**RESEARCH ARTICLE**

# Synthesis and *In-vivo* Anticancer Evaluation of N-(4-oxo-2- (4-((5-aryl-1,3,4 thiadiazole-2yl) amino) Phenyl thiazolidine-3-yl) Benzamide derivative

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**ABSTRACT**

The development of novel antitumor agents is paramount in the fight against drug resistance and its associated cardiotoxicity. The present study carried out a thorough anticancer evaluation of N- (4-oxo-2-(4-((5-aryl-1,3,4 thiadiazole-2yl) amino) phenyl thiazolidine-3-yl) benzamide (TH08) in Ehrlich ascites carcinoma (EAC) in Swiss albino mice. Our assessment included the measurement of tumor weight, the duration of survival for mice with tumors, and tumor cell growth inhibition. Additionally, we analyzed hematological parameters, like white and red blood cells and hemoglobin levels. The results we obtained clearly demonstrate that TH08 is a potent anticancer agent that exerts a positive effect on EAC cells. To determine TH08's effectiveness, We compared our findings to those achieved by using the standard medication, bleomycin. Our findings provide compelling evidence that TH08 has significant potential as a novel antitumor agent.

**Keywords:** Anticancer, Antitumor, Cancer, EAC, Ehrlich ascites carcinoma, Synthesis, Thiadiazole.

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## INTRODUCTION

The prevalence of cancer as a health issue is a matter of great global concern, given that it has now risen to become the second leading cause of mortality worldwide, with the only other cause surpassing it being cardiovascular diseases. This highlights the urgent need for effective strategies to tackle this issue and improve the overall health outcomes of populations across the globe. This complex disease manifests in various forms and can result in various consequences at the cellular and molecular levels. Cancer is characterized by uncontrolled cell proliferation, which can occur at varying rates, depending on the specific cancer type. Despite significant progress in developing anticancer drugs, much remains to be learned about the biology and pharmacology of this disease. Unfortunately, in some cases, traditional medical treatments may prove insufficient. According to the World Cancer Report, from 2008, there were around 12 million new cases of cancer diagnosed, resulting in roughly seven million deaths. As of now, an estimated 25 million people worldwide are struggling with cancer.[1,2]

Thiadiazole is a commonly used heterocyclic compound in the pharmaceutical industry. It exists in 4 different isomeric forms found in nature, namely 1,3,4-thiadiazole, 1,2,5-thiadiazole, 1,2,4-thiadiazole, and 1,2,3-thiadiazole. This thiadiazole compound contains the five-member ring and nitrogen and sulfur. Thiadiazole is the center of attraction for many researchers to modify and synthesize new compounds. So far, cytotoxicity against cancer cell lines by modifying the thiadiazole ring has proven to have improved potency and lesser toxicity.[3] Thiadiazole-containing compounds have demonstrated remarkable biological activity due to the ring's potent aromaticity, which guarantees good *in-vivo* stability and low toxicity.[4] The observed positive characteristics of these substances include antimicrobial, analgesic, anticancer, anti-inflammatory, anti-tubercular, anticonvulsant, diuretic, anti-depressant, and anti-*Helicobacter pylori* activity.[5,6]

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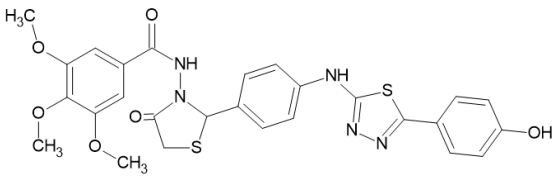

**Figure 1:** Structure of TH08

In the ongoing fight against drug resistance and the adverse side effects it can cause, the development of novel cancer treatments is of paramount importance. Thiadiazole has been the subject of extensive research for more than a century due to its therapeutic benefits. Thiadiazoles' propensity to dissolve in lipids is improved by the sulfur atom in the ring, and their mesoionic makeup enables them to interact with biological targets and traverse cell membranes with various degrees of affinity.[7] Thiadiazole-based anticancer agents have displayed potent effects on a wide array of human cancer cell lines, inhibiting a diverse range of molecular targets such as focal adhesion kinase, Abl tyrosine kinase, Akt, histone deacetylase, and tubulin polymerization. These findings suggest that thiadiazole-based substances constitute an exciting opportunity for the development of new cancer therapies.[8]

Around the world, cancer research uses EAC cell lines as experimental tumor models. Paul Ehrlich first identified these cells in the white mouse's breast gland in 1907, and they were later given his name.[9] In the current investigation, we selected N-(4-oxo-2- (4-((5-aryl-1,3,4 thiadiazole-2yl) amino) phenyl thiazolidine-3-yl) benzamide (TH08) as the test compound and examined it's *in-vivo* anticancer property in EAC cells.

## MATERIALS AND METHODS

### Synthesis TH08 Compound

The method used for synthesizing TH08 was identical to that described in the earlier paper.[10] By measuring melting points and doing IR, NMR, and HRMS spectral analyses, it was possible to validate the compound's purity and synthesis. The derivative has the following structure (Figure 1).

### Anticancer Activity

*Animals*

For this study, we used male Swiss albino mice that weighed between 19 and 26 grams. They were maintained under consistent environmental conditions, receiving a standardized pellet diet and having continuous access to water throughout the study. Before the experiment began, the mice were acclimated for 10 days to laboratory conditions. The Institutional Animal Ethical Committee of the University reviewed and approved all procedures described in this study.

*Median lethal doses Determination ($LD_{50}$)*

To estimate the $LD_{50}$ values, an acute toxicity test was conducted as explained in another source.[11] The TH08 was solubilized in dimethyl sulfoxide (3%) and given to various groups with ascending doses intraperitoneally. Each group contained 4 animals, and death was observed after 24 hours of therapy. The dosage at which fifty percent of mice survived the substance was determined to have an $LD_{50}$ value.

*Cancer cell line*

The Cancer Research Centre in Amala, Kerala, provided the EAC cell lines used in this work. The mice were regularly maintained through weekly inoculation of $10^6$ cells, each intraperitoneally.

*Tumor transplantation*

A type of cancer called Ehrlich's ascites carcinoma was kept alive by repeatedly transplanting it from tumor-bearing mice. Ascetic fluid was taken from the mice at the tumor cells' log phase (which occurred on day 78). The tumor cell count was then adjusted to 2 million cells/mL ($2 \times 10^6$ cells/mL). Only samples with a more than 90% viability rate were chosen for transplantation. Every animal was given 0.2 mL of the tumor cell suspension comprising 2 million cells per mL ($2 \times 10^6$ cells/mL) via intraperitoneal injection.[12]

*Drug therapy schedule*

Six male Swiss albino mice were given an intraperitoneal injection of EAC cells (0.2 mL, $2x10^6$ cells/mouse) and divided into five groups. Day 0 was marked as the injection day. Group 1 (cancer control) received intraperitoneal administration of propylene glycol 5 mL/kg for 14 days starting from the first day. Group 2 was given bleomycin as a standard (0.3 mg/kg/day/mice). Groups 3 to 5 were administered TH08 at several doses (5, 15, and 25 mg/kg/mouse/day). Three mice from each group were euthanized following the final dose to measure anticancer potential and hematological parameters. The mice remaining in every group were observed for MST and the percentage of hosts who lived longer after exposure to tumors. The study evaluated various parameters, including body weight, life span, cytological studies of cell lines, and hematological parameters (WBC, RBC, hemoglobin, and differential count) to assess the anticancer activity of TH08. Changes in ascitic tumor volume, body weight, packed cell volume, nonviable and viable tumor cell count, MST, and %ILS were observed.[12,13]

*Packed cell volume and tumor cell volume*

We dissected the animal to obtain ascitic fluid from the peritoneal cavity. We then centrifuged the fluid at 1000 rpm for five minutes to measure the packed cell volume. We carefully collected the transplantable murrain tumor in order to determine its volume.

*Nonviable and viable cell count*

We used tryptophan blue staining (normal saline 0.4%) and the dye exclusion test to count viable and nonviable cells in the ascetic cell. We determined the count using a Neubauer counting chamber. Nonviable cells absorbed the pigment, while viable cells did not.

*MST and percent ILS*

The impact of TH08 on tumor growth was evaluated using the %ILS and MST methods. The MST was measured by

monitoring the mortality of four mice in each group daily for six weeks. The %ILS was calculated utilizing a specific equation.[14-16]

MST = (Day of first death + day of last death)/2. %ILS = (MST of treated group/MST of control group)1 × 100.

*Effect of TH08 on hematological parameters*

Blood was obtained from every mouse through intracardial puncture using heparin as a blood anticoagulant. The resulting sample was analyzed to determine the presence of WBCs, RBCs, hemoglobin, and differential count.

**Statistical Analysis**

The investigational finding was expressed as mean ± SEM. The Student t-test analyzed data. Significant values are *p < 0.05, **p < 0.01, and ***p < 0.001.

## RESULTS

The synthesis of the target compound (TH08) was carried out as per our previously published work. The compound's melting point was 162–164, the Rf value was 0.73 using solvent system ethanol (70%) and ethyl acetate (30%) and the percentage yield was 66%.

**TH08**

mp 162–164°C. Yield: 66%; IR $cm^{-1}$ (KBr Pelletes): 3219.34 (NH, str.); 3081.18 (C-H, str., aromatic); 1781.18 (C=O, str., ketonic); 1723.02 (C=N, str., Aromatic); 1481.59 (C=C, str., aromatic); 1239.99 (str., C-N); 753.36 (C-S. str., aromatic);. $^{1}$H-NMR δ ppm (400 MHz, DMSO): 11.94 (m, 1H, CONH**);** 10.31 (m, 1H, NH-Ar); 7.61 (m, 2H, ArH); 7.43 (m, 2H, ArH); 7.21 (m, 2H, ArH); 6.96 (m, 2H, ArH); 5.86 (m, 1H, SCH); 3.93 (s, 2H, $CH_2S$); 3.84 (s, 6H, $OCH_3$); 3.75 (s, 3H, $OCH_3$);**.** $^{13}$C-NMR δ ppm (100 MHz, DMSO): 35.81 ($SCH_2$); 56.54 ($C_{3,5}$-$OCH_3$); 60.80 ($C_4$-$OCH_3$); 64.30 (SCH); 116.66 (C4-ArNH);; 120.54 ($C_1$-$Ar_1$); 127.54 ($C_2$-$Ar_2$); 128.96 ($C_6$-$Ar_2$); 129.25 ($C_{2,6}$-ArNH); 138.41($C_4$-$Ar_1$); 152.82($C_5$-$Ar_1$); 158.42($C_4$-$Ar_2$); 168.26 (CO-thiazole). HRMS (ESI) *m/z* (%): calcd for $C_{27}H_{25}N_5O_6S_2$ $[M+H]^+$: 579.12; found 579.25. Anal. Calcd for $C_{27}H_{25}N_5O_6S_2$: C, 55.95; H, 4.35; N, 12.08. Found: C, 56.09; H, 4.38; N, 12.05.

Typically, we obtained the mean values from repeated experiments. Our findings indicate that the lethal dose of TH08 for intraperitoneal treatment of mice is 110 mg/kg. We have presented the impact of bleomycin and TH08 on EAC cell growth on the sixth day after tumor transplantation as given in Table 1.

The outcomes of the study indicated that all the anticancer drugs administered significantly impacted the survival time of mice with EAC cells. In particular, the effects of TH08 at various dosages were found to be significant, as demonstrated in Table 2. Notably, treatment with TH08 led to a considerable increase in the lifespan of the mice, with the percentage of increase varying based on the dose. Specifically, the lifespan of the mice was extended by 27.15, 48.65, and 70.52% for doses of 5, 15, and 25 mg/kg (i.p.) of TH08, correspondingly. It is worth noting that the survival time increased as the dose of TH08 increased, which indicates the dose-dependent nature of the drug's effect.

Furthermore, the study compared the efficacy of TH08 with that of bleomycin (3 mg/kg i.p.). The findings showed that bleomycin significantly lifespan increased of mice by 86.54% compared to bleomycin. Interestingly, the percentage of lifespan increase at 25 mg/kg i.p. of TH08 was similar to that of bleomycin, emphasizing TH08 as a promising anticancer drug. Overall, these findings highlight the potential of TH08 to increase the lifespan of mice with EAC cells in a dose-dependent manner, making it a promising drug for further research and development.

In comparison to the control mice with EAC, it has been observed that the administration of TH08 has a dose-dependent impact on packed cell volume, tumor volume, and viable tumor cell count. This is demonstrated in Table 3, which displays a reduction in these parameters with increasing doses of TH08.

Figure 2 illustrates the impact of TH08 on the hematological parameters of mice with tumors. As tumor growth progressed, the hematological parameters differed from their typical values. Upon inoculation of EAC cells, the WBC counts increased while the hemoglobin level and RBC counts decreased. However, after administering TH08 at the recommended doses, the parameters were moderately restored. Although TH08 exhibited slight toxicity during treatment, the parameters almost returned to normal after the therapy.

**Table 2:** MST and %ILS

| *Experimental group* | *Dose, mg/kg (i.p.)* | *MST, day (Mean ± SEM)* | *Percent increase of lifespan* |
|---|---|---|---|
| Control | - | 23.56 ± 0.86 | |
| Bleomycin | 0.3 | 45.63 ± 1.18** | 86.54 |
| TH08 | 5 | 28.25 ± 1.47* | 27.15 |
| TH08 | 15 | 35.61 ± 2.14** | 48.65 |
| TH08 | 25 | 40.06 ± 1.56* | 70.52 |

**Table 1:** Effects of the bleomycin and TH08 on EAC cell growth on 6$^{th}$ day after the tumor transplantation

| *Experimental Group* | *Dose, mg/kg (i.p.)* | *EAC cells on 6$^{th}$ day after tumour cell inoculation* | *Inhibition of cell growth(%)* |
|---|---|---|---|
| Control | - | $(2.517 \pm 0.182)\times10^7$ | |
| Bleomycin | 0.3 | $(0.297 \pm 0.012)\times10^7$*** | 88.20 |
| TH08 | 5 | $(1.766 \pm 0.004)\times10^7$*** | 29.82 |
| TH08 | 15 | $(0.962 \pm 0.017)\times10^7$** | 61.80 |
| TH08 | 25 | $(0.498 \pm 0.007)\times10^7$*** | 80.20 |

n=6, Results are depicted as Average ± SEM. Significant values are ***p < 0.001, **p < 0.01, and.*p < 0.05.

**Table 3:** Packed cell volume, tumour volume, and viable tumor cell count

| *Parameters* | *Control* | *Bleomycin* | *TH08 (05 mg/kg)* | *TH08 (15 mg/kg)* | *TH08 (25 mg/kg)* |
|---|---|---|---|---|---|
| Body weight | 26.23 ± 0.24 | 23.9 ± 0.02 | 25.16 ± 0.19 | 24.34 ± 0.16 | 23.28 ± 0.13 |
| Tumor volume (mL) | 5.71 ± 0.032 | 2.42 ± 0.13 | 4.22 ± 0.051 | 3.42 ± 0.082 | 3.02 ± 0.056 |
| Packed cell volume (mL) | 2.34 ± 0.23 | 1.15 ± 0.03 | 1.87 ± 0.068 | 1.75 ± 0.043 | 1.56 ± 0.092 |
| Viable tumor cell count, $\%10^6$ cells /mL | 11.27 ± 0.097 | 4.90 ± 0.015 | 8.54 ± 0.058 | 7.78 ± 0.18 | 4.85 ± 0.23 |
| Nonviable tumor cell count, $\times10^6$ cells/mL | 0.5 ± 0.017 | 1.23 ± 0.81 | 0.75 ± 0.068 | 0.92 ± 0.023 | 1.47 ± 0.021 |

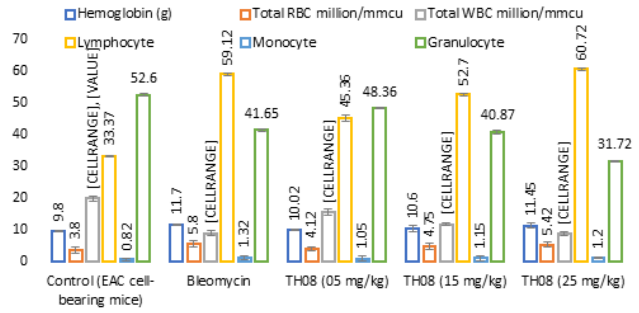


**Figure 2:** Hematological parameters of the tumor-bearing mice

## DISCUSSION

The efficacy of TH08 in combatting cancer has been evaluated through the measurement of three crucial factors. These include a decrease in tumor mass, hindrance of cell proliferation, and an augmentation in the average lifespan of mice afflicted with EAC.[13] In mice with EAC-bearing tumors, it has been observed that the weight of the tumors increases quickly over time. However, treatment with TH08 has been shown to slow down the rate of tumor growth. This trend is also reflected in the ability of TH08 to inhibit cell growth. Additionally, the lifespan of mice was significantly increased when treated with TH08. When determining the potency of an anticancer drug, it is necessary to observe the lifespan of mice with cancer. This is a reliable and crucial criterion, as per Clarkson *et al*.[14] In order to ensure the effectiveness of a TH08 on mice with EAC, it is important to monitor changes in both hematological and biological parameters. This monitoring process discovered that control mice's RBC and hemoglobin content slowly decreased over time, similar to the results found in normal mice. This could be due to iron deficit in hemolytic or myelopathic conditions.[15] The research on mice with EAC tumors has shown that the compound treatment has been effective in bringing the RBC and hemoglobin levels back to normal while also slowing down the increase of WBC levels. As a precautionary measure, experiments were conducted on normal mice to detect any potential toxic effects that the compound may have on the host. The results of these experiments showed a slight deterioration in some parameters, but it was not significant. It is worth noting that similar toxic effects were found in mice treated with bleomycin at a dosage of 0.3 mg/kg (i.p.).

After extensive research, it has been determined that introducing trimethoxy or dimethoxy groups to the phenyl-Ar1 molecule is crucial for enhancing its efficacy as an anticancer agent. It is worth noting that Ar1 refers to the aryl ring connected to the thiazole ring, while Ar2 is the aryl ring linked with the thiadiazole. TH08 demonstrated the most potent activity against the EAC cell line among the compounds tested. This could be attributed to incorporating trimethoxy or electron donating group on the phenyl-Ar1 ring and para substituting the phenyl ring-Ar2 with a robust electron donating moiety (OH). These results offer significant insights into the design and development of novel anticancer agents for improved therapeutic outcomes.

## CONCLUSION

According to the study's results, the anticancer agent TH08 displays significant potential in combatting EAC cells in Swiss albino mice. However, while these findings are promising, they do not yet meet the criteria for implementing a novel treatment in clinical settings. More research is necessary to determine the effectiveness of TH08 against various types of cancer cells and animal models. It is imperative that additional investigations be carried out to establish the safety and efficacy of TH08 before it can be considered as a viable option for cancer treatment.

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