4. Kinetic Studies on Some Xanthine Oxidase Inhibitors

The kinetic behavior of compounds which inhibit superoxide anion production by the inhibition of xanthine oxidase was studied. Six compounds were selected on the basis of lowest IC_{50} values against xanthine oxidase to investigate the mechanism of inhibition. These include three new natural inhibitors (compounds **11, 38** and **39**) and three new synthetic inhibitors (compounds **61, 62-63**) along with propyl gallate. The type of inhibition was determined by the Lineweaver Burk and Dixon plots. The reciprocals of the velocity of reactions were plotted against the reciprocals of various substrate concentrations and the various concentrations of inhibitors in the Lineweaver Burk and Dixon plots, respectively. The secondary plots of both the Lineweaver Burk and Dixon plots were also plotted.

4.1. Mechanistic Studies on Cinnamic Acid Derivative and Stilbenes

The xanthine oxidase inhibitors, cinnamic acid derivative (**11**) and stilbene derivatives **38** and **39**, were studied to determine the effect on K_m and V_{max} by plotting the Lineweaver Burk and Dixon plots.

Based on the kinetic analysis, the mode of action of compounds **11** and **39** was found to be a mixed type inhibition with the K_i values of 12 and 45 μM, respectively. The mixed-type inhibition is a combination of partial competitive and pure non-competitive inhibition. The high K_m and low V_{max} of xanthine oxidase reaction with compound **11** was observed.

Compound **38** in contrast showed an un-competitive mode of inhibition as inferred from the decreased K_m and V_{max} values. The K_i value of compound **38** was found to be 98 μM. In uncompetitive inhibition, the inhibitor binds to the enzyme only after

substrate binding. In this case, first the substrate (S) binds with the enzyme (E) to form the ES complex. The inhibitor (I) then approaches this ES complex, and the ESI complex is formed which is catalytically inactive.

The structures of compounds **38** and **39** differ only in the sugar moieties. This difference results in the different modes of inhibition of these compounds. The IC_{50} and K_i values of natural compounds are presented in Table **4.1**. Kinetic analysis of compound **11** by the Lineweaver Burk and Dixon plots and their secondary plots is presented in Figures **4.1-4.2**.

4.2. Kinetic Studies on 1,3,4-Oxadiazole-2-(3*H*)-Thione Derivatives

Mechanistic studies on three new synthetic inhibitors (compounds **61**, **62** and **63**) belonging to the 1,3,4-oxadiazole-2-(3*H*)-thione class, were conducted. The mode of action of compounds **61** and **63** was found to be uncompetitive inhibition of xanthine oxidase as indicated by decreased K_m and V_{max} values. The kinetic analysis of compound **63** against xanthine oxidase enzyme is represented in Figures **4.3-4.4**. However, the interaction of compound **62** to the enzyme was found to be a mixed-type inhibition on the basis of higher K_m and low V_{max} values. Propyl gallate was also found to be an uncompetitive inhibitor of the enzyme with the K_i value 510 μM.

Table 4.1: Results of *in vitro* xanthine oxidase inhibition studies on selected compounds.

Compounds No.	IC_{50} (μM)	*Ki* (μM)	Type of inhibition
11	181 ± 1.60	12	Mixed-type
38	178 ± 4.62	98	Uncompetitive
39	129 ± 3.00	45	Mixed-type
61	160 ± 1.44	125	Uncompetitive
62	139 ± 0.27	130	Mixed-type
63	110 ± 1.18	73	Uncompetitive
Propyl gallate	628 ± 5.00	510	Uncompetitive

SEM is the mean of three values.
K_i is calculated using Dixon plot and Lineweaver Burk secondary plots.

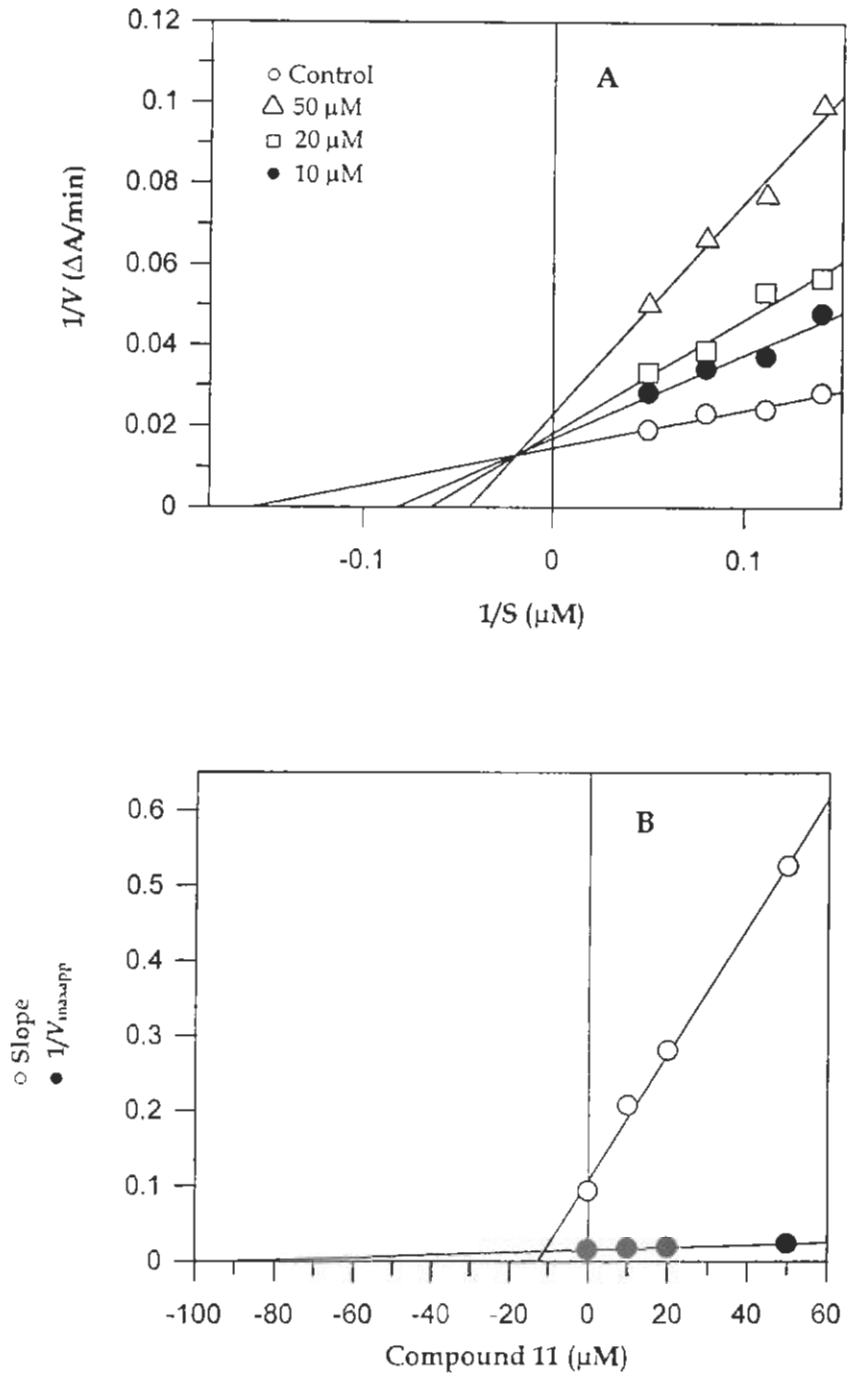

Figure 4.1: Steady state inhibition of xanthine oxidase by compound 11.
(A) Lineweaver Burk plot of reciprocal of the initial velocities versus reciprocal of xanthine. (B) Respective secondary plots of the Lineweaver Burk plot: $1/V_{maxapp}$ and slope versus compound concentrations. Each point in these graphs represents the mean of three replicates.

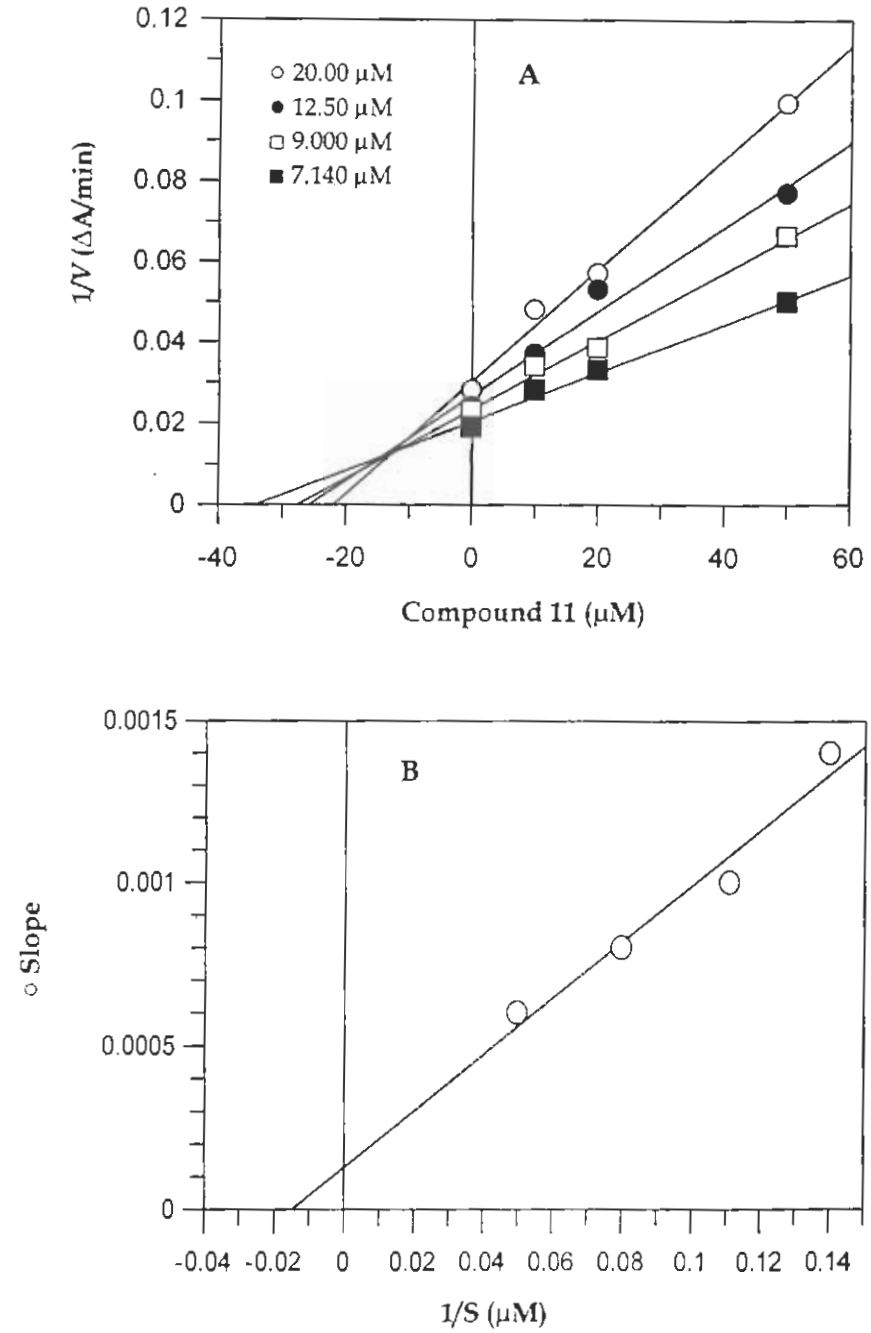

Figure 4.2: Steady state inhibition of xanthine oxidase by compound **11**.
(A) Dixon plot of reciprocal of the initial velocities versus concentrations of compound **11**. (B) Respective secondary plots of the Dixon plot: slope versus reciprocal of the various concentrations of xanthine. Each point in these graphs represents the mean of three replicates.

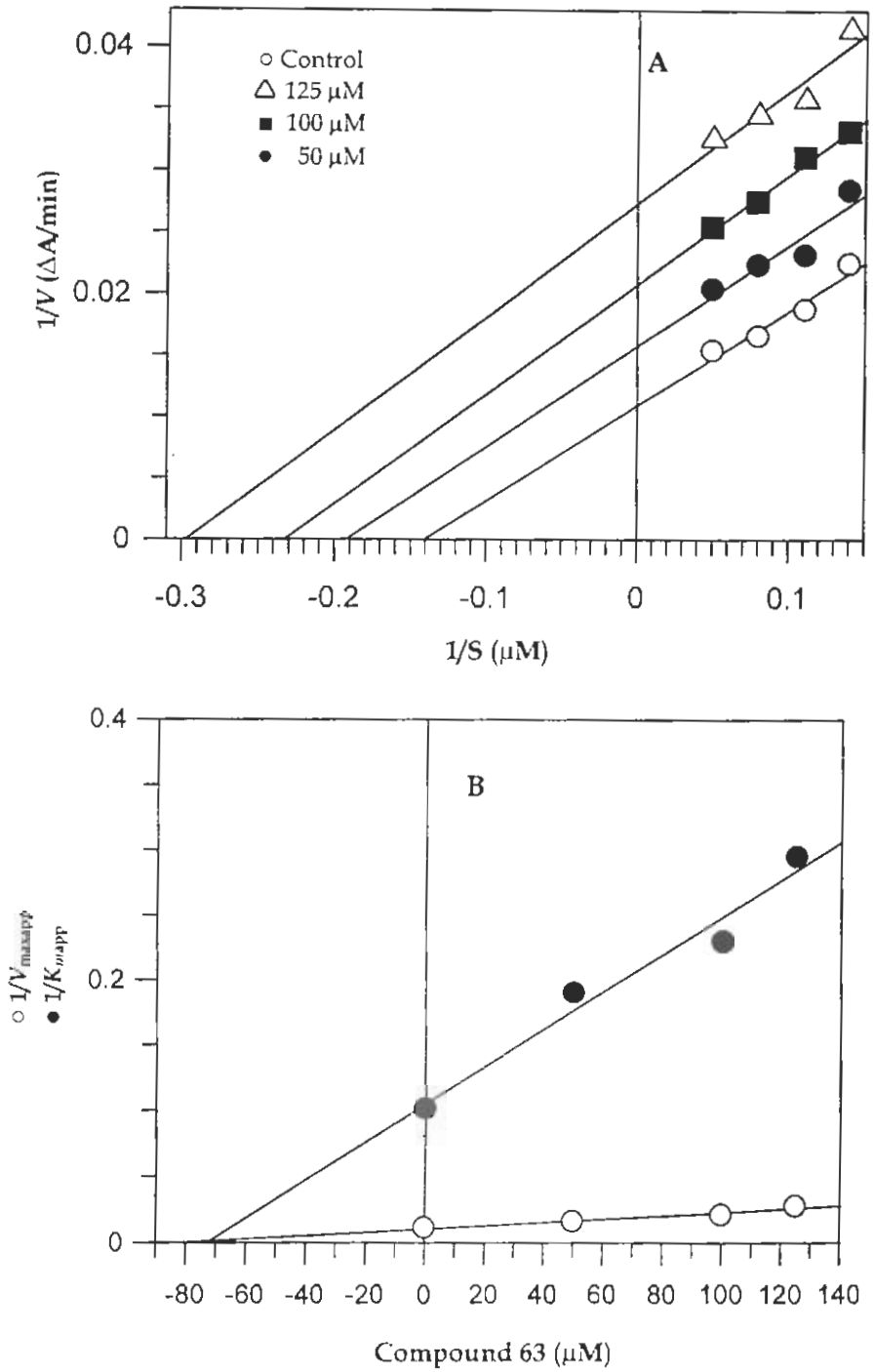

Figure 4.3: Steady state inhibition of xanthine oxidase by compound **63**.
(A) Lineweaver Burk plot of reciprocal of the initial velocities versus reciprocal of xanthine. (B) Respective secondary plots of the Lineweaver Burk plot: $1/V_{maxapp}$ and $1/K_{mapp}$ versus compound concentrations. Each point in these graphs represents the mean of three replicates.

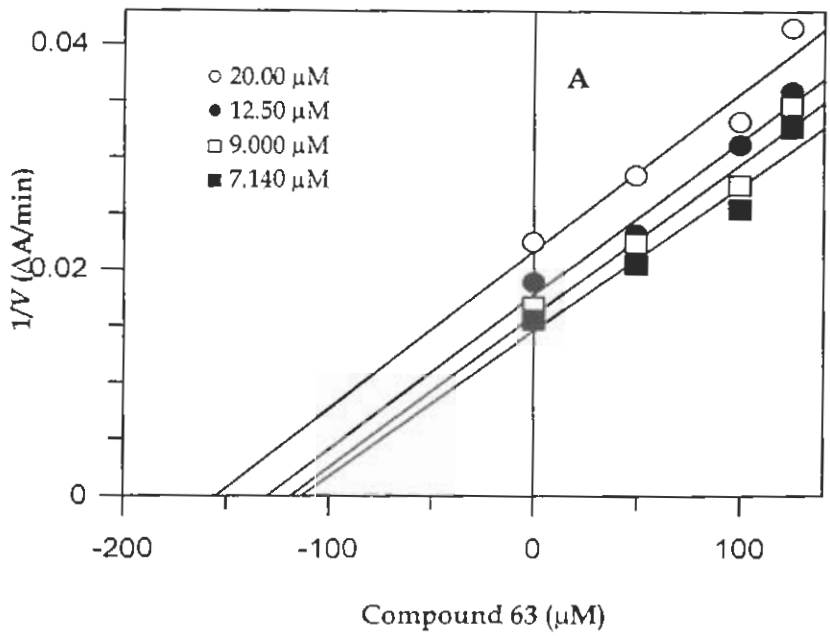

Figure 4.4: Steady state inhibition of xanthine oxidase by compound **63**.
(A) Dixon plot of reciprocal of the initial velocities versus concentrations of compound **63**. Each point in the graph represents the mean of three replicates.