activity on 5α-reductase, as shown in Figure 2. With this assay, the more potent inhibitory compounds were shown to be associated with analogues that are unsaturated at C-24 and C-25. For example, three most potent inhibitors (**1**, **2**, and **8**) contain sp^2 hybridization from C-24 to C-25, while the fully saturated triterpenoids are less potent. In addition, the C-26-hydroxy (**12**) is less potent than the C-26-carboxy (**1**), indicating that the presence of an acidic functionality at the 26-carbon is imperative for potent enzyme inhibition within this series of compounds. Among ganoderic acids, **1** and **2** were the only ones that have $\Delta^{24,25}$ and showed stronger 5α-reductase inhibitory activity. Among ganoderma alcohol, **8**, **9**, **10**, and **13**, which have $\Delta^{24,25}$, showed stronger 5α-reductase inhibitory activity than those having no $\Delta^{24,25}$ (**11**, **12**) in the 17β-side chain.

The pH dependence of 5α-reductase inhibitory activity of **2** is presented in Figure 3. A high pH is required for 5α-reductase inhibition. Within the pH range (6–8) in the 5α-reductase assay, the conversion from testosterone to DHT by the 5α-reductase was at the same level. These data indicate that the inhibition of 5α-reductase by the 26-carboxy triterpenoids depended on the carboxylate anion of the triterpenoid inhibitor. The protonated, electrophilic form of this functionality might coordinate to the carboxylate anion of the triterpenoid inhibitor through an ionic interaction, thereby stabilizing the enzyme-bound complex.

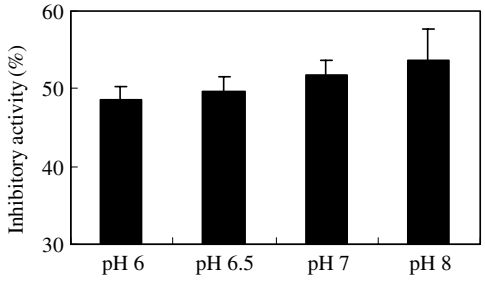

Figure 3. The pH dependence of **2** on 5α-reductase inhibitory activity. $n = 2$.

As a working model, the chemical mechanism of 5α-reductase is proposed to involve the direct hydride transfer from NADPH to C-5 of testosterone, whereby an enzyme-associated electrophile could stabilize the resulting 3,4-enolate (Fig. 4a). The NADPH is the first substance to bind, and the $NADP^+$ is the last product to be released from the enzyme surface. Consequently, both NADPH and $NADP^+$ independently interact with free enzyme, giving rise to binary enzyme complexes to which steroidal inhibitors could associate.[45] Formation of a triterpenoid species with cationic character at C-26 by initial activation of the enone substrate through coordination to this electrophilic center would facilitate the hydride transfer. Upon subsequent C-4 protonation of the enolate intermediate, DHT would be formed. According to Wolfenden,[46] chemically stable structural and electronic mimics of enzyme-bound reaction intermediates should demonstrate high enzyme affinity via exploitation of the specific interactions involved in the stabilization of the active site-associated transition or intermediate states. To optimize the interaction with the binding site of 5α-reductase, the α,β-unsaturated 17β-side chain should therefore be taken into account. Thus, the unsaturated 26-carboxyl triterpenoids should be designed as enzyme-bound compounds (Fig. 4b).

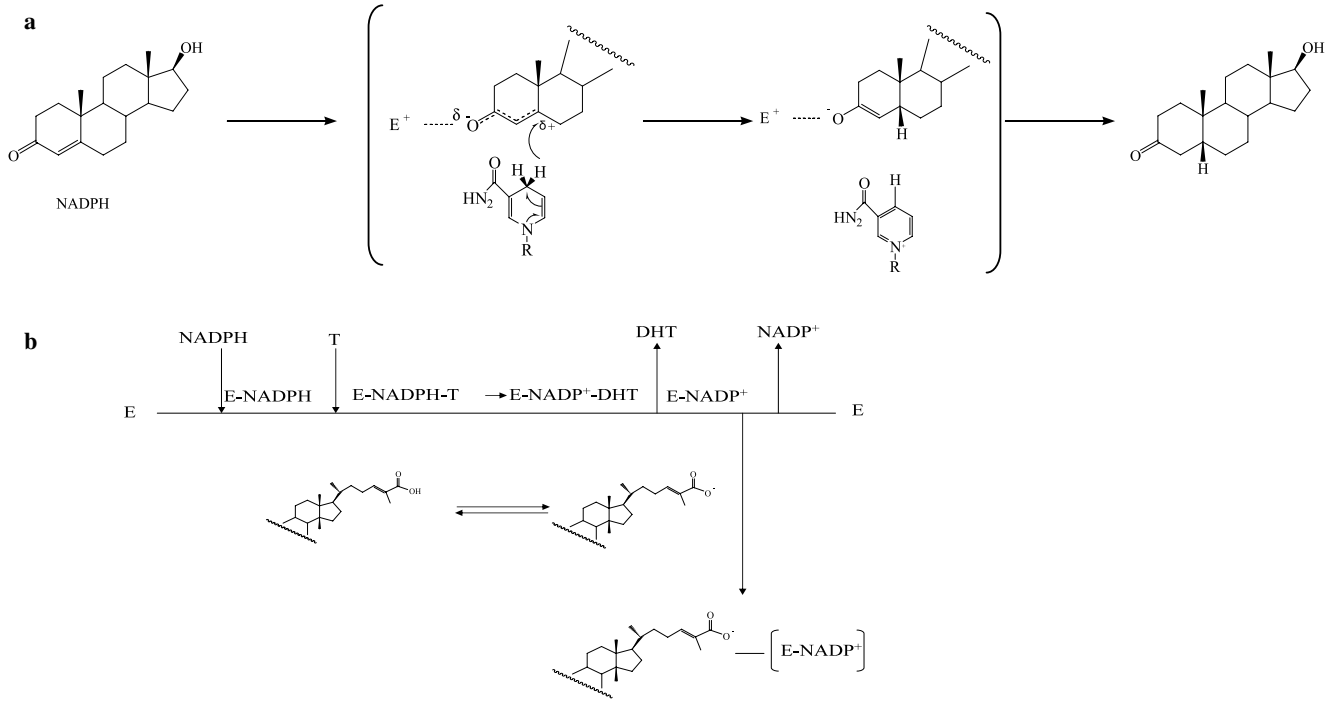

Figure 4. Proposed chemical mechanism of steroid 5α-reductase.