]. Thirty minutes after pretreating the mice with the MRESL (at doses ranging from 150 to 600 mg/kg, administered intraperitoneally) or a vehicle (10 ml/kg), hexobarbitone (85.0 mg/kg, intraperitoneally) was given. This study used hexobarbitone-induced sleeping time to assess the hypnotic or sedative effects of various drugs, natural products, and chemical agents[9,10]. This evaluation method measures how effectively a drug or chemical can extend the duration of sleep induced by hexobarbitone. The loss of the righting reflex was used to determine the onset of hypnosis, while the recovery of this reflex served as an indicator of awakening[11**].**

**Picrotoxin- or pentelenetetrazole-induced Convulsion**

Picrotoxin-induced convulsion models are utilized to investigate the anticonvulsive effects of drugs, natural compounds, or chemical substances in animals. In this study, mice were randomly assigned to five groups (n = 6) and received intraperitoneal pretreatment with either normal saline (vehicle) at a dosage of 10 ml/kg, phenobarbital (40 mg/kg i.p.), which is a standard anticonvulsant agent, or methanol root extract of Securidaca longepedunculata at doses of 150, 300, and 600 mg/kg. Thirty minutes later, seizures were induced by the intraperitoneal administration of picrotoxin (10 mg/kg i.p.) or pentelenetetrazole (85 mg/kg, i.p) following the methodology described by Elisah et al. (1988)[12**].** After administration, each mouse was placed in an observation box. Convulsions were scored based on clonic contractions, and the onset and duration of convulsive episodes measured the anticonvulsant activity. Mice that did not exhibit convulsions within 30 minutes after strychnine administration were considered protected. In the case of picrotoxin-induced seizures, a delay in the onset of action and the survival of the animals was indicative of anticonvulsant properties.[13]

**Evaluation of the Effect of Flumazenil or amphetamine or chlorpromazine on the Anticonvulsant Activity of methanol root extract of *Securidaca longepedunculata* in picrotocin-induced convulsion**

To investigate the potential involvement of benzodiazepine receptors, we examined how flumazenil, a benzodiazepine receptor antagonist, or amphetamine (3 mg/kg) or chlorpromazine (5 mg/kg) affects the anticonvulsant activity of methanol root extract of *Securidaca longepedunculata*.

The animals were randomly assigned into six groups (n = 6) as follows:

-Group 1 (Control): Received normal saline (10 mL/kg, intraperitoneally) 30 minutes before the administration of Picrotoxin (10 mg/kg, intraperitoneally).

- Group 2: Received flumazenil (3 mg/kg), or amphetamine (3 mg/kg) or chlorpromazine (5 mg/kg) intraperitoneally, 30 minutes before the administration of Picrotoxin (10 mg/kg, intraperitoneally).

- Group 3: Received phenobarbitone (40 mg/kg, intraperitoneally) 30 minutes before the administration of Picrotoxin (10 mg/kg, intraperitoneally).

- Group 4: Received flumazenil (3 mg/kg, intraperitoneally) 15 minutes before the injection of phenobarbitone (40 mg/kg, intraperitoneally) and 30 minutes before the injection of Picrotoxin.

- Group 5: Received methanol root extract of *Securidaca longepedunculata* (600 mg/kg, intraperitoneally) 30 minutes before the administration of Picrotoxin.

- Group 6: Received flumazenil (3 mg/kg, intraperitoneally) 15 minutes before the injection of  *Securidaca longepedunculata* (600 mg/kg, intraperitoneally) and 30 minutes before the injection of Picrotoxin (10 mg/kg, intraperitoneally).

Following the injections, seizure latency, seizure duration, and the percentage of seizure protection were recorded 30 minutes after the administration of Picrotoxin as previously reported **[14].** The anticonvulsant activity of methanol root extract of *Securidaca longepedunculata* and phenobarbitone in mice pretreated with flumazenil or amphetamine or chlorpromazine were evaluated and compared with the control.

**RESULTS**

**Effect of Methanol root Extract of  *Securidaca longepedunculata* on the potentiation of Hexobarbitone-induced Sleeping Time**

In the control-treated group, when hexobarbitone was administered, the onset of sleep was 6.14 ± 0.71 minutes, and its duration was 32.72 ± 0.36 minutes (Table 1). The onset of sleep in the group treated with methanol extract of *Securidaca longepedunculata* (150-600 mg kg, i.p) was significantly (p<0.05) reduced, while the duration of sleep induced by hexobarbitone was significantly (p<0.05) prolonged in a dose-dependent manner. The peak hypnotic effect was observed in the group treated with 600 mg/kg of *Securidaca longepedunculata*. In this group, the onset of sleep was significantly increased (p<0.05), compare with the control group. Additionally, the duration of sleep significantly increased (p<0.05) compare with the control group. Similarly, in the diazepam-treated group, there was a significant reduction (p<0.05) in the onset of sleep, and a prolongation of the duration of sleep compared to the control group. The hypnotic effect produced by diazepam was comparable to that of *Securidaca longepedunculata* at the dose of 600 mg/kg. Moreover, the effect of the extract on hexobarbitone-induced sleep duration was reversed upon intraperitoneal administration of flumazenil, a GABA-A antagonist. This indicates that the extracts contain GABA-A agonists.

**Table 1:. Effect of methanol root extract of*****Securidaca longepedunculata* on the potentiation of hexobarbitone-induced sleeping time**.

|  |
| --- |
| **Treatments Doses (mg/kg) Onset of sleep(min)\*\* Duration of sleep(min)\*\*** |
| Control 0 6.14 ± 0.71 32.72 ± 0.36 |
| MRESL 150 4.95 ± 0.50 48.52 ± 1.03\* |
| MRESL 300 3.75 ± 0.53 57.20 ± 3.51\* |
| MRESL 600 3.01 ± 0.06 97.43 ± 0.60\* |
| Diazepam 1 2.34 ± 0.05 110.45±7.42\* |

\*\*Values are recorded as means ± SEM (n = 6).

\*Values are statistically significant (p<0.05) with control. One-way ANOVA follows

by Newman-Keuls Multiple Comparison tests.

**Picrotoxin-Induced Seizure**

Intraperitoneal injection of picrotoxin (10 mg/kg) induced seizures in control subjects, with an average onset time of 207.3 ± 13.7 seconds and a duration of clonic seizures lasting 187.1 ± 6.4 seconds, resulting in 100% mortality. However, when methanol root extract of *Securidaca longepedunculata* was administered intraperitoneally at doses of 150, 300, and 600 mg/kg 30 minutes prior to the injection of the convulsant agent, there was a significant prolongation (p < 0.05) in the onset time of seizures and a reduction in seizure duration, following a dose-dependent pattern. The protective effect observed was comparable to that of phenobarbital, a standard anticonvulsant drug, which showed a significantly shorter seizure duration of 75.8 ±5.1 seconds when compared to the group treated with 600 mg/kg of *Securidaca longepedunculata* methanol root extract (refer to Table 2). Chlorpromazine and amphetamine were each co-administered with methanol root extract of *Securidaca longepedunculata* . Picrotoxin (10 mg/kg) was administered after 30 minutes. The animals were observed for 2 hours for clonic-tonic seizures.

**Table 2. Effect of methanol extract of *Securidaca longepedunculata* on picrotoxin-induced seizure in mice.**

|  |
| --- |
| **Treatments Doses (mg/kg) Onset of seizure(s)\*\* Duration of seizure(s)\*\* %Protection** |
| Control 0 207.3 ± 13.1 187.1± 6.4 0 |
| MRESL 150 261.5 ± 6.1 101.5 ± 9.3 40 |
| MRESL 300 285.0 ± 17.6 91.2 ± 3.16 40 |
| MRESL 600 312.2 ± 23.5 83.3 ± 8.6 60 |
| Phenobarb 40 314.5 ± 13.3 75.4± 5.1 80 |

\*\*Values are recorded as means±SEM (n=6).

\*Values are statistically significant (p<0.05) with control. One-way ANOVA follows by Newman-Keuls Multiple Comparison tests.

**The influence of Flumazenil or amphetamine, or chlorpromazine on the effect of *Securidaca longepedunculata* in picrotoxin-induced convulsion**

Administration of flumazenil (3 mg/kg) or amphetamine (3 mg/kg) or chlorpromazine (5 mg/kg), 15 minutes before methanol root extract of *Securidaca longepedunculata* (600 mg/kg) and 30 minutes before the injection of picrotoxin. Table 3 revealed a reversed of anticonvulsant activity by flumazenil, while, amphetamine potentiates anticonvulsant activity with complete protection and chlorpromazine significantly increased the seizure activity of picrotoxin. Similarly effect was observed with phenobarbitone .

Table 3. **The influence of Flumazenil or amphetamine, or chlorpromazine on the effect of *Securidaca longepedunculata* in picrotoxin-induced convulsion**

|  |
| --- |
| **Treatments Dose mg/kg Onset of seizure(s)\*\* Duration of seizure(s)\*\* %Protection** |
| Control 0 205.7 ± 18.1 196.1 ± 13.7 0 |
| MRESL 600 320.3 ± 17.5 81.2 ± 11.6 4 60 |
| MRESL+FLU 600&3 231.6 ± 13.1\* 211.7 ± 11.6\* 0 |
| Phenobarb 40 323.5 ± 17.1 76.1 ± 4. 8 80 |
| Phenobarb+FLU 40&3 245.5 ± 21.5\* 203.8 ± 13.1\* 0 |
| MRESL + Amp 600&3 251.6 ± 21.1\* 243.3 ± 19.6\* 100 |
| Phenobarb+Amp 40&3 267.5 ± 23.5\* 265.8 ± 21.1\* 100 |
| MRESL +  Chlopromazine 600 & 5 105.4 ± 1.08\* 120.8 ± 7.1\* 0 |

\*\*Values are recorded as means±SEM (n=6).

\*Values are statistically significant (p<0.05) with control. One-way ANOVA follow by

Newman-Keuls Multiple Comparison tests

**Effect of methanol root extract on Pentylenetetrazol -induced seizure in mice.**

The methanol root extract of Securidaca long pedunculate, administered at a dose of 600 mg/kg body weight, provided 40% protection against seizures induced by Pentylenetetrazol (PTZ) in mice. Additionally, amphetamine enhanced the anticonvulsant effects of the extract. In contrast, chlorpromazine did not show any significant impact on the anticonvulsant activity of the extract against PTZ-induced seizures in mice (see Tables 4 and 5).

**Table 4. Effect of methanol root extract on Pentylenetetrazol -induced seizure in mice.**

|  |
| --- |
| **Treatments Doses (mg/kg) Onset of seizure(s)\*\* Duration of seizure(s)\*\* %Protection** |
| Control 0 195.3 ± 15.1 191.1± 11.4 0 |
| MRESL 150 198.5 ± 11.1 187.5 ± 11.3 0 |
| MRESL 300 201.0 ± 18.1 189.2 ± 14.1 0 |
| MRESL 600 217.2 ± 19.5 102.3 ± 11.6 40 |
| Phenobarb 40 311.5 ± 23.7 79.1± 4.8 80 |

\*\*Values are recorded as means±SEM (n=6).

\*Values are statistically significant (p<0.05) with control. One-way ANOVA follows by Newman-Keuls Multiple Comparison tests.

**Table 5 : The influence of Flumazenil or amphetamine, or chlorpromazine on the effect of *Securidaca longepedunculata* in Pentylenetetrazol -induced convulsion**

|  |
| --- |
| **Treatments Dose mg/kg Onset of seizure(s)\*\* Duration of seizure(s)\*\* %Protection** |
| Control 0 225.1 ± 17.5 186.1 ± 15.7 0 |
| MRESL 600 312.3 ± 15.1 89.2 ± 9.36 40 |
| MRESL+FLU 600&3 212.6 ± 13.3\* 234.7 ± 18.6\* 0 |
| Phenobarb 40 315.5 ± 21.1 85.1 ± 4. 07 80 |
| Phenobarb+FLU 40&3 252.5 ± 19.5\* 203.8 ± 13.1\* 0 |
| MRESL + Amp 600&3 242 ± 11.7\* 233.8 ± 17.3\* 100 |
| MRESL +  Chlopromazine 600 & 5 220.4 ± 3.13 115.8 ± 9.5 40 |

\*\*Values are recorded as means±SEM (n=6).

\*Values are statistically significant (p<0.05) with control. One-way ANOVA follow by

Newman-Keuls Multiple Comparison tests

**DISCUSSION**

The claims of therapeutic successes on central nervous system disorders by traditional medicine practitioners using methanol root extract of *Securidaca longepedunculata* have not been exhaustively subjected to scientific investigation. In this study, the potential mechanism of anticonvulsant action of methanol root extract of *Securidaca longepedunculata*was determined using an animal model. The present study evaluated the effects of the methanol root extract of *Securidaca longepedunculata* on hexobarbitone-induced sleeping time. The methanol root extract of *Securidaca longepedunculata*exhibited sedative effects, as shown by their ability to prolong hexobarbitone-induced sleeping time. Several neurotransmitters and endogenous molecules are involved in regulating sleep and wakefulness. The neurons that promote sleep are located in the anterior hypothalamus, and this releases gamma-aminobutyric acid (GABA), suppressing the activity of wake-inducing areas of the brain [15]. Barbiturate is known to act at GABA receptors ionophore complex, thus enhancing the binding of GABA. Similarly, diazepam, a benzodiazepine agonist, enhances the affinity of GABA for its receptor and hence prolongs hexobarbital-induced sleep duration [16]. Meanwhile, some medicinal plants have been reported to interact with the GABAergic system to induce their hypnotic effect [17]. The hypnotic activity of medicinal plants has been attributed to various phytochemical compounds, including flavonoids, terpenes, and saponins, as previously reported [18, 19]. Studies have also shown that the potentiation of barbiturate hypnosis is an index for the central nervous system depressant effect [20] and that the drugs with sedative properties prolonged the time of sleep produced by barbiturates [21]. Two parameters were measured in this experiment: onset (sleep latency) and duration of sleep. The time it takes the animals to lose their righting reflex (unconsciousness) from the time of drug administration is referred to as latency or onset of sleep. In contrast, the period of sleep is defined as the number of times in a minute it takes the animals from loss of righting reflex to regaining of righting reflex (recovery of consciousness), as reported by Ayoka et al. (2006) [22]. It is, therefore, suggested that the ability of the extracts to prolong barbiturate-induced sleeping time indicates that it might possess central nervous system depressant properties. Diazepam belongs to the benzodiazepine group, which has a binding site on GABA receptor type ionophore complex (GABA A ) [23], and this mechanism can be useful in the onset of sleep and increase sleep duration**.** *Securidaca longepedunculata* administration at doses of 150-600 mg/kg produced a sedative effect similar to that observed with 1 mg/kg of diazepam. This effect observed may be mediated via the GABA-ergic system. Therefore, the drugs that influence these systems can be necessary for insomnia disorder. Studies have shown that an increase in catecholamines can enhance anticonvulsant activity[24]. Therefore, the aqueous extract of Securidaca longepedunculata may be linked to neurohumoral transmission processes. The enhancement of central dopaminergic transmission plays a key role in anticonvulsant activity [25]. In this study, the concurrent administration of amphetamine with the extract provided protection to mice against picrotoxin-induced seizures. Conversely, the administration of chlorpromazine, a dopamine antagonist, led to an increase in seizure activity. This data suggests a potential involvement of the dopaminergic mechanism in the modulation of seizures in mice. The enhancement of central dopaminergic transmission plays a key role in anticonvulsant activity. In this study, the concurrent administration of amphetamine with the extract provided protection to mice against picrotoxin-induced seizures. Conversely, the administration of chlorpromazine, a dopamine antagonist, led to an increase in seizure activity. This data suggests a potential involvement of the dopaminergic mechanism in the modulation of seizures in mice.

Moreover, some studies have indicated that picrotoxin diminishes the GABAergic tone, probably by acting as a competitive antagonist on the BZD receptors. Similarly, drugs that enhance GABA A receptor neurotransmission, such as BZDs [26], can block seizures induced by picrotoxin. Picrotoxin, a GABA A receptor antagonist, produces seizures by blocking the chloride-ion channels linked to GABA A receptors, thus preventing the entry of chloride ions into the neurons. This results decreased GABA transmission and brain activity. Thus, convulsions arising from picrotoxin are due to the decreased GABA-A receptor-mediated inhibition, which tips the balance in favor of glutamate-mediated excitatory transmission [27]. The ability of the stem bark extract of PP to attenuate seizures induced by picrotoxin may be due to an interaction with GABA-A-receptors and GABA transmission. Phenobarbitone, a reference anticonvulsant, produced similar effects on picrotoxin-induced seizures. Moreover, it is known to enhance GABAergic neurotransmission by increasing chloride ion flux through the chloride channels of GABA-A receptors. Since stem bark extract of P. staudtii mimicked, to some extent, the anticonvulsant actions of phenobarbitone, the plant may antagonize picrotoxin-induced seizure by opening the chloride channel associated with GABA- A receptors. It is also possible to achieve these effects by suppressing glutamate-mediated excitation. Our findings from this study revealed the anticonvulsant and hypnotic effects of methanol root extract of *Securidaca longepedunculata*when compared to the effect of the standard drug used for the management of epileptic seizures. The onset and duration of the seizure were significantly prolonged. Additionally, the plant protects mice against mortality at various doses of the extract. The effect produced may suggest that the extract acts to oppose the selective non-competitive antagonism of picrotoxin on gamma-aminobutyric acid (GABA), which is mediated by the GABA A receptor; this has been widely implicated in epilepsy, as previously reported [28]. The primary inhibitory neurotransmitter in the brain is gamma-aminobutyric acid, and suppressing it results in increased activity of excitatory neurotransmitter (glutamate), forming the underlying factor in epilepsy [29, 30].

The findings of this study indicated that methanol root extract of *Securidaca longepedunculata* was more potent in delaying myoclonic and clonic seizures and more effective in protecting animals from picrotoxin-induced death, at least to some extent. The above statement may be due to the type and amounts of chemical compositions in the plant. Future studies on evaluating the anticonvulsant potential of *Securidaca longepedunculata*should focus on its fractions and isolate the possible compound(s) that might be responsible for the said action.

To determine the possible involvement of the GABAergic pathway in the mechanisms of action of *Securidaca longepedunculata* , flumazenil, a GABA receptor antagonist, amphetamine and chlorpromazine were used. It produced its inhibiting effect at the benzodiazepine recognition site on the GABA/benzodiazepine receptor complex competitively. From this finding, flumazenil antagonized the anticonvulsant effect of *Securidaca longepedunculata* significantly when compared to both vehicle control and phenobarbitone. Similarly, flumazenil significantly blocked the anticonvulsant effect of phenobarbitone by reducing the onset of convulsion, producing 100% mortality. It has been noted that an increase in catecholamines can enhance anticonvulsant activity. Therefore, the methanol root extract of  *Securidaca longepedunculata* could be linked to the processes involved in neurohumoral transmission. The enhancement of central dopaminergic transmission is linked to anticonvulsant activity. In this study, the concurrent administration of amphetamine with the extract protected against picrotocin-induced seizures in mice. Conversely, the administration of chlorpromazine, a dopamine antagonist, resulted in increased seizure activity. This data suggests that the dopaminergic mechanism may play a role in seizure modulation in mice.

**CONCLUSION**

In conclusion, the results of the present study demonstrated the anticonvulsant activity of the methanol root extract of *Securidaca longepedunculata* . Also, our results suggest that the anticonvulsant activity of *Securidaca longepedunculata* may be mediated via benzodiazepine pathways. These results support the traditional use of the plant in the treatment of convulsions. However, further research is required to isolate and characterize the active compound(s) that may be responsible for the observed activity.

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