CHAPTER -7

IN VIVO ANTICANCER STUDIES OF SCHIFF BASES
AND THIADIAZOLES

7.1 Introduction

Three Schiff bases, 3e, 3g, 3h and three thiadiazoles, 7a, 7m, 7n were selected for the in vivo anticancer studies depending on their low IC$_{50}$ in MTT assay. The above six triazole derivatives in two different concentrations were evaluated for their antitumor activity in EAC (Ehrlich ascites carcinoma) bearing Swiss albino mice. The anti-tumor activity was assessed in vivo by measuring the mean survival time, biochemical as well as hematological parameters [1]. The histopathological studies to assess the toxicity of these compounds on vital organs also have been carried out. The tumor progression was assessed by the extent of angiogenesis and increase in body weight.

7.2 In vivo toxicological assay

Nine groups of mice were formed for the in vivo assay. Each group comprised of twelve female Swiss albino mice [2].

Group -1: Normal mice.
Group -2: EAC bearing mice (Control).
Group -3: EAC bearing mice treated with a single dose of Cisplatin (Standard).
Groups 4-9: The study group, treated with Schiff bases / thiadiazoles as depicted below:
Group- 4: \(3g\) (50 mg/Kg) / \(7a\) (50 mg/Kg).

Group-5: \(3g\) (100 mg/Kg) / \(7a\) (150 mg/Kg).

Group-6: \(3h\) (50 mg/Kg) / \(7m\) (50 mg/Kg).

Group-7: \(3h\) (150 mg/Kg) / \(7m\) (100 mg/Kg).

Group-8: \(3e\) (50 mg/Kg) / \(7n\) (50 mg/Kg).

Group-9: \(3e\) (100 mg/Kg) / \(7n\) (150 mg/Kg).

7.2.1 Mean survival time (MST) [1]

A single dose Cisplatin of 3.5 mg/kg body weight, intra-peritoneal (i.p.) significantly enhanced the MST. Schiff bases, \(3g\) and \(3e\) in both concentrations were found to increase the survival time of animals. However, \(3e\) at an optimal dose of 100 mg/kg showed remarkable enhancement in the survival time of mice by 24 %. \(3h\) in both concentrations was found to be inactive. The detailed data showing the mean survival time is given in Fig. 7.1.

Both the thiadiazoles \(7m\) and \(7n\) in two different concentrations were found to escalate the survival time of animals. However, \(7m\) at an optimal dose of 50 mg/kg showed notable improvement in the survival time of mice by 33 %. \(7a\) in both concentrations was not effective. The mean survival time study results of thiadiazoles are depicted in Fig. 7.2.

7.2.2 Body weight [3]

A consistent gain in body weight was observed in the control mice, with a maximum of 36.12 %. In the case of \(3e\) (100 mg/kg) treated mice, gain in body weight was 11.11 %, indicative of its efficacy in bringing down the progression of cancer. The graph depicting the body weight changes of animals on treatment with Schiff bases are shown in Fig. 7.3.

In the case of \(7m\) (50 mg/kg) treated mice, gain in body weight was only 15.33 % indicative of its efficiency in bringing down the advancement of cancer. The graph representing the body weight changes of animals when treated with thiadiazoles are shown in Fig. 7.4.
**Fig. 7.1:** Effect of Schiff bases on the Kaplan Meier’s estimate of survival of EAC bearing mice

**Fig. 7.2:** Effect of thiadiazoles on the Kaplan Meier’s estimate of survival of EAC inoculated mice
Fig. 7.3: Effect of Schiff bases on body weight changes in EAC inoculated mice. *P < 0.05 compared to control and †P < 0.05 compared to standard.

Fig. 7.4: Effect of thiadiazoles on body weight changes in EAC inoculated mice. *P < 0.05 compared to control and †P < 0.05 compared to standard.
7.3 Hematological studies [4-6]

The detailed data obtained for each hematological parameter on treatment with Schiff bases are given in Fig. 7.5, Fig. 7.6 and Fig. 7.7. The RBC count was almost restored to normal range on treatment with 3e (100 mg/kg). The Hemoglobin levels were in the normal range in the 3e (100 mg/kg) treated groups. 3e at an optimal dose of 100 mg/kg could bring down the WBC level, but not as efficiently as Cisplatin.

The detailed data obtained for each hematological parameter when treated with thiadiazoles are given in Fig. 7.8, Fig. 7.9 and Fig. 7.10. The RBC count and hemoglobin content returned to normal level on treatment with 7m (50 mg/kg). None of the three thiadiazoles could bring down the WBC count to normal. But 7m (50 mg/kg) could reduce the count to an appreciable extent when compared to control.
Fig. 7.6: Effect of Schiff bases on WBC count in EAC induced mice. $^a$P < 0.05 compared to normal, $^b$P < 0.05 compared to control and $^c$P < 0.05 compared to standard.

Fig. 7.7: Effect of Schiff bases on hemoglobin count in EAC inoculated mice. $^a$P < 0.05 compared to normal, $^b$P < 0.05 compared to control and $^c$P < 0.05 compared to standard.
Fig. 7.8: Effect of thia diazoles on RBC count in EAC challenged mice. \(^a\)P < 0.05 compared to normal and \(^c\)P < 0.05 compared to standard.

Fig. 7.9: Effect of thia diazoles on WBC count in EAC induced mice. \(^a\)P < 0.05 compared to normal, \(^b\)P < 0.05 compared to control and \(^c\)P < 0.05 compared to standard.
**Fig. 7.10**: Effect of thiadiazoles on hemoglobin count in EAC inoculated mice. a P < 0.05 compared to normal, b P < 0.05 compared to control and c P < 0.05 compared to standard.

### 7.4 Biochemical studies [5]

The ALP (alkaline phosphatases) values were in normal range in 3e (100 mg/kg) treated group which is shown in Fig. 7.11. The SGPT (Serum glutamic pyruvic transaminase) and SGOT (Serum glutamic oxaloacetic transaminase) values remained in the normal range in 3e (100 mg/kg) treated group, suggestive of its less toxic effects when compared with the standard. In the case of 3e (100 mg/kg) treated mice, the protein levels did not vary significantly from normal (Fig. 7.12). Serum bilirubin which is indicative of hepatotoxicity, was also in the normal range in 3e (100 mg/kg) treated mice as shown in Fig. 7.13.
Fig. 7.11: Effect of Schiff bases on transaminases and ALP in EAC challenged mice. \(^aP < 0.05\) compared to normal, \(^bP < 0.05\) compared to control and \(^cP < 0.05\) compared to standard.

Fig. 7.12: Effect of Schiff bases on protein content in EAC induced mice. \(^aP < 0.05\) compared to normal, \(^bP < 0.05\) compared to control and \(^cP < 0.05\) compared to standard.
**Fig. 7.13:** Effect of Schiff bases on bilirubin count in EAC induced mice. \(^a\)P < 0.05 compared to normal, \(^b\)P < 0.05 compared to control and \(^c\)P < 0.05 compared to standard.

The ALP values were in normal range in 7m (50 mg/kg) treated group which is represented in Fig. 7.14. The SGPT and SGOT values remained in the normal range in 7m (50 mg/kg) treated group, suggestive of its less toxic effects when compared with the standard. In the case of 7m (50 mg/kg) treated mice, the protein levels did not vary significantly from normal (Fig. 7.15). The total bilirubin levels were also in the normal range in 7m (50 mg/kg) treated mice as shown in Fig. 7.16.

### 7.5 Angiogenesis

The dissection of animals was done on day-15 (Fig. 7.17). The inner peritoneal lining was examined for angiogenesis (Fig. 7.18). There were no signs of angiogenesis in the normal and Cisplatin treated mice. Formation of new blood vessels was observed in the control mice.

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**Fig. 7.14:** Effect of 6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-3-[(naphthalen-2-yloxy) methyl] [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7m) on Transaminases and ALP in EAC challenged mice. \(^a_P < 0.05\) compared to normal and \(^b_P < 0.05\) compared to control.

**Fig. 7.15:** Effect of 6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-3-[(naphthalen-2-yloxy) methyl] [1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7m) on protein content in EAC induced mice. \(^a_P < 0.05\) compared to normal and \(^b_P < 0.05\) compared to control.
**Fig. 7.16:** Effect of 6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-3-[(naphthalen-2-yloxy) methyl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7m) on bilirubin count in EAC induced mice. \(^a\)P < 0.05 compared to normal and \(^b\)P < 0.05 compared to control.

**Fig. 7.17:** Dissected-ventral view of mice

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Fig. 7.18: Inner peritoneal lining of mice
The inner peritoneal lining of 3e (100 mg/kg) and 7m (50 mg/kg) treated mice showed very mild angiogenesis.

### 7.6 Histopathology studies [7]

The histopathology studies of liver (Fig. 7.19) revealed less toxic nature of 3e (100 mg/kg) as compared to the control and standard group when viewed under light microscope of magnification 40x. The histology studies of liver of standard group showed cellular infiltration (inflammation), congestion and mild central vein dilatation. Whereas 3e (100 mg/kg) treated group showed only mild central vein dilatation suggesting less hepatotoxicity compared to the standard group. 7m (50 mg/kg) treated group showed only mild central vein dilation.

The histopathology of the kidney (Fig. 7.20) of control group showed mild cellular infiltration, while the standard group showed signs of nephrotoxicity due to cellular and glomerular infiltration. The mice treated with 3e (100 mg/kg) showed only mild glomerular infiltration. There were no signs of tubular necrosis, casts and glomerular congestion, which were indicative of mild nephrotoxicity on treatment with 3e (100 mg/kg). The 7m (50 mg/kg) treated group also showed mild glomerular infiltration. There were no signs of tubular necrosis, casts or glomerular congestion on treatment with 7m (50 mg/kg).

The standard group showed loss of splenic architecture and congestion. The 3e (100 mg/kg) and 7m (50 mg/kg) treated group also showed mild loss of splenic architecture, however congestion was not observed indicating their lower splenic toxicity. The splenic histopathology is depicted in Fig. 7.21.
Fig. 7.19: Histopathology of liver (40x)
Fig. 7.20: Histopathology of kidney (40x)
Fig. 7.21: Histopathology of spleen (40x)
7.7 Statistical analyses

The statistical analyses were performed by one-way ANOVA, followed by Tukey’s post hoc test using Graph Pad Prism 5.02. The results were expressed as the mean ± S.E.M. Differences were considered significant with a p value < 0.05.

7.8 Conclusion

In the light of the in vitro studies, compounds 4-((3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylidene)amino)-5-[(2-methylphenoxy)methyl]-1,2,4-triazole-3-thiol (3e), 4-((3-(4-chlorophenyl)-1H-pyrazol-4-yl)methylidene)amino)-5-[(4-methylphenoxy)methyl]-1,2,4-triazole-3-thiol (3g), 4-((3-(4-fluorophenyl)-1H-pyrazol-4-yl)methylidene)amino)-5-[(4-methylphenoxy)methyl]-1,2,4-triazole-3-thiol (3h), 6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-3-[phenoxy methyl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7a), 6-[3-(4-chlorophenyl)-1H-pyrazol-4-yl]-3-[(naphthalen-2-yloxy)methyl][1,2,4]triazolo[3,4-b][1,3,4]thiadiazole (7m) and 6-[3-(4-fluorophenyl)-1H-pyrazol-4-yl]-3-[(naphthalen-2-yloxy)methyl][1,2,4]triazolo [3,4-b][1,3,4]thiadiazole (7n) were subjected to in vivo anticancer studies against EAC cells in mice. It can be concluded that Schiff base 3e and thiadiazole 7m, at an optimal dose of 100 and 50 mg/kg body weight respectively are effective antineoplastic agents with comparatively less toxicity. They increased the mean survival time and decreased the body weight rise in mice indicating reduced tumor progression. The hematological and biochemical parameters also displayed the less toxic nature of triazole derivatives, 3e and 7m. The histopathology studies of liver, kidney and spleen also revealed minimum side effects with 3e and 7m treatment. Moreover, there were less signs of angiogenesis when the EAC bearing mice were treated with the above two compounds. Thus the results of the in vitro anticancer studies were confirmed in the subsequent in vivo studies.
Methylation can drastically enhance the bioavailability of molecules and thereby increase its efficacy. The introduction of a methyl group on phenyl ring usually increases the lipophilicity, but sometimes it renders a molecule more compact or globular, thereby leading to more solubility in biological fluids [8]. Greater solubility could also result from a decrease of the crystal lattice energy, the methyl group hindering the various intermolecular interactions like hydrogen bonds, dipole-dipole bonds etc. The oxidation of the methyl group might have given rise to an active metabolite, thus contributing to a reasonable half-life to the Schiff base, 3e. Also, the presence of a methyl group especially on aromatic ring, represents often a good means of detoxification as it is readily oxidized to an inactive and easy-to-eliminate carboxylic group. The presence of halogen atoms might have favored the passage of biomembranes in both 3e and 7m. The naphthyl group present in 7m could have brought in additional interactions with the target receptors thereby enhancing its anticancer properties.
References


