

Article

Establishing the Thermodynamic Cards of Dipine Models' Oxidative Metabolism on 21 Potential Elementary Steps

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Abstract: Dipines are a type of important antihypertensive drug as L-calcium channel blockers, whose core skeleton is the 1,4-dihydropyridine structure. Since the dihydropyridine ring is a key structural factor for biological activity, the thermodynamics of the aromatization dihydropyridine ring is a significant feature parameter for understanding the mechanism and pathways of dipine metabolism in vivo. Herein, 4-substituted-phenyl-2,6-dimethyl-3,5-diethyl-formate-1,4-dihydropyridines are refined as the structurally closest dipine models to investigate the thermodynamic potential of dipine oxidative metabolism. In this work, the thermodynamic cards of dipine models' aromatization on 21 potential elementary steps in acetonitrile have been established. Based on the thermodynamic cards, the thermodynamic properties of dipine models and related intermediates acting as electrons, hydrides, hydrogen atoms, protons, and two hydrogen ions (atoms) donors are discussed. Moreover, the thermodynamic cards are applied to evaluate the redox properties, and judge or reveal the possible oxidative mechanism of dipine models.

Keywords: thermodynamic cards; dipine oxidative metabolism; elementary steps



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1. Introduction

1,4-dihydropyridines are a type of important antihypertensive drug known as L-calcium channel blockers [1–6]. Until now, over a dozen 1,4-dihydropyridine drugs have been extensively used in clinic, such as Nifedipine, Felodipine, Amlodipine, etc. (Figure 1) [7,8]. Because their drug names all contain the word “dipine”, they are also well-known as dipine drugs or dipines. The core skeleton of dipines is the 1,4-dihydropyridine structure, and the dihydropyridine ring is a key structural factor for their biological activity [7–10]. If the dihydropyridine ring is oxidized or aromatized into a pyridine structure in vivo, the resulting oxidative metabolites would lose the biological activity of blocking the L-calcium ion channel to lower blood pressure (Figure 2a) [1–10]. There exists various oxidoreductases in vivo (Figure 2b), such as CYP450 [11,12], flavin coenzyme [13–15], heme iron coenzyme [16–19], and coenzyme Q [20–23], as well as NADH coenzyme [24–26], and they are excellent electron, hydrogen atom, or hydride acceptors [27–31]. For example, it was reported that dipines were oxidated by cytochrome P-450 in human liver microsomes, and the metabolism process experienced a successive $e + H^+ + e + H^+$ mechanism [30]. Some highly active intermediates, including a radical cation, a radical, and a protonated pyridine, were involved in the oxidative process [30]. Therefore, the aromatized metabolism process of dipines may involve 21 possible thermodynamic elementary steps and six potential active intermediates (Figure 3), whose thermodynamic driving forces are the significant physical parameters for understanding and revealing the possible oxidative pathways of dipine metabolism in vivo.

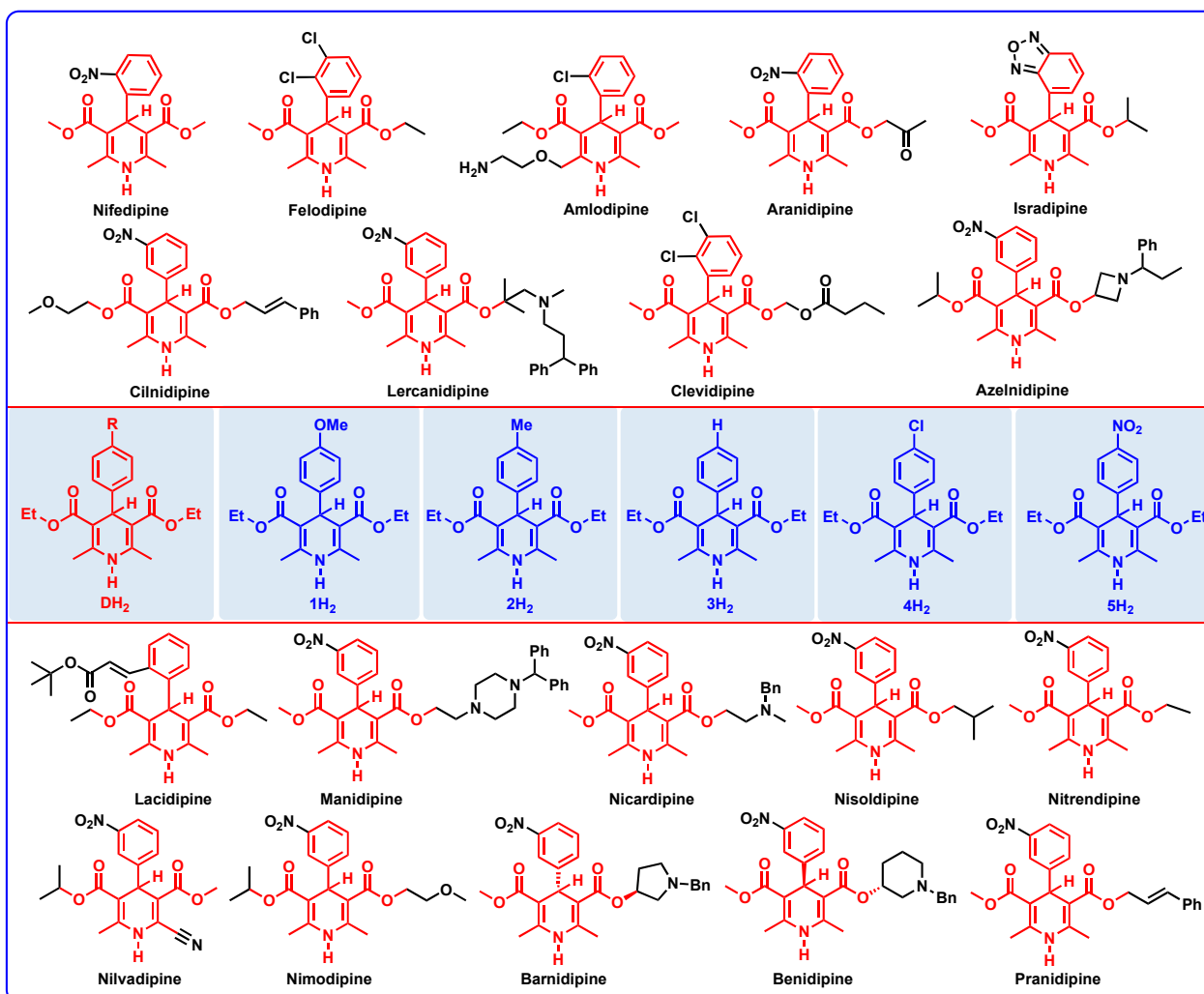


Figure 1. The chemical structures of diltiazem and refined diltiazem models (DH₂).

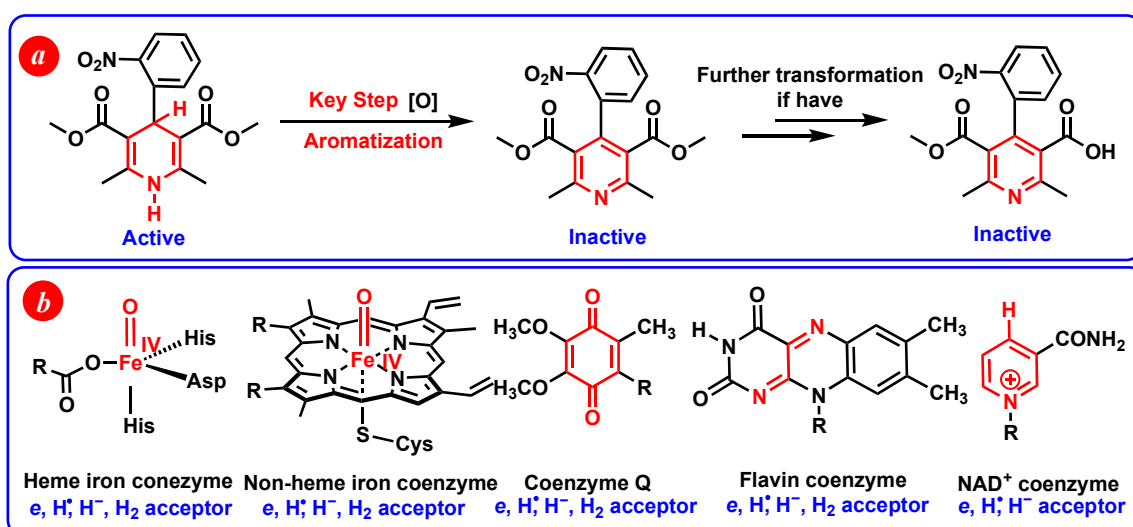


Figure 2. (a) The oxidative metabolism process of Nifedipine in vivo. (b) Some common oxidoreductases in vivo.

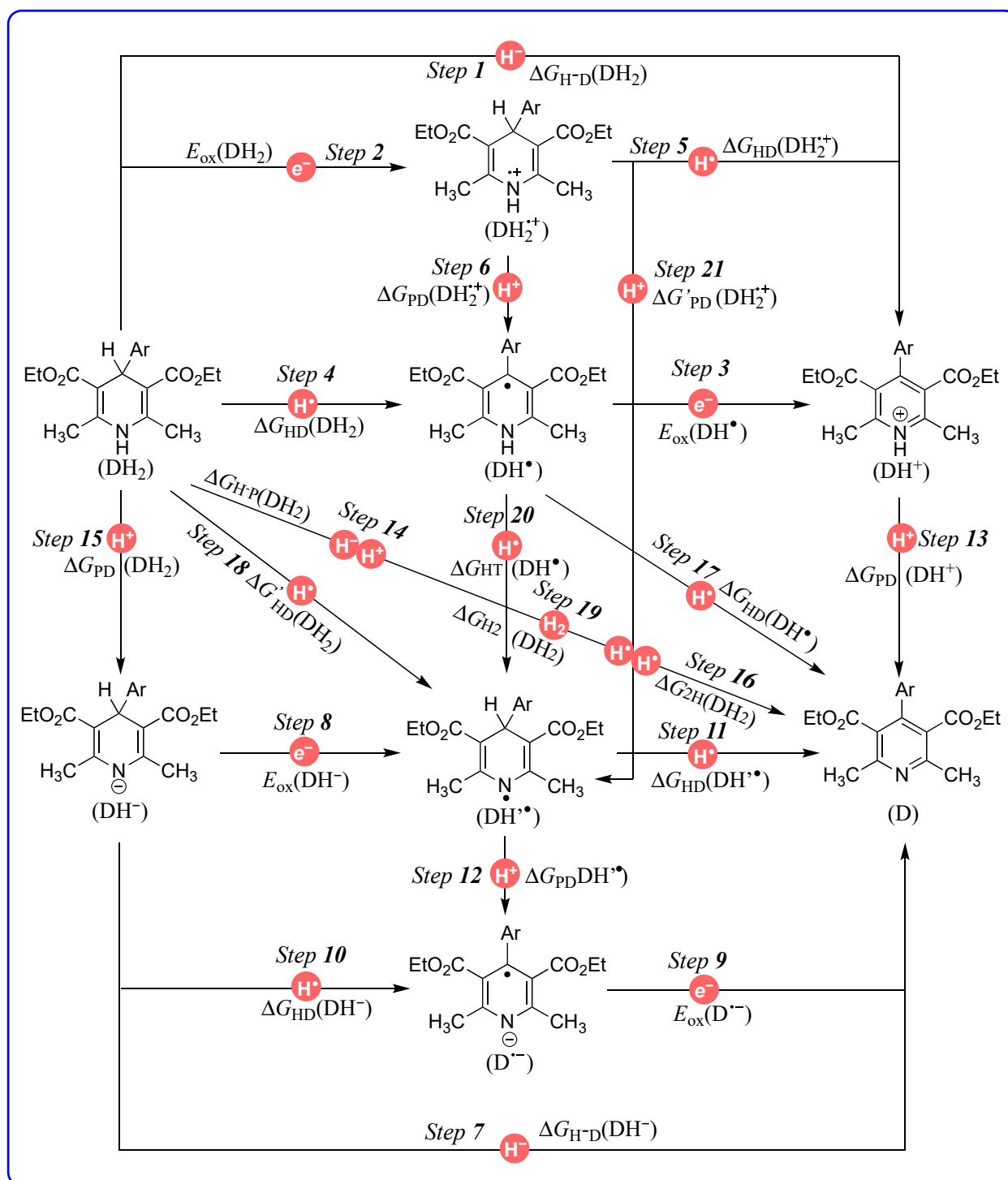


Figure 3. Thermodynamic cards of dipine model (DH₂) aromatization on 21 potential elementary steps during oxidative metabolism.

From the view of structure, dipines are the analogues of the Hantzsch ester (YH₂) [31] with the same core structure of 1,4-dihydropyridine, so dipines and the Hantzsch ester (YH₂) all belong to NADH models. Further examining the structural characteristics of dipines in Figure 1, it can be discovered that they generally have the same parent structure of 4-aromatic-2,6-dimethyl-3,5-diformate-1,4-dihydropyridines. In this work, 4-substituted-phenyl-2,6-dimethyl-3,5-diethyl-formate-1,4-dihydropyridines (DH₂) are refined as the

structurally closest dipine models and represented all of the dipines to investigate the thermodynamic parameters of dipine oxidative metabolism (Figure 1).

Our group has long been committed to thermodynamic research on hydrogen transfer for NADH models, and determining the thermodynamic driving forces of over 200 organic hydrides releasing hydrides in non-aqueous media [27,31]. The previous works inspire us to further investigate and clarify the thermodynamic parameters of dipine aromatization in solution. Herein, the thermodynamic cards of dipine models' aromatization on 21 potential elementary steps has been established (Figure 3). From the thermodynamic cards, the thermodynamic properties of dipine models and related intermediates acting as electrons, hydrides, hydrogen atoms, protons, and two hydrogen ions (atoms) donors are discussed. Furthermore, the thermodynamic cards are utilized to measure the redox properties, and diagnose the possible oxidative mechanism of dipine models.

2. Results and Discussion

2.1. Thermodynamic Parameters and Results

The thermodynamics of five 4-substituted-phenyl-2,6-dimethyl-3,5-diethyl-formate-1,4-dihydropyridines (DH₂), including 4-(4-methoxyphenyl)-2,6-dimethyl-3,5-di(ethylformate)-1,4-dihydropyridine (1H₂), 4-(4-methylphenyl)-2,6-dimethyl-3,5-di(ethylformate)-1,4-dihydropyridine (2H₂), 4-phenyl-2,6-dimethyl-3,5-di(ethylformate)-1,4-dihydropyridine (3H₂), 4-(4-chlorophenyl)-2,6-dimethyl-3,5-di(ethylformate)-1,4-dihydropyridine (4H₂), and 4-(4-nitrophenyl)-2,6-dimethyl-3,5-di(ethylformate)-1,4-dihydropyridine (5H₂) in acetonitrile were investigated, along with the thermodynamics of the Hantzsch ester (YH₂) for better comparison.

The thermodynamic potentials of 21 elementary steps are defined as the corresponding Gibbs free energies for *Step 1*, *Steps 4–7*, *Steps 10–21* or potentials for *Step 2*, *Step 3*, *Step 8*, and *Step 9*, which are shown in Figure 3.

As can be seen from Figure 3, *Step 1* and *Step 7* are the hydride-releasing chemical processes, $\text{DH}_2 \rightarrow \text{DH}^- + \text{H}^-$ and $\text{DH}^- \rightarrow \text{D} + \text{H}^-$, and their thermodynamic potentials are described by the related Gibbs free energies of DH₂ and DH[−] releasing hydrides, respectively, $\Delta G_{\text{H}^-/\text{D}}(\text{DH}_2)$ and $\Delta G_{\text{H}^-/\text{D}}(\text{DH}^-)$, which are also called hydricities [32–34]. Since the entropy change (TΔS) of organic hydrides releasing hydrides is estimated as 4.9 kcal/mol [35] in acetonitrile, so the $\Delta G_{\text{H}^-/\text{D}}(\text{DH}_2)$ and $\Delta G_{\text{H}^-/\text{D}}(\text{DH}^-)$ are derived from their corresponding enthalpy changes, $\Delta H_{\text{H}^-/\text{D}}(\text{DH}_2)$ and $\Delta H_{\text{H}^-/\text{D}}(\text{DH}^-)$, by Equations (1) and (5) in Table 1. The reliability was proved in previous literature [36,37]. $\Delta H_{\text{H}^-/\text{D}}(\text{DH}_2)$ and $\Delta H_{\text{H}^-/\text{D}}(\text{DH}^-)$ were available from our previous work [27,38].

Steps 2–3 and *Steps 8–9* are the electron-releasing chemical processes, and their thermodynamic potentials are described by the related oxidation potentials, $E_{\text{ox}}(\text{DH}_2)$ for *Step 2*, $E_{\text{ox}}(\text{DH}^\bullet)$ for *Step 3*, $E_{\text{ox}}(\text{DH}^-)$ for *Step 8*, and $E_{\text{ox}}(\text{D}^\bullet)$ for *Step 9*, respectively. The oxidation potentials were determined in our previous work [27,38].

Step 4–5, *Steps 1–11*, and *Steps 17–18* are the hydrogen-atom-releasing chemical processes, and their thermodynamic potentials are described by the related Gibbs free energies, $\Delta G_{\text{HD}}(\text{DH}_2)$ for *Step 4*, $\Delta G_{\text{HD}}(\text{DH}_2^{\bullet+})$ for *Step 5*, $\Delta G_{\text{HD}}(\text{DH}^-)$ for *Step 10*, $\Delta G_{\text{HD}}(\text{DH}^\bullet)$ for *Step 11*, $\Delta G_{\text{HD}}(\text{DH}^\bullet)$ for *Step 17*, and $\Delta G'_{\text{HD}}(\text{DH}_2)$ for *Step 18*, respectively. Based on Hess' law [27,37,38], $\Delta G_{\text{HD}}(\text{DH}_2)$, $\Delta G_{\text{HD}}(\text{DH}_2^{\bullet+})$, $\Delta G_{\text{HD}}(\text{DH}^-)$, $\Delta G_{\text{HD}}(\text{DH}^\bullet)$, $\Delta G_{\text{HD}}(\text{DH}^\bullet)$, and $\Delta G'_{\text{HD}}(\text{DH}_2)$ were calculated using Equations (2)–(3), (6)–(7), and (13)–(14), respectively, by constructing corresponding thermodynamic cycles, cycle *Step 4–Step 3–Step 1* for $\Delta G_{\text{HD}}(\text{DH}_2)$, cycle *Step 1–Step 2–Step 5* for $\Delta G_{\text{HD}}(\text{DH}_2^{\bullet+})$, cycle *Step 10–Step 9–Step 7* for $\Delta G_{\text{HD}}(\text{DH}^-)$, cycle *Step 7–Step 8–Step 11* for $\Delta G_{\text{HD}}(\text{DH}^\bullet)$, cycle *Step 4–Step 17–Step 16* for $\Delta G_{\text{HD}}(\text{DH}^\bullet)$, and cycle *Step 18–Step 11–Step 16* for $\Delta G'_{\text{HD}}(\text{DH}_2)$.

Step 6, *Steps 12–13*, *Step 15*, and *Step 21* are the proton-releasing chemical processes, and their thermodynamic potentials are described by the related Gibbs free energies, $\Delta G_{\text{PD}}(\text{DH}_2^{\bullet+})$ for *Step 6*, $\Delta G_{\text{PD}}(\text{DH}^\bullet)$ for *Step 12*, $\Delta G_{\text{PD}}(\text{DH}^+)$ for *Step 13*, $\Delta G_{\text{PD}}(\text{DH}_2)$ for *Step 15*, and $\Delta G'_{\text{PD}}(\text{DH}_2^{\bullet+})$ for *Step 21*, respectively. $\Delta G_{\text{PD}}(\text{DH}^+)$ for *Step 13* could be calculated by Equation (10) using the $\text{p}K_{\text{a}}(\text{DH}^+)$ value, $\Delta G_{\text{PD}}(\text{DH}^+) = 1.37\text{p}K_{\text{a}}(\text{DH}^+)$ [39].

In this work, the $pK_a(DH^+)$ values were predicted using the method developed by Luo and coworker at 2020 [40]. Based on Hess' law [27,37,38], the $\Delta G_{PD}(DH_2^{\bullet+})$, $\Delta G_{PD}(DH'^{\bullet})$, $\Delta G_{PD}(DH_2)$, and $\Delta G'_{PD}(DH_2^{\bullet+})$ were calculated using Equations (4), (8), (11), and (17) in Table 1, respectively, by constructing corresponding thermodynamic cycles, cycle Step 4–Step 2–Step 6 for $\Delta G_{PD}(DH_2^{\bullet+})$, cycle Step 8–Step 12–Step 10 for $\Delta G_{PD}(DH'^{\bullet})$, cycle Step 14–Step 15–Step 7 for $\Delta G_{PD}(DH_2)$, and cycle Step 6–Step 20–Step 21 for $\Delta G'_{PD}(DH_2^{\bullet+})$.

Step 14 is the chemical process of DH_2 releasing two hydrogen ions ($H^- + H^+$), and the thermodynamic potential is described by the Gibbs free energy of DH_2 releasing two hydrogen ions, $\Delta G_{H^-P}(DH_2)$. $\Delta G_{H^-P}(DH_2)$ could be obtained from $\Delta G_{H^-D}(DH_2)$ and $\Delta G_{PD}(DH^+)$ via Equation (10) in Table 1, $\Delta G_{H^-P}(DH_2) = \Delta G_{H^-D}(DH_2) + \Delta G_{PD}(DH^+)$ (Equation (10)), by constructing the thermodynamic cycle [27] Step 1–Step 13–Step 14.

Step 16 is the chemical process of DH_2 releasing two hydrogen atoms, and the thermodynamic potential is described by the Gibbs free energy of DH_2 releasing two hydrogen atoms, $\Delta G_{2H}(DH_2)$. $\Delta G_{2H}(DH_2)$ could be obtained by Equation (12) in Table 1, $\Delta G_{2H}(DH_2) = \Delta G_{H^-P}(DH_2) + F[E_{red}(H^{\bullet}) - E_{ox}(H^{\bullet})]$ (Equation (12)). Among Equation (12), $E_{red}(H^{\bullet})$ and $E_{ox}(H^{\bullet})$ were reported as -1.137 V and -2.307 V (vs. Fc) [27,38] in acetonitrile.

Step 19 is the chemical process of DH_2 releasing hydrogen gas (H_2), and the thermodynamic potential is described by the Gibbs free energy of DH_2 releasing H_2 , $\Delta G_{H_2}(DH_2)$. $\Delta G_{H_2}(DH_2)$ could be obtained by Equation (15) in Table 1, $\Delta G_{H_2}(DH_2) = \Delta G_{2H}(DH_2) - \Delta G_{H^-D}(H_2)$ (Equation (15)). Among Equation (15), $\Delta G_{H^-D}(H_2)$ refers to the Gibbs free energy of H_2 releasing hydrides, which was reported as 76.0 kcal/mol [41,42] in acetonitrile.

Step 20 is the chemical process of a hydrogen atom transfer within a molecule from the N_1 -position to the C_4 -position, and the thermodynamic potential is described by the related Gibbs free energy, $\Delta G_{HT}(DH^{\bullet})$. $\Delta G_{HT}(DH^{\bullet})$ could be obtained by Equation (16) in Table 1, $\Delta G_{HT}(DH^{\bullet}) = \Delta G_{HD}(DH^{\bullet}) - \Delta G_{HD}(DH'^{\bullet})$ (Equation (16)) by constructing the thermodynamic cycle [27] Step 20–Step 11–Step 17.

Table 1. Chemical processes, thermodynamic potentials, and computed equations or data sources of 21 elementary steps for DH_2 aromatization.

Step X	Chemical Process	Potentials	Computed Equations or Data Sources	Equation
Step 1	$DH_2 \rightarrow DH^+ + H^-$	$\Delta G_{H^-D}(DH_2)$	$\Delta G_{H^-D}(DH_2) = -\Delta H_{H^-D}(DH_2) - 4.9$	(1)
Step 2	$DH_2 \rightarrow DH_2^{\bullet+} + e^-$	$E_{ox}(DH_2)$	[38]	-
Step 3	$DH^{\bullet} \rightarrow DH^+ + e^-$	$E_{ox}(DH^{\bullet})$	[38]	-
Step 4	$DH_2 \rightarrow DH^{\bullet} + H^{\bullet}$	$\Delta G_{HD}(DH_2)$	$\Delta G_{HD}(DH_2) = \Delta G_{H^-D}(DH_2) - F[E_{red}(DH^+) - E_{ox}(H^-)]$	(2)
Step 5	$DH_2^{\bullet+} \rightarrow DH^{\bullet} + H^{\bullet}$	$\Delta G_{HD}(DH_2^{\bullet+})$	$\Delta G_{HD}(DH_2^{\bullet+}) = \Delta G_{H^-D}(DH_2) - F[E_{ox}(DH_2) - E_{red}(H^{\bullet})]$	(3)
Step 6	$DH_2^{\bullet+} \rightarrow DH^{\bullet} + H^+$	$\Delta G_{PD}(DH_2^{\bullet+})$	$\Delta G_{PD}(DH_2^{\bullet+}) = \Delta G_{HD}(DH_2) - F[E_{ox}(DH_2) - E_{red}(H^+)]$	(4)
Step 7	$DH^- \rightarrow D + H^-$	$\Delta G_{H^-D}(DH^-)$	$\Delta G_{H^-D}(DH^-) = \Delta H_{H^-D}(DH^-) - 4.9$	(5)
Step 8	$DH^- \rightarrow DH^{\bullet} + e^-$	$E_{ox}(DH^-)$	[38]	-
Step 9	$D^{\bullet-} \rightarrow D + e^-$	$E_{ox}(D^{\bullet-})$	[38]	-
Step 10	$DH^- \rightarrow D^{\bullet-} + H^{\bullet}$	$\Delta G_{HD}(DH^-)$	$\Delta G_{HD}(DH^-) = \Delta G_{H^-D}(DH^-) - F[E_{red}(D) - E_{ox}(H^-)]$	(6)
Step 11	$DH'^{\bullet} \rightarrow D + H^{\bullet}$	$\Delta G_{HD}(DH'^{\bullet})$	$\Delta G_{HD}(DH'^{\bullet}) = \Delta G_{H^-D}(DH^-) - F[E_{ox}(DH^-) - E_{red}(H^{\bullet})]$	(7)
Step 12	$DH'^{\bullet} \rightarrow D^- + H^+$	$\Delta G_{PD}(DH'^{\bullet})$	$\Delta G_{PD}(DH'^{\bullet}) = \Delta G_{HD}(DH^-) - F[E_{ox}(DH^-) - E_{red}(H^+)]$	(8)
Step 13	$DH^+ \rightarrow D + H^+$	$\Delta G_{PD}(DH^+)$	$\Delta G_{PD}(DH^+) = 1.37 pK_a(DH^+)$	(9)
Step 14	$DH_2 \rightarrow D + H^- + H^+$	$\Delta G_{H^-P}(DH_2)$	$\Delta G_{H^-P}(DH_2) = \Delta G_{H^-D}(DH_2) + \Delta G_{PD}(DH^+)$	(10)
Step 15	$DH_2 \rightarrow DH^- + H^+$	$\Delta G_{PD}(DH_2)$	$\Delta G_{PD}(DH_2) = \Delta G_{H^-P}(DH_2) - \Delta G_{H^-D}(DH^-)$	(11)
Step 16	$DH_2 \rightarrow D + H^{\bullet} + H^{\bullet}$	$\Delta G_{2H}(DH_2)$	$\Delta G_{2H}(DH_2) = \Delta G_{H^-P}(DH_2) + F[E_{red}(H^{\bullet}) - E_{ox}(H^{\bullet})]$	(12)
Step 17	$DH^{\bullet} \rightarrow D + H^{\bullet}$	$\Delta G_{HD}(DH^{\bullet})$	$\Delta G_{HD}(DH^{\bullet}) = \Delta G_{2H}(DH_2) - \Delta G_{HD}(DH_2)$	(13)
Step 18	$DH_2 \rightarrow DH'^{\bullet} + H^{\bullet}$	$\Delta G'_{HD}(DH_2)$	$\Delta G'_{HD}(DH_2) = \Delta G_{2H}(DH_2) - \Delta G_{HD}(DH'^{\bullet})$	(14)
Step 19	$DH_2 \rightarrow D + H_2$	$\Delta G_{H_2}(DH_2)$	$\Delta G_{H_2}(DH_2) = \Delta G_{2H}(DH_2) - \Delta G_{H^-D}(H_2)$	(15)
Step 20	$DH^{\bullet} \rightarrow DH'^{\bullet}$	$\Delta G_{HT}(DH^{\bullet})$	$\Delta G_{HT}(DH^{\bullet}) = \Delta G_{HD}(DH^{\bullet}) - \Delta G_{HD}(DH'^{\bullet})$	(16)
Step 21	$DH_2^{\bullet+} \rightarrow DH'^{\bullet} + H^+$	$\Delta G'_{PD}(DH_2^{\bullet+})$	$\Delta G'_{PD}(DH_2^{\bullet+}) = \Delta G_{PD}(DH_2^{\bullet+}) + \Delta G_{HT}(DH^{\bullet})$	(17)

Note: The unit of potentials is V vs. Fc. The unit of Gibbs free energies is kcal/mol. $E_{ox}(H^-) = E_{red}(H^{\bullet}) = -1.137$ V vs. Fc in acetonitrile [38]. $E_{red}(H^+) = E_{ox}(H^{\bullet}) = -2.307$ V vs. Fc in acetonitrile [38]. $\Delta G_{H^-D}(H_2) = 76.0$ kcal/mol in acetonitrile [41].

Step 21 is the chemical process of $\text{DH}_2^{\bullet+}$ releasing protons from the N_1 -position, and the thermodynamic potential is described by the related Gibbs free energy, $\Delta G'_{\text{PD}}(\text{DH}_2^{\bullet+})$. $\Delta G'_{\text{PD}}(\text{DH}_2^{\bullet+})$ could be obtained by Equation (17) in Table 1, $\Delta G'_{\text{PD}}(\text{DH}_2^{\bullet+}) = \Delta G_{\text{PD}}(\text{DH}_2^{\bullet+}) + \Delta G_{\text{HT}}(\text{DH}^{\bullet})$ (Equation (17)) by constructing the thermodynamic cycle [27] Step 6–Step 20–Step 21.

All of the chemical processes, thermodynamic potentials, computed equations, or data sources of the 21 elementary steps for DH_2 aromatization are listed in Table 1. Moreover, the thermodynamic results of 1H_2 – 5H_2 and YH_2 aromatization are shown in Table 2. Accordingly, the thermodynamic cards [27,31,38] of 1H_2 – 5H_2 (Figures S1–S5) and YH_2 (Figure S6) on 21 potential elementary steps are established and presented in the Supporting Information to make them more convenient to query and use. Obviously, the thermodynamic cards (Figures 3 and S1–S5) can visually exhibit the mutual transformations among dipine models DH_2 , aromatic products D, and the related six active intermediates, as well as the thermodynamic driving forces of the related 21 potential elementary steps. Naturally, the thermodynamic cards could be employed to quantitatively measure and predict the characteristic chemical or thermodynamic properties of the dipine models and involved intermediates.

Table 2. Thermodynamic results of dipine models (DH_2) and Hantzsch ester (YH_2) aromatization on 21 elementary steps.

Step X	Parameters	1H_2	2H_2	3H_2	4H_2	5H_2	YH_2
Step 1	$\Delta G_{\text{H}^- \text{D}}(\text{DH}_2)$	63.9	64.7	66.0	66.9	69.0	64.4
Step 2	$E_{\text{ox}}(\text{DH}_2)$	0.712	0.721	0.731	0.745	0.781	0.479
Step 3	$E_{\text{ox}}(\text{DH}^{\bullet})$	−0.980	−0.932	−0.856	−0.775	−0.655	−1.112
Step 4	$\Delta G_{\text{HD}}(\text{DH}_2)$	60.3	60.0	59.5	58.6	57.9	63.8
Step 5	$\Delta G_{\text{HD}}(\text{DH}_2^{\bullet+})$	21.3	21.9	22.9	23.5	24.9	27.1
Step 6	$\Delta G_{\text{PD}}(\text{DH}_2^{\bullet+})$	−9.3	−9.9	−10.5	−11.8	−13.2	−0.4
Step 7	$\Delta G_{\text{H}^- \text{D}}(\text{DH}^-)$	31.8	32.2	33.2	34.6	37.9	34.3
Step 8	$E_{\text{ox}}(\text{DH}^-)$	−0.497	−0.491	−0.479	−0.444	−0.382	−0.695
Step 9	$E_{\text{ox}}(\text{D}^{\bullet-})$	−2.611	−2.580	−2.528	−2.453	−2.289	−2.446
Step 10	$\Delta G_{\text{HD}}(\text{DH}^-)$	65.7	65.4	65.2	64.9	64.4	64.4
Step 11	$\Delta G_{\text{HD}}(\text{DH}^{\bullet})$	17.1	17.3	18.0	18.6	20.5	24.1
Step 12	$\Delta G_{\text{PD}}(\text{DH}^{\bullet})$	24.1	23.6	23.1	22.0	20.1	27.4
Step 13	$\Delta G_{\text{PD}}(\text{DH}^+)$	19.9	19.5	18.7	17.6	17.6	18.7
Step 14	$\Delta G_{\text{H}^- \text{P}}(\text{DH}_2)$	83.8	84.2	84.7	84.5	86.6	83.1
Step 15	$\Delta G_{\text{PD}}(\text{DH}_2)$	52.0	52.0	51.5	49.9	48.7	48.8
Step 16	$\Delta G_{2\text{H}}(\text{DH}_2)$	110.8	111.2	111.7	111.5	113.6	110.1
Step 17	$\Delta G_{\text{HD}}(\text{DH}^{\bullet})$	50.5	51.2	52.2	52.9	55.7	46.3
Step 18	$\Delta G'_{\text{HD}}(\text{DH}_2)$	93.7	93.9	93.7	92.9	93.1	86.0
Step 19	$\Delta G_{\text{H}_2}(\text{DH}_2)$	7.8	8.2	8.7	8.5	10.6	7.1
Step 20	$\Delta G_{\text{HT}}(\text{DH}^{\bullet})$	33.4	33.9	34.2	34.3	35.2	22.2
Step 21	$\Delta G'_{\text{PD}}(\text{DH}_2^{\bullet+})$	24.1	24.0	23.7	22.5	22.0	21.8

Note: The unit of potentials is V vs. Fc, and the unit of Gibbs free energies is kcal/mol. $E_{\text{ox}}(\text{DH}_2)$, $E_{\text{ox}}(\text{DH}^{\bullet})$, $E_{\text{ox}}(\text{DH}^-)$, and $E_{\text{ox}}(\text{D}^{\bullet-})$ values are taken from [38].

2.2. The Acidities of DH^+ in Acetonitrile

In our previous work [39], the pK_a of 78 protonated pyridines in acetonitrile were predicted using the method developed by Luo and coworker at 2020 [40], and the predicted accuracy was estimated as $\pm 1 \text{ pK}_a$. Herein, the pK_a of DH^+ and YH^+ with typical structures of protonated pyridine in acetonitrile are also predicted using the same method. To better understand the acidities of DH^+ and YH^+ in acetonitrile, the pK_a values of DH^+ and YH^+ , along with the pK_a of common organic acids in acetonitrile, are displayed in Figure 4. From Figure 4, it is found that the $\text{pK}_a(\text{DH}^+)$ scale ranges from 12.83 for 1H^+ to 14.54 for 5H^+ , and the acidities of DH^+ (12.83–14.54) are between PyH^+ (12.3) [43–45] and protonated 2,4,6-trimethylpyridine (15.0) [43–45], which further verify the accuracy of the predicted $\text{pK}_a(\text{DH}^+)$ values.

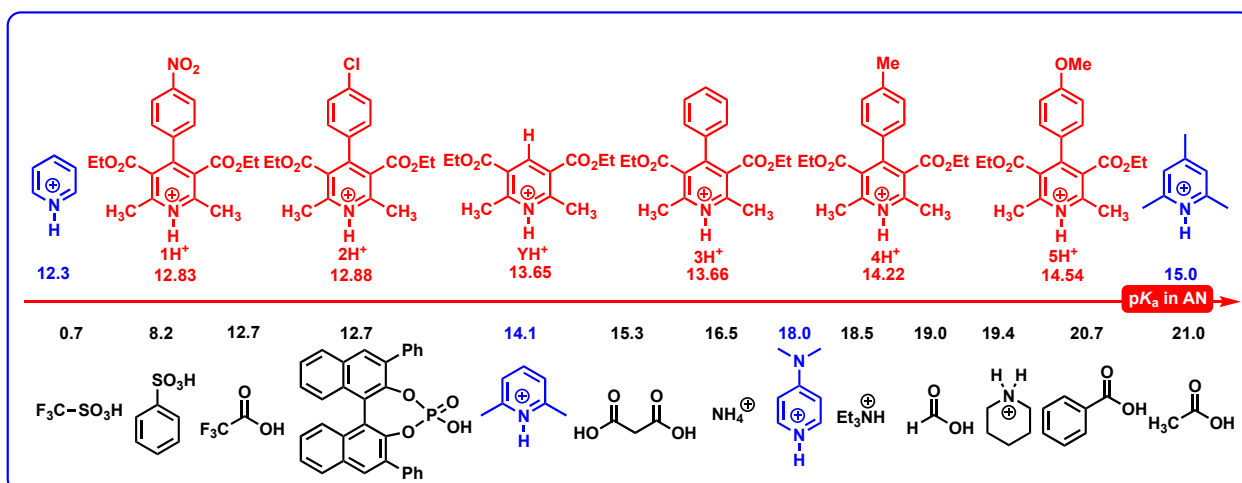


Figure 4. The pK_a of DH^+ and YH^+ , along with the pK_a of common organic acids in acetonitrile.

What is more, several interesting conclusions could be drawn from Figure 4. (1) Since the pK_a of BINOL-derived phosphoric acids (BPA) are reported as 12–14 in MeCN [46–49], DH^+ (12.83–14.54) have comparable acidities to BPA. (2) DH^+ (12.83–14.54) generally belongs to medium–strong organic acids, which could be applied in acid-catalyzed chemical reactions. (3) The acidity of $3H^+$ (13.66) is almost equal to that of YH^+ (13.65), and when the 4-phenyl group of DH^+ is substituted by an electron-withdrawing group, the acidity of new DH^+ is stronger than $3H^+$ (13.66) or YH^+ (13.65). In contrast, if the 4-phenyl group of DH^+ is substituted by an electron-donating group, the acidity of new DH^+ is weaker than $3H^+$ (13.66) or YH^+ (13.65). (4) Sometimes, after DH_2 are oxidized by hydride acceptors, the acidities of resulting DH^+ should be considered when the organic bases are involved in the reaction system. (5) D ($pK_a(DH^+) = 12.83 - 14.54$) could be used as alternative organic bases of pyridine (pK_a of conjugate acid is 12.3) [43–45], 2,6-trimethylpyridine (pK_a of conjugate acid is 14.1) [43–45], and 2,4,6-trimethylpyridine (pK_a of its conjugate acid is 15.0) [43–45] in acetonitrile.

2.3. Thermodynamic Properties of DH_2 and Related Intermediates Acting as Electrons, Hydrides, Hydrogen Atoms, Protons, and Two Hydrogen Ions (Atoms) Donors in Acetonitrile

2.3.1. Electron-Donating Properties

To clearly compare the electron-donating properties of DH_2 and related intermediates (DH^- , $DH^\bullet$, and $D^{\bullet-}$), the oxidation potentials (E_{ox} vs. Fc) of DH_2 , DH^- , $DH^\bullet$, and $D^{\bullet-}$, as well as the reduction potentials (E_{red} vs. Fc) of common coenzyme models (BNA⁺ for NADH coenzyme, PQ for coenzyme Q, Asc for oxidized ascorbate, Fl⁺ for flavin coenzyme, and Ru^{IV}O²⁺ for heme enzyme) [40,42] and electron acceptors (H^+ , $H^\bullet$, O_2 , and PTZ^{•+}) [29,50] in acetonitrile are exhibited in Figure 5. From Figure 5, it is clear that the oxidation potential scale is from 0.712 V to 0.781 V for DH_2 , from −0.382 V to −0.497 V for DH^- , from −0.980 V to −0.655 V for $DH^\bullet$, and from −2.611 V to −2.289 V for $D^{\bullet-}$, indicating that $DH^{\bullet-}$ are the thermodynamically best electron donors, even better than $H^\bullet$ ($E_{ox} = -2.307$ V) [38], and DH_2 are the thermodynamically worse electron donors than BNAH (0.219 V) [27]. The electron-donating abilities increase in the following order of $DH_2 < DH^- < DH^\bullet < D^{\bullet-}$. According to their thermodynamic range, DH_2 (0.712–0.781 V), DH^- (−0.382–−0.497 V), $DH^\bullet$ (−0.980–−0.655 V), and $D^{\bullet-}$ (−2.611–−2.289 V) are recognized as the weak electron donors, medium–strong electron donors, strong electron donors, and very strong electron donors, respectively.

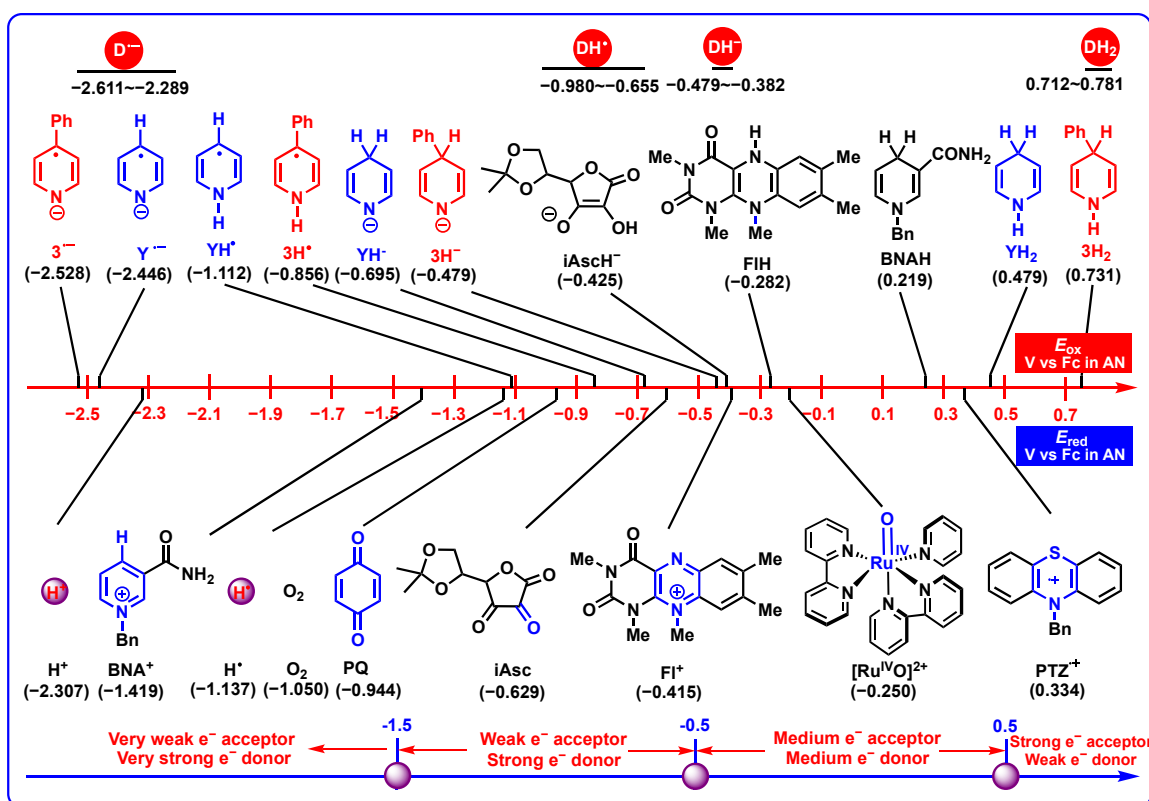


Figure 5. Oxidation potentials (E_{ox}) of DH_2 , DH^- , $DH^{\bullet}$, and $D^{\bullet-}$, as well as the reduction potentials (E_{red}) of common coenzyme models and electron acceptors in acetonitrile (V vs. Fc).

Since ascorbate is a good single-electron donor in vivo, the $E_{ox}(DH_2)$ (0.712–0.781 V) is greater than 5,6-isopropylidene ascorbate ($iAsCH^{\bullet-}$, -0.425 V) [27], and the $E_{ox}(DH^{\bullet})$ (-0.980–0.655 V), $E_{ox}(DH^-)$ (-0.382–0.497 V), and $E_{ox}(D^{\bullet-})$ (-2.611–2.289 V) are more negative than $iAsCH^{\bullet-}$ (-0.425 V) [27], which means that DH_2 are thermodynamically worse electron donors than $iAsCH^{\bullet-}$, and $DH^{\bullet}$, DH^- , and $D^{\bullet-}$ are thermodynamically better electron donors than $iAsCH^{\bullet-}$. In addition, $Ru^{IV}O^{2+}$ is known as the model of high-valence metal oxides, such as heme or non-heme iron coenzyme [16–19], and cytochrome P450 [11–15]. The reduction potential of $Ru^{IV}O^{2+}$ is determined as -0.250 V (vs. Fc) [51] in acetonitrile; therefore, the electron transfers from DH^- (-0.382–0.497 V), $DH^{\bullet}$ (-0.980–0.655 V), and $D^{\bullet-}$ (-2.611–2.289 V) to $Ru^{IV}O^{2+}$ are thermodynamically favorable with large thermodynamic driving forces, while the electron transfers from DH_2 (0.71–0.781 V) to $Ru^{IV}O^{2+}$ are thermodynamically unfavorable with $\Delta G_{ET}(DH_2/Ru^{IV}O^{2+}) \geq 22.2$ kcal/mol.

It is well-known that O_2 often functions as the electron acceptor in vivo, and the reduction potential of O_2 was determined as -1.050 V (vs. Fc) [29] in acetonitrile. The thermodynamic analyses indicate that the electron transfers from DH^- (-0.382–0.497 V), $DH^{\bullet}$ (-0.980–0.655 V) and DH_2 (0.712–0.781 V) to O_2 are thermodynamically unfavorable, while electron transfers from $D^{\bullet-}$ (-2.611–2.289 V) to O_2 are thermodynamically feasible with $\Delta G_{ET}(D^{\bullet-}/O_2) \leq -28.6$ kcal/mol. Further considering the thermodynamic data, the Gibbs free energies of electron transfers from DH_2 (0.712–0.781 V) to O_2 , $DH_2 + O_2 \rightarrow DH_2^{\bullet+} + O_2^{\bullet-}$, are estimated to be as high as 40.6–42.2 kcal/mol [$\Delta G_{ET}(DH_2/O_2)$], meaning that DH_2 is stable in the air, and the direct electron transfer from DH_2 to O_2 is thermodynamically unfeasible. In contrast, the Gibbs free energies of electron transfers from DH^- (-0.832–0.479 V) and $DH^{\bullet}$ (-0.980–0.655 V) to O_2 , are calculated as 5.0–13.2 kcal/mol and 1.6–9.1 kcal/mol separately, which are slightly large energy barriers to surpass.

2.3.2. Hydride-Donating Properties

For a better comparison, the hydride-donating properties of DH_2 and DH^- and the hydricities of DH_2 , DH^- , and common hydride donors (iAsch $^-$, BNAH, and AcrH), as well as the hydride-affinities of common coenzyme models (BNA $^+$ for NADH coenzyme, PQ for coenzyme Q, iAsc for oxidated ascorbic acid, Fl $^+$ for flavin coenzyme, and Ru $^{\text{IV}}\text{O}^{2+}$ for heme enzyme) and hydride acceptors (H^+ and PTZ $^{\bullet+}$) in acetonitrile are displayed in Figure 6. As can be seen from Figure 6, it is found that the hydricity ranges from 63.9 to 69.0 kcal/mol for DH_2 , and from 31.8 to 37.9 kcal/mol for DH^- . If the hydricities between DH_2 and DH^- are compared, it is easy to discover that the hydricities of DH^- are greatly improved by the negative charge at the N_1 -atom compared with their parents DH_2 , and the hydride-donating abilities of DH^- (31.8–37.9 kcal/mol) are greater than DH_2 (63.9–69.0 kcal/mol) by more than 30 kcal/mol. Based on their thermodynamic range, DH_2 (63.9–69.0 kcal/mol) belong to thermodynamically medium–strong hydride donors, while DH^- (31.8–37.9 kcal/mol) belong to thermodynamically strong hydride donors, respectively.

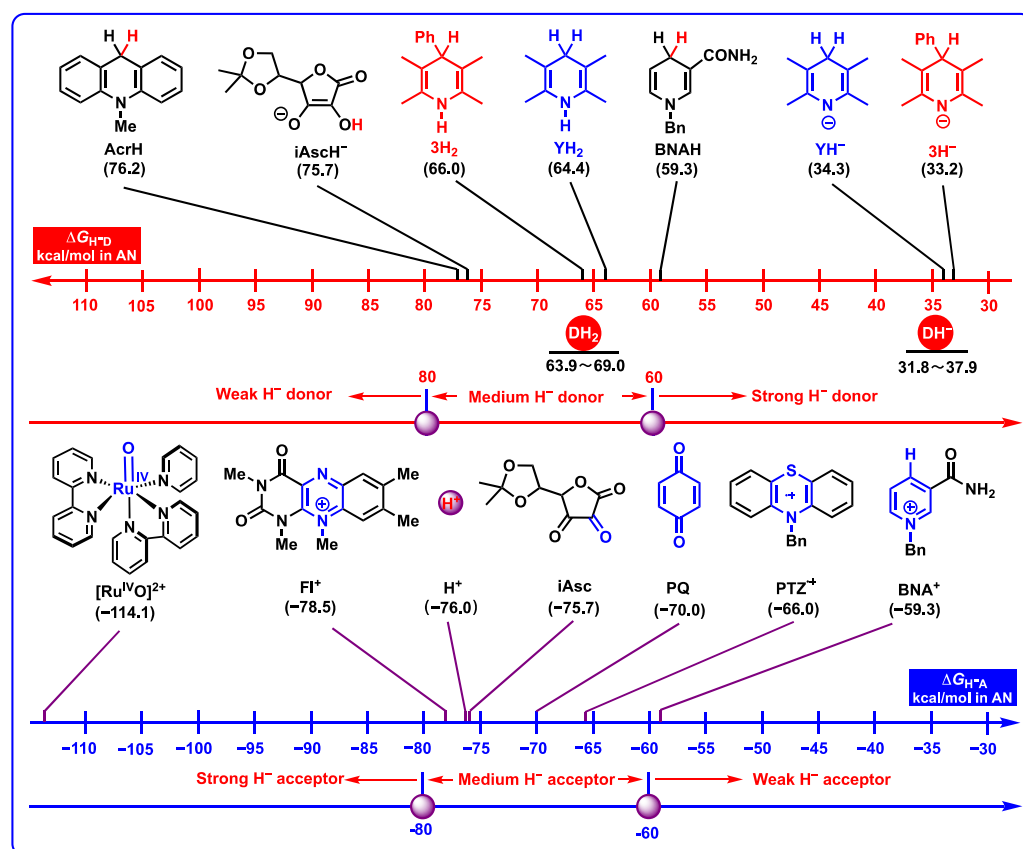


Figure 6. Hydricities of DH_2 and DH^- , as well as H^+ -affinities of common coenzyme models and hydride acceptors in acetonitrile (kcal/mol).

DH_2 are thermodynamically worse hydride donors than BNAH and thermodynamically better hydride donors than iAsch $^-$, due to the hydricities of DH_2 (63.9–69.0 kcal/mol) being more negative than BNAH (59.3 kcal/mol) [27] by 4.6–9.7 kcal/mol, and greater than iAsch $^-$ (75.7 kcal/mol) [27] by 6.7–11.8 kcal/mol. DH^- are much better hydride donors than BNAH and iAsch $^-$, and the hydricities of DH^- (31.8–37.9 kcal/mol) are much greater than BNAH (59.3 kcal/mol) and iAsch $^-$ (75.7 kcal/mol) by more than 20 kcal/mol. The above thermodynamic data indicate that DH_2 could not be oxidized by BNA $^+$ through hydride transfer with related Gibbs free energies greater than 0, $4.6 \text{ kcal/mol} \leq \Delta G_{\text{H-T}}(\text{DH}_2/\text{BNA}^+) \leq 9.7 \text{ kcal/mol}$, but the anion intermediates of DH_2 (DH^-) could be oxidized by BNA $^+$ through hydride transfer with Gibbs free energies less than 0,

$-27.5 \text{ kcal/mol} \leq \Delta G_{\text{H-T}}(\text{DH}^-/\text{BNA}^+) \leq -21.4 \text{ kcal/mol}$. In addition, DH_2 and DH^- could be oxidized by iAsc (-75.7 kcal/mol) by hydride transfer with Gibbs free energies less than 0, $-9.7 \text{ kcal/mol} \leq \Delta G_{\text{H-T}}(\text{DH}_2/\text{iAsc}) \leq -4.6 \text{ kcal/mol}$ and $-43.9 \text{ kcal/mol} \leq \Delta G_{\text{H-T}}(\text{DH}^-/\text{iAsc}) \leq -37.8 \text{ kcal/mol}$. What is more, since all of the Gibbs free energies of hydride transfer processes from DH_2 to $\text{Ru}^{\text{IV}}\text{O}^{2+}$ (-114.1 kcal/mol), from DH_2 to Fl^+ (-78.5 kcal/mol), from DH_2 to PQ (-70.0 kcal/mol), and from DH_2 to H^+ (-76.0 kcal/mol) are less than 0, it can be inferred that dipines may be oxidized by heme enzyme, cytochrome P450, flavin coenzyme, coenzyme Q, and H^+ under suitable oxidoreductase by hydride oxidation in vivo.

2.3.3. Hydrogen-Atom-Donating Properties

Due to the N-H bond and C-H bond at the 1-position and 4-position of DH_2 , there are six possible elementary steps to release hydrogen atoms during the aromatization. Herein, the thermodynamic hydrogen-atom-donating abilities of DH_2 , DH^- , $\text{DH}_2^{\bullet+}$, $\text{DH}^\bullet$, and $\text{DH}^{\bullet+}$, as well as the hydrogen-atom affinities of 12 common radicals (involving $\text{H}^\bullet$, $^t\text{BuO}^\bullet$, $\text{CumO}^\bullet$, $\text{PhCH}_2^\bullet$, $\text{PINO}^\bullet$, $^t\text{BuO}_2^\bullet$, $\text{CumO}_2^\bullet$, $\text{PhO}^\bullet$, $\text{DPPH}^\bullet$, $\text{PhS}^\bullet$, $2,4,6\text{-}^t\text{Bu}_3\text{PhO}^\bullet$, and $\text{TEMPO}^\bullet$) [29] and coenzyme models (BNA^+ for NADH coenzyme, PQ for coenzyme Q, iAsc for oxidated ascorbic acid, Fl^+ for flavin coenzyme, and $\text{Ru}^{\text{IV}}\text{O}^{2+}$ for heme enzyme) in acetonitrile are shown in Figure 7.

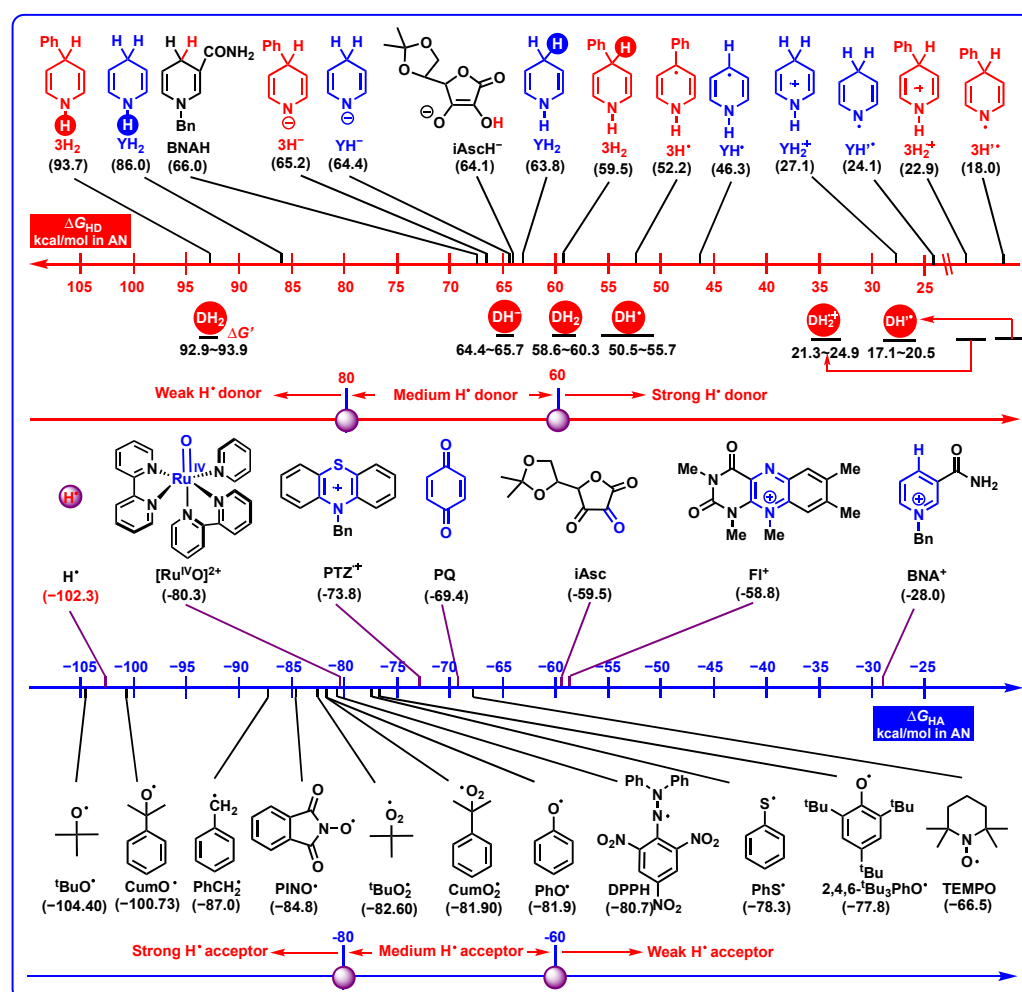


Figure 7. Thermodynamic hydrogen-atom-donating abilities of DH_2 , DH^- , $\text{DH}_2^{\bullet+}$, $\text{DH}^\bullet$, and $\text{DH}^{\bullet+}$, as well as hydrogen atom affinities of common radicals and coenzyme models in acetonitrile (kcal/mol).

From Figure 7, the thermodynamic hydrogen-atom-donating ability scale ranges from 92.9 to 93.9 kcal/mol for DH_2 releasing hydrogen atoms from $\text{N}_1\text{-H}$, from 58.6 to 60.3 kcal/mol for DH_2 releasing hydrogen atoms from $\text{C}_4\text{-H}$, from 64.4 to 65.7 kcal/mol for DH^- , from 21.3 to 34.9 kcal/mol for $\text{DH}_2^{\bullet+}$, from 50.5 to 55.7 kcal/mol for $\text{DH}^\bullet$, and from 17.1 to 20.5 kcal/mol for $\text{DH}'^\bullet$, and the thermodynamic hydrogen-atom-donating abilities increase in the order of DH_2 ($\text{N}_1\text{-H}$, 92.9–93.9 kcal/mol) < DH^- (64.4–65.7 kcal/mol) < DH_2 ($\text{C}_4\text{-H}$) (58.6–60.3 kcal/mol) < $\text{DH}^\bullet$ (50.5–55.7 kcal/mol) < $\text{DH}_2^{\bullet+}$ (21.3–34.9 kcal/mol) < $\text{DH}'^\bullet$ (17.1–20.5 kcal/mol). According to their thermodynamic ranges, $\text{DH}^\bullet$, $\text{DH}_2^{\bullet+}$, and $\text{DH}'^\bullet$ belong to thermodynamically strong hydrogen atom donors, DH^- and DH_2 generally belong to thermodynamically medium–strong hydrogen atom donors to break $\text{C}_4\text{-H}$ bonds, while DH_2 belong to thermodynamically weak hydrogen atom-donors to break $\text{N}_1\text{-H}$ bonds. Based on the above analysis, whether DH_2 are medium–strong hydrogen atom donors or weak hydrogen atom donors depends on which hydrogen atoms DH_2 releases, $\text{N}_1\text{-H}$ bonds or $\text{C}_4\text{-H}$ bonds. Generally, DH_2 prefer to release hydrogen atoms from $\text{C}_4\text{-H}$ bonds (58.6–60.3 kcal/mol) instead of $\text{N}_1\text{-H}$ bonds (92.9–93.9 kcal/mol) from thermodynamics.

Since ascorbate is an excellent antioxidant to quench radicals in vivo [52], it is necessary to compare the thermodynamic properties of iAsCH^- , DH_2 , and related intermediates releasing hydrogen atoms. Several interesting conclusions could be made as follows. It is revealed that (1) the thermodynamic antioxidant potentials of DH^- (64.4–65.7 kcal/mol) are comparable to iAsCH^- (64.1 kcal/mol) [27,52]. (2) DH_2 ($\text{C}_4\text{-H}$ homolysis, 58.6–60.3 kcal/mol), $\text{DH}^\bullet$ (50.5–55.7 kcal/mol), $\text{DH}_2^{\bullet+}$ (21.3–34.9 kcal/mol), and $\text{DH}'^\bullet$ (17.1–20.5 kcal/mol) are thermodynamically better antioxidants than iAsCH^- (64.1 kcal/mol). (3) Unlike hydricities, the thermodynamic hydrogen-atom-donating abilities of DH^- decrease as a result of the negative charge at the N_1 -atom compared with their parents DH_2 , and the thermodynamic hydrogen-atom-donating abilities of DH^- (64.4–65.7 kcal/mol) are more negative than DH_2 (58.6–60.3 kcal/mol) by 4.1–7.1 kcal/mol. (4) Because the final products of $\text{DH}^\bullet$ and $\text{DH}'^\bullet$ releasing hydrogen atoms are the same (D and $\text{H}^\bullet$), $\text{DH}^\bullet \rightarrow \text{D} + \text{H}^\bullet$ and $\text{DH}'^\bullet \rightarrow \text{D} + \text{H}^\bullet$. When the hydrogen-atom-donating Gibbs free energies of $\text{DH}^\bullet$ (50.5–55.7 kcal/mol) and $\text{DH}'^\bullet$ (17.1–20.5 kcal/mol) are compared, it can also be deduced that $\text{DH}^\bullet$ are more thermodynamically stable radicals than $\text{DH}'^\bullet$, and the Gibbs free energies of hydrogen atom transfer within $\text{DH}^\bullet$ from the N_1 -position to the C_4 -position [$\Delta G_{\text{HT}}(\text{DH}^\bullet)$ for Step 20 in Figure 3] are computed as 33.4–35.2 kcal/mol, which further verify the relative stability of $\text{DH}^\bullet$ and $\text{DH}'^\bullet$ in solution.

BNAH is the structurally closest model of the NADH coenzyme, and the hydrogen-atom-donating Gibbs free energy of BNAH is 66.0 kcal/mol [27]. It is clear that DH_2 ($\text{C}_4\text{-H}$ homolysis, 58.6–60.3 kcal/mol), $\text{DH}^\bullet$ (50.5–55.7 kcal/mol), $\text{DH}_2^{\bullet+}$ (21.3–34.9 kcal/mol), and $\text{DH}'^\bullet$ (17.1–20.5 kcal/mol) are thermodynamically better hydrogen atom donors than BNAH (66.0 kcal/mol), while DH_2 are thermodynamically much weaker hydrogen atom donors than BNAH (66.0 kcal/mol) if the DH_2 break $\text{N}_1\text{-H}$ bonds (92.9–93.9 kcal/mol).

As for the common 11 organic radicals collected in Figure 7, involving $^t\text{BuO}^\bullet$, $\text{CumO}^\bullet$, $\text{PhCH}_2^\bullet$, $\text{PINO}^\bullet$, $^t\text{BuO}_2^\bullet$, $\text{CumO}_2^\bullet$, $\text{PhO}^\bullet$, DPPH , $\text{PhS}^\bullet$, $2,4,6\text{-}^t\text{Bu}_3\text{PhO}^\bullet$, and TEMPO [29,53,54], the hydrogen atom affinity scale ranges from −66.5 for TEMPO to −104.40 kcal/mol for $^t\text{BuO}^\bullet$. All of the 11 radicals (−66.5–−104.40 kcal/mol) could thermodynamically oxidize DH^- (64.4–65.7 kcal/mol), DH_2 (breaking $\text{C}_4\text{-H}$) (58.6–60.3 kcal/mol), $\text{DH}^\bullet$ (50.5–55.7 kcal/mol), $\text{DH}_2^{\bullet+}$ (21.3–34.9 kcal/mol), and $\text{DH}'^\bullet$ (17.1–20.5 kcal/mol) via hydrogen atoms transfer. Only $^t\text{BuO}^\bullet$ (−104.40 kcal/mol) and $\text{CumO}^\bullet$ (−110.73 kcal/mol) could oxidize DH_2 by $\text{N}_1\text{-H}$ bonds homolysis (92.9–93.9 kcal/mol) from the point of thermodynamics. In addition, $\text{H}^\bullet$ (−102.3 kcal/mol) [28,29], $\text{Ru}^{\text{IV}}\text{O}_2^{2+}$ (−80.3 kcal/mol) [51], and PQ (−69.4 kcal/mol) [29] have the thermodynamic abilities to oxidize DH^- (64.4–65.7 kcal/mol), DH_2 (breaking $\text{C}_4\text{-H}$) (58.6–60.3 kcal/mol), $\text{DH}^\bullet$ (50.5–55.7 kcal/mol), $\text{DH}_2^{\bullet+}$ (21.3–34.9 kcal/mol), and $\text{DH}'^\bullet$ (17.1–20.5 kcal/mol) via hydrogen atoms transfer from thermodynamics, meaning that dipines may be oxidized by heme enzyme, cytochrome P450, coenzyme Q (Co Q), and $\text{H}^\bullet$ under suitable oxidoreductase, by hydrogen atoms oxidation in vivo.

2.3.4. Proton-Donating Properties

In order to better understand the acidities of DH_2 and related active intermediates, the thermodynamic proton-donating abilities of DH_2 , DH^+ , $DH_2^{\bullet+}$, and $DH^{\bullet}$, as well as the proton-donating abilities of common acids ($PhSO_3H$, $iAscH_2$, Et_3NH^+ , $PhCO_2H$, $AcOH$, $AcrH$, and H_2) [43–45] in acetonitrile are presented in Figure 8.

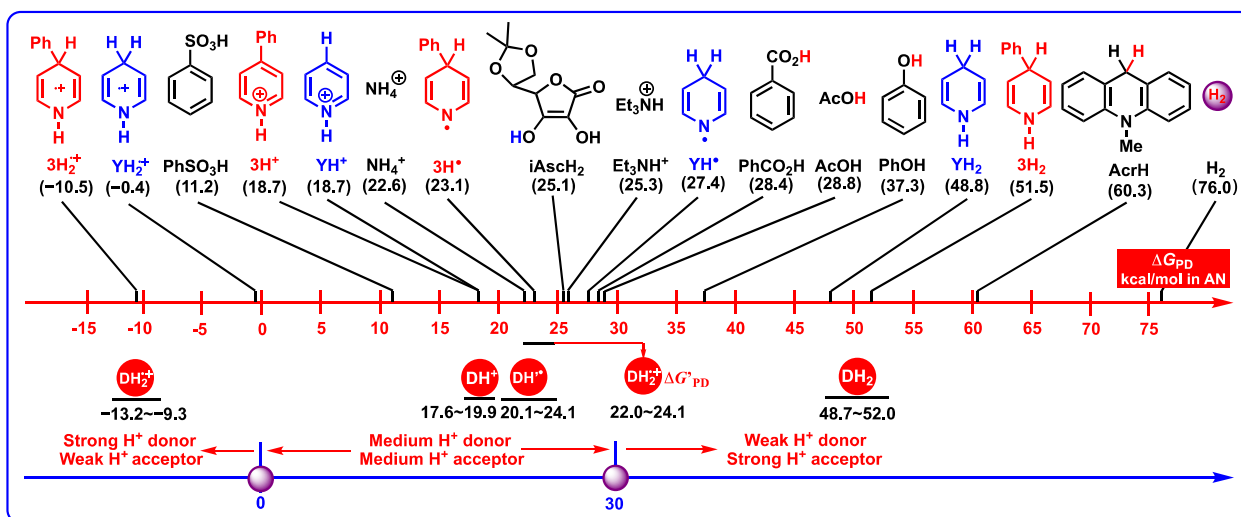


Figure 8. Thermodynamic proton-donating abilities of DH_2 , DH^+ , $DH_2^{\bullet+}$, and $DH^{\bullet}$, as well as proton-donating abilities of common acids in acetonitrile (kcal/mol).

Obviously, the proton-donating Gibbs free energy scale ranges from -13.2 to -9.3 kcal/mol for DH_2^{+} releasing protons from C_4 -H bonds, from 22.0 to 24.1 kcal/mol for $DH_2^{\bullet+}$ releasing protons from N_1 -H bonds, from 17.6 to 19.9 kcal/mol for DH^+ , from 20.1 to 24.1 kcal/mol for $DH^{\bullet}$, and from 48.7 to 52.0 kcal/mol for DH_2 , which discloses that the thermodynamic proton-donating abilities increase according to the order of DH_2 (N_1 -H, 48.7 – 52.0 kcal/mol) $<$ $DH_2^{\bullet+}$ (N_1 -H, 22.0 – 24.1 kcal/mol) $\approx$ $DH^{\bullet}$ (20.1 – 24.1 kcal/mol) $<$ DH^+ (17.6 – 19.9 kcal/mol) $<$ DH_2^{+} (C_4 -H, -13.2 – -9.3 kcal/mol). Based on their thermodynamic ranges, it could be suggested that DH_2 (N_1 -H, 48.7 – 52.0 kcal/mol) belong to weak proton donors, $DH_2^{\bullet+}$ (N_1 -H, 22.0 – 24.1 kcal/mol), $DH^{\bullet}$ (20.1 – 24.1 kcal/mol), and DH^+ (17.6 – 19.9 kcal/mol) belong to medium–strong proton donors, and DH_2^{+} (C_4 -H, -13.2 – -9.3 kcal/mol) belong to strong proton donors.

The above thermodynamic analyses result in the following conclusions.

- (1) DH_2^{+} (C_4 -H, -13.2 – -9.3 kcal/mol) are the strongest organic acids among them. After the single-electron oxidation of DH_2 (0.712 – 0.781 V vs. Fc), DH_2^{+} are extremely unstable intermediates that spontaneously release protons from C_4 -H bonds with significant thermodynamic potentials (-13.2 – -9.3 kcal/mol).
- (2) DH_2 (48.7 – 52.0 kcal/mol) are the weakest organic acids in acetonitrile among them, indicating that the N_1 -H bond in DH_2 is the weak polar bond. Since the proton affinity of hydride ions (H^-) is determined as -76.0 kcal/mol in acetonitrile [41,42], the proton-abstracting reaction from N_1 -H bonds in DH_2 molecules to H^- ($DH_2 + H^- \rightarrow DH^- + H_2$) is thermodynamically favorable and extremely exothermic with $-27.3 \leq \Delta G_{PT}(DH_2/H^-) \leq 24.0$ kcal/mol.
- (3) From Figure 8, the thermodynamic proton-abstracting abilities of common organic bases [43–45], consisting of $iAscH^-$ (-25.1 kcal/mol), Et_3N (-25.3 kcal/mol), NH_3 (-22.6 kcal/mol), $PhCO_2^-$ (-28.4 kcal/mol), AcO^- (-28.8 kcal/mol), and PhO^- (-37.3 kcal/mol), are generally smaller than -40 kcal/mol. Although the proton abstraction reactions from DH_2 to common organic bases are thermodynamically unfeasible ($\Delta G_{PT} > 0$), the resulting DH^- (31.8 – 37.9 kcal/mol) are very excellent hydride donors, and the hydride transfers process from DH^- to hydride acceptors is

extremely exothermic with $\Delta G_{H^-T}(DH^-/H^- \text{-acceptor}) \ll 0$ kcal/mol. Therefore, it is reasonable to suppose that the concerted or successive proton and hydride ($H^+ + H^-$) transfer mechanism is thermodynamically feasible for the oxidation of dipines by hydride-acceptor/base pair in vivo.

- (4) The common bases, such Et_3N (−25.3 kcal/mol), NH_3 (−22.6 kcal/mol) or amino acids, $iAscH^-$ (−25.1 kcal/mol), $PhCO_2^-$ (−28.4 kcal/mol), AcO^- (−28.8 kcal/mol), and PhO^- (−37.3 kcal/mol) in vivo could easily absorb protons from the related intermediates of DH_2 , i.e., $DH_2^{\bullet+}$ ($C_4\text{-H}$, −13.2–−9.3 kcal/mol), $DH_2^{\bullet+}$ ($N_1\text{-H}$, 22.0–24.1 kcal/mol), $DH'^{\bullet}$ (20.1–24.1 kcal/mol), and DH^+ (17.6–19.9 kcal/mol).
- (5) According to structural features, $DH_2^{\bullet+}$ ($N_1\text{-H}$, 22.0–24.1 kcal/mol) are typical aromatic radical cations, while $DH'^{\bullet}$ ($C_4\text{-H}$, 20.1–24.1 kcal/mol) are N-radical structures. However, they have thermodynamically similar proton-donating abilities for $DH_2^{\bullet+}$ releasing protons from N_1H bonds and $DH'^{\bullet}$ releasing protons from $C_4\text{-H}$ bonds. Interestingly, $DH_2^{\bullet+}$ ($C_4\text{-H}$, −13.2–−9.3 kcal/mol) are much stronger C-acids than $DH'^{\bullet}$ ($C_4\text{-H}$, 20.1–24.1 kcal/mol) in acetonitrile.

2.3.5. Two Hydrogen Ions (Atoms) Donating Properties

Examining the structural characteristics of NADH models, they are generally N-alkyl-1,4-dihydropyridines and act as hydride carriers [27,55]. Unlike common NADH models, the biggest difference between common NADH models (such as BNAH and AcrH) and DH_2 or the Hantzsch ester (YH_2) is that DH_2 and YH_2 have two hydrogen atoms at the N_1 -position and C_4 -position. During the aromatization process, DH_2 and YH_2 could release two hydrogen ions (atoms) or hydrogen gas from both $N_1\text{-H}$ and $C_4\text{-H}$ bonds. Therefore, the Hantzsch ester (YH_2) has been widely used as an excellent hydrogenation reagent to reduce unsaturated compounds by offering two hydrogen ions (atoms) [56–58]. Furthermore, many studies also focused on the hydrogen storage properties of YH_2 and related N-heterocycles [42]. In addition, some oxidoreductases, for example flavin coenzyme, coenzyme Q (Co Q), heme or nonheme iron coenzyme, and pyrroloquinoline quinone (PQQ) are also the two hydrogen ions (atoms) carriers in vivo. As a result, the thermodynamic abilities of DH_2 releasing two hydrogen ions (atoms) or H_2 , $\Delta G_{H^-P}(DH_2)$, $\Delta G_{2H}(DH_2)$, and $\Delta G_{H_2}(DH_2)$, are vital thermodynamic parameters to evaluate the comprehensive reduction properties, and judge the oxidation feasibility of DH_2 by two hydrogen ions (atoms) carrier enzymes in vivo.

The thermodynamic abilities of DH_2 and common hydrogen carriers (HCO_2H , H_2 , YH_2 , $F420H_2$, PQH_2 , and $iAscH_2$) releasing two hydrogen ions ($H^- + H^+$), two hydrogen atoms ($2H^{\bullet}$), or H_2 in acetonitrile are shown in Figure 9 [27,43]. It is found from Figure 9 that the Gibbs free energies of DH_2 releasing two hydrogen ions range from 83.8 to 86.6 kcal/mol, which belong to medium–strong two hydrogen ion donors.

According to the thermodynamic data, several valuable conclusions could be drawn. (1) The $\Delta G_{H^-P}(DH_2)$ values (83.8–86.6 kcal/mol) are greater than those of H_2 (76.0 kcal/mol) [41,42], meaning that DH_2 are thermodynamically worse two hydrogen ions (atoms) reductants than H_2 , and H_2 could hydrogenate D to regenerate DH_2 in solution under suitable catalysts. (2) The $\Delta G_{H^-P}(DH_2)$ values (83.8–86.6 kcal/mol) are slightly greater than $\Delta G_{H^-P}(YH_2)$ (83.1 kcal/mol), illustrating that DH_2 are thermodynamic alternatives of YH_2 as two hydrogen ions (atoms) reductants in chemical reactions. (3) Since *p*-hydroquinone (PQH_2) is a close model of Co Q, if the $\Delta G_{H^-P}(DH_2)$ values (83.8–86.6 kcal/mol) are compared with the $\Delta G_{H^-P}(PQH_2)$ value (96.2 kcal/mol) [29], it is discovered that DH_2 are thermodynamically better two hydrogen ion donors than PQH_2 , and DH_2 may be oxidated by Co Q via two hydrogen ions (atoms) transfer in vivo. (4) Due to the fact that $\Delta G_{H^-P}(DH_2)$ values (83.8–86.6 kcal/mol) are much more negative than $\Delta G_{H^-P}(iAscH_2)$ (100.8 kcal/mol) [27], DH_2 are thermodynamically worse two hydrogen ions (atoms) donors, and DH_2 may be oxidated by the oxidation state of ascorbic acid (Asc) in vivo. (5) Because the $\Delta G_{H_2}(DH_2)$ values (7.8–10.6 kcal/mol) are greater than $\Delta G_{H_2}(H_2)$ (0.0 kcal/mol), H_2 release from DH_2

is thermodynamically unfavorable, and DH_2 are not hydrogen storage chemicals unless extra energy is being provided, such as light, electron, heat, or gas pressure.

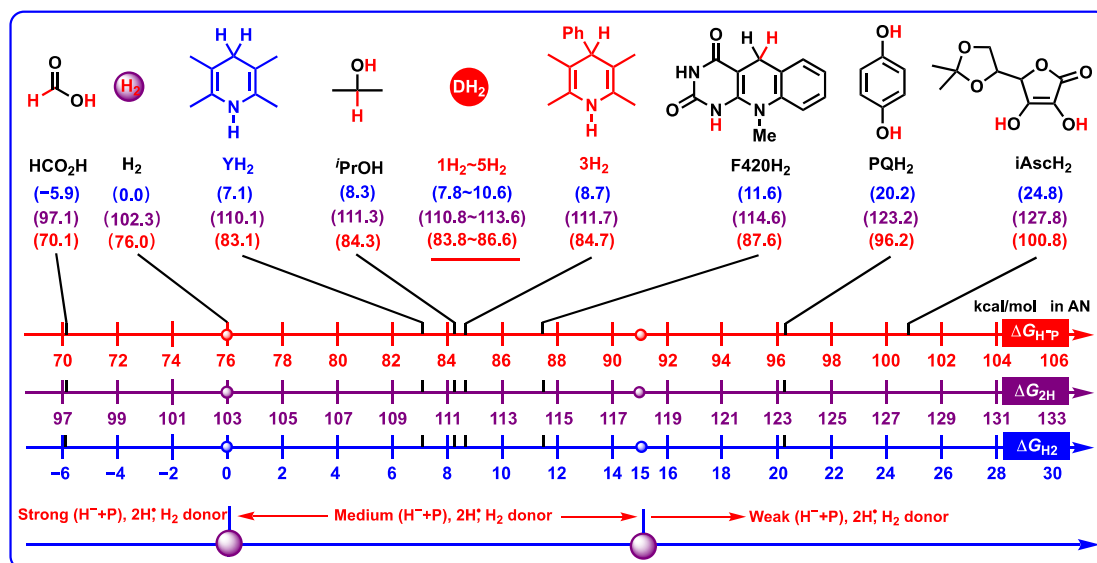


Figure 9. Thermodynamic abilities of DH_2 and common hydrogen carriers releasing two hydrogen ions ($\text{H}^- + \text{H}^+$) in the red brackets, releasing two hydrogen atoms ($2\text{H}^\bullet$) in the purple brackets, and releasing H_2 in the blue brackets in acetonitrile (kcal/mol).

2.4. Application of Thermodynamic Data to Evaluate the Redox Properties of DH_2

Without doubt, the thermodynamic cards of DH_2 display the redox properties of DH_2 to reveal the possible oxidative process of DH_2 (Figure 3). For DH_2 , there are five possible initial oxidation pathways (Figure 10), including hydride oxidation (Step 1), single-electron oxidation (Step 2), hydrogen atom oxidation from the $\text{C}_4\text{-H}$ bond (Step 4), hydrogen atom oxidation from the $\text{N}_1\text{-H}$ bond (Step 18), and proton release from the $\text{N}_1\text{-H}$ bond (Step 15). As hydrogen atom donors or antioxidants, DH_2 could release hydrogen atoms from the $\text{C}_4\text{-H}$ (Step 4) or $\text{N}_1\text{-H}$ (Step 18) bond. The $\Delta G_{\text{HD}}(\text{DH}_2)$ values of Step 18 ($\text{N}_1\text{-H}$, 92.9–93.9 kcal/mol) are ~30.0 kcal/mol larger than the $\Delta G'_{\text{HD}}(\text{DH}_2)$ values of Step 4 ($\text{C}_4\text{-H}$, 58.6–60.3 kcal/mol). Thereby, it is reasonable to deduce that DH_2 generally release hydrogen atoms from $\text{C}_4\text{-H}$ instead of $\text{N}_1\text{-H}$ during the antioxidant processes from thermodynamics.

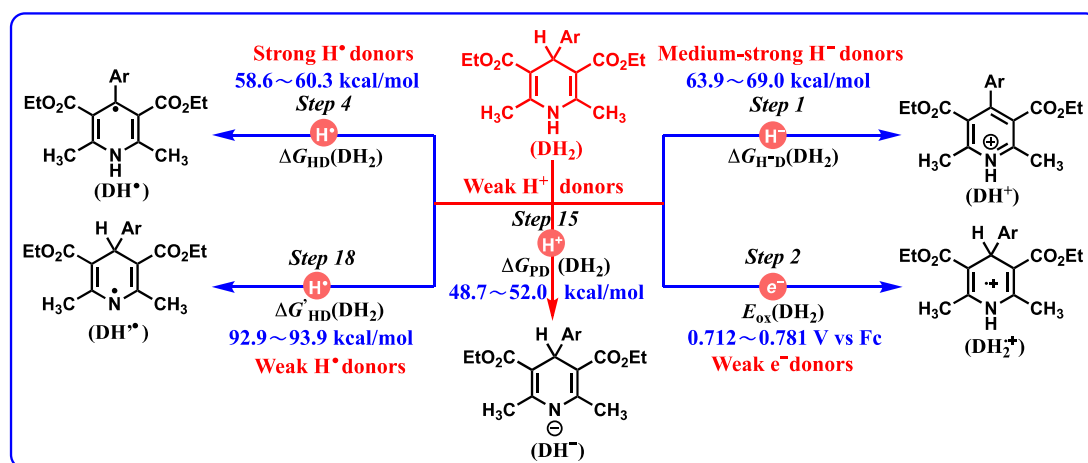


Figure 10. Thermodynamic abilities of five possible elementary steps for DH_2 oxidation.

Based on their thermodynamic results (*Steps 1–2, Step 4, Step 18, and Step 15*), DH_2 are medium–strong hydride donors (*Step 1*: 63.9–69.0 kcal/mol), weak single electron donors (*Step 2*: 0.712–0.781 V vs. Fc), strong hydrogen atom donors for $\text{C}_4\text{-H}$ bond breaking (*Step 4*: 58.6–60.3 kcal/mol), weak hydrogen atom donors for $\text{N}_1\text{-H}$ bond breaking (*Step 18*: 92.9–93.9 kcal/mol), and weak proton donors (*Step 15*: 48.752.0 kcal/mol). It is supposed that (a) DH_2 generally release hydrogen atoms from the $\text{C}_4\text{-H}$ bond instead of the $\text{N}_1\text{-H}$ bond in the radical oxidation process. (b) Generally, DH_2 undergoing hydride or radical oxidations is thermodynamically favorable. (c) Since DH_2 are thermodynamically very weak proton donors, the concerted or successive proton and hydride ($\text{H}^+ + \text{H}^-$) transfer mechanism is thermodynamically practicable. (d) The single-electron oxidation of DH_2 is thermodynamically disadvantageous, and a high single-electron oxidant is needed.

2.5. Application of Thermodynamic Data of Important Intermediates to Possible Oxidative Mechanism Judgement

The thermodynamic cards of DH_2 also could reveal the redox properties of key intermediates (Figure 3). For example, if the oxidation of DH_2 is initiated by single oxidation, the thermodynamic data of resulting $\text{DH}_2^{\bullet+}$ are noteworthy physical parameters to diagnose possible oxidation pathways. As organic acids, $\text{DH}_2^{\bullet+}$ have two different pathways to donate protons (Figure 11), consisting of releasing protons from $\text{C}_4\text{-H}$ bonds (*Step 6*) and $\text{N}_1\text{-H}$ bonds (*Step 21*). When the thermodynamic potentials of *Step 6* and *Step 21* are compared, it is easy to find that the $\Delta G_{\text{PD}}(\text{DH}_2^{\bullet+})$ values (−9.3–−13.2 kcal/mol) are 3 and −35 kcal/mol more negative than $\Delta G'_{\text{PD}}(\text{DH}_2^{\bullet+})$ (22.0–24.1 kcal/mol), indicating that, as proton donors, $\text{DH}_2^{\bullet+}$ thermodynamically prefer to release protons from $\text{C}_4\text{-H}$ bonds instead of $\text{N}_1\text{-H}$ bonds, and the proton release from $\text{C}_4\text{-H}$ bonds in $\text{DH}_2^{\bullet+}$ occurs spontaneously.

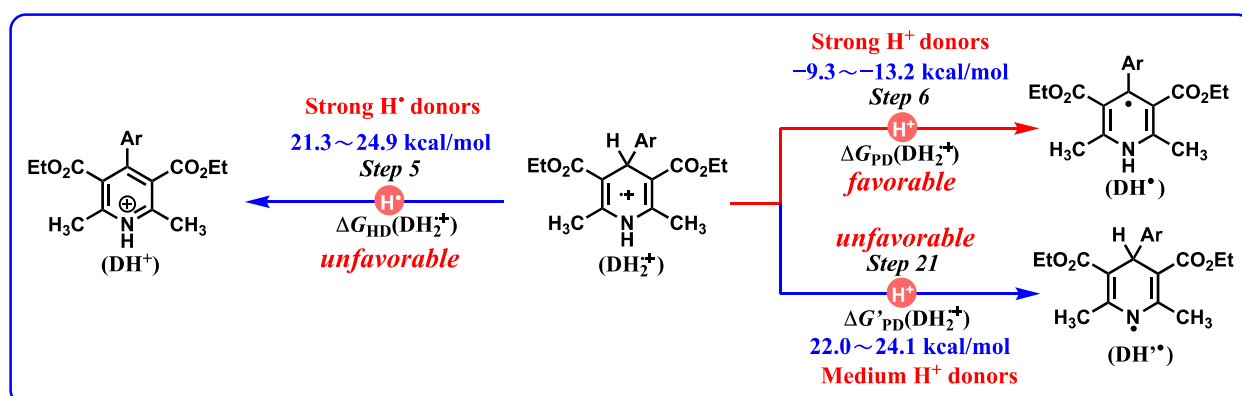


Figure 11. Thermodynamic analysis of possible oxidative process for intermediate $\text{DH}_2^{\bullet+}$.

For $\text{DH}_2^{\bullet+}$, there are three possible pathways to release hydrogen ions or atoms in all (Figure 11), including proton release from $\text{C}_4\text{-H}$ bonds (*Step 6*), proton release from $\text{N}_1\text{-H}$ bonds (*Step 21*), and hydrogen atom release from $\text{C}_4\text{-H}$ bonds (*Step 5*). According to their thermodynamic results (*Step 6, Step 21, and Step 5*), $\text{DH}_2^{\bullet+}$ belong to strong proton donors for $\text{C}_4\text{-H}$ bond breaking (*Step 6*: −9.3–−13.2 kcal/mol), medium–strong proton donors for $\text{N}_1\text{-H}$ bond breaking (*Step 21*: 22.0–24.1 kcal/mol), and strong hydrogen atom donors for $\text{C}_4\text{-H}$ bond breaking (*Step 5*: 21.3–24.9 kcal/mol).

$\text{DH}_2^{\bullet+}$ may act as proton donors or hydrogen atom donors, which depends on the properties of substrates. If the substrates are radicals, $\text{DH}_2^{\bullet+}$ act as hydrogen atom donors. If the substrates are bases, $\text{DH}_2^{\bullet+}$ act as proton donors. Most importantly, with a combination of the oxidation potentials of DH_2 and the properties of resulting $\text{DH}_2^{\bullet+}$ releasing hydrogen ions (atoms), the initial slight energy barrier (10–20 kcal/mol) of electron oxidation could be overcome by the subsequent protons transfer or hydrogen atoms transfer owning extreme thermodynamic driving forces ($\Delta G \ll 0$).

2.6. Application of Thermodynamic Data of DH_2 to Oxidative Mechanism Diagnosis between DH_2 and $iAsc$

Since the thermodynamic cards of dipine models' (DH_2) aromatization on 21 potential elementary steps have been established, they provide a precious opportunity to investigate the possible oxidative mechanism of DH_2 in vivo. Ascorbic acid ($AscH_2$) is known as an excellent electron and hydrogen atom donor [52]; however, the oxidated ascorbic acid (Asc) is actually a potential two hydrogen ions (atoms) oxidant. Because of the ortho dicarbonyl feature, Asc has the property of *o*-benzoquinone [59], which is confirmed by the fact that $\Delta G_{H^-P}(iAscH_2)$ (100.8 kcal/mol) [27] is slightly greater than $\Delta G_{H^-P}(PQH_2)$ (96.2 kcal/mol) [29].

Herein, the oxidative mechanism between $3H_2$ and $iAsc$ is taken as an example to represent the application of the thermodynamic cards of DH_2 to oxidative mechanism diagnosis. First of all, the thermodynamic card of $iAsc$ accepting two hydrogen ions (atoms) on nine potential elementary steps is constructed based on our previous work (Figure 12). Subsequently, according to the thermodynamic cards of $3H_2$ and $iAsc$, a thermodynamic analysis platform of elementary steps for the redox process between $3H_2$ and $iAsc$ without any catalyst in acetonitrile is established and shown in Figure 13.

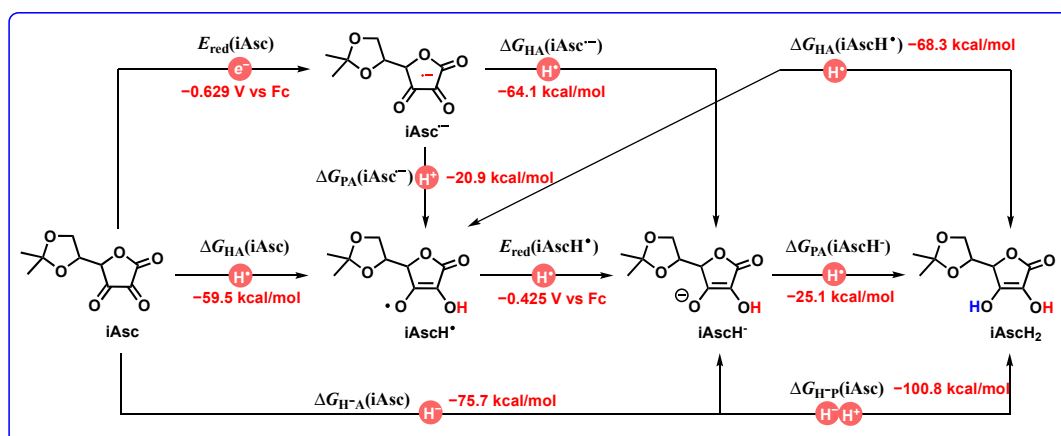


Figure 12. Thermodynamic card of $iAsc$ accepting two hydrogen ions (atoms) on nine potential elementary steps.

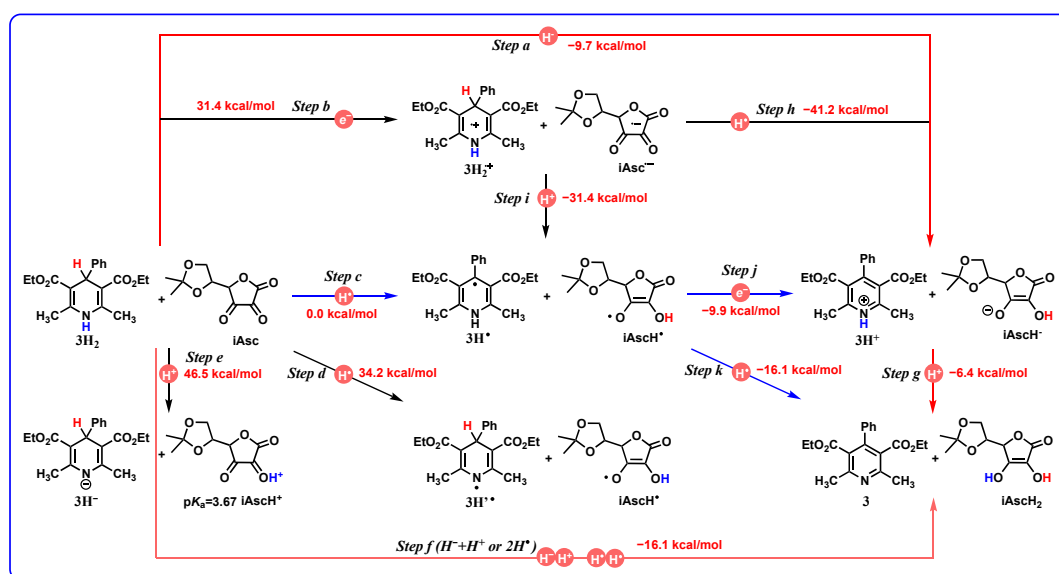


Figure 13. Thermodynamic analysis platform of elementary steps for the redox process between $3H_2$ and $iAsc$ without any catalyst in acetonitrile.

From Figure 13, Step 13 is the concerted two hydrogen ions (atoms) transfer step from 3H_2 to iAsc, $3\text{H}_2 + \text{iAsc} \rightarrow 3 + \text{iAscH}_2$, and the overall Gibbs free energy of two hydride ions (atoms) transfer from 3H_2 to iAsc for Step 13 is -16.1 kcal/mol, which means the oxidation of 3H_2 by iAsc is thermodynamically feasible. Moreover, as can be seen from Figure 13, six possible elementary steps are involved in the initial oxidation of 3H_2 by iAsc. Step a is the hydride transfer step from 3H_2 to iAsc, $3\text{H}_2 + \text{iAsc} \rightarrow 3\text{H}^+ + \text{iAscH}^-$, and the Gibbs free energy of Step a is -9.7 kcal/mol. Step b is the electron transfer step from 3H_2 to iAsc, $3\text{H}_2 + \text{iAsc} \rightarrow 3\text{H}^{\bullet+} + \text{iAsc}^{\bullet-}$, and the Gibbs free energy of Step b is 31.4 kcal/mol. Steps c and d are the hydrogen atom transfer step from $\text{C}_4\text{-H}$ and $\text{N}_1\text{-H}$ bonds in 3H_2 to iAsc, respectively, $3\text{H}_2 + \text{iAsc} \rightarrow 3\text{H}^{\bullet} + \text{iAscH}^{\bullet}$ and $3\text{H}_2 + \text{iAsc} \rightarrow 3\text{H}^{\bullet} + \text{iAscH}^{\bullet}$, and the Gibbs free energy of Steps c and d are 0.0 and 34.2 kcal/mol. Step e is the proton transfer step from 3H_2 to iAsc, $3\text{H}_2 + \text{iAsc} \rightarrow 3\text{H}^- + \text{iAscH}^+$, and the Gibbs free energy of Step e is 46.5 kcal/mol. Finally, Step f is the concerted two hydrogen ions (atoms) transfer step from 3H_2 to iAsc, and the Gibbs free energy of Step f is -16.1 kcal/mol.

According to the thermodynamic potentials of Steps a–f, the Gibbs free energies of Step b (31.4 kcal/mol), Step d (34.2 kcal/mol), and Step e (46.8 kcal/mol) are greater than 30 kcal/mol, which implies that the oxidation of 3H_2 could not occur through electron transfer (Step b), hydrogen transfer from the $\text{N}_1\text{-H}$ bond (Step d), and proton transfer (Step e). Since the Gibbs free energies of Step a (-9.7 kcal/mol), Step c (0.0 kcal/mol), and Step f (-16.1 kcal/mol) are less than or equal to zero, the oxidation of 3H_2 may experience an initial hydride (Step a), hydrogen atom from $\text{C}_4\text{-H}$ (Step c), and concerted two hydrogen ions (atoms) transfer step (Step f).

Further investigating the thermodynamics of possible hydrogen atom oxidation processes, there are two different pathways for the oxidation of 3H_2 by iAsc overall. The first is the $\text{H}^{\bullet} + \text{e} + \text{H}^+$ pathway (Step c–Step j–Step g), and the second is the $\text{H}^{\bullet} + \text{H}^{\bullet}$ pathway (Step c–Step k). Due to the fact that all of the Gibbs free energies of the above five elementary steps are less than or equal to zero (-16.1 – 0.0 kcal/mol), it can be assumed that these two hydrogen-atom-initiated pathways, Step c–Step j–Step g and Step c–Step k, are thermodynamically feasible.

Additionally, the Gibbs free energies of direct hydride transfer from 3H_2 to iAsc and proton transfer from 3H^+ to iAscH^- , Step a (hydride transfer, -9.7 kcal/mol) and Step g (proton transfer, -6.4 kcal/mol), are much less than zero, and it can be inferred that the oxidation process of 3H_2 and iAsc undergoing successive hydride (Step a, -9.7 kcal/mol) and proton transfer (Step g, -6.4 kcal/mol) is thermodynamically feasible.

Similarly, the oxidation of 3H_2 by iAsc processing concerted two hydrogen ions (atoms) transfer (Step f, -16.1 kcal/mol) is also thermodynamically feasible. Although the concerted two hydrogen ions (atoms) transfer (Step f) is thermodynamically feasible, 3H_2 and iAsc molecules do not completely match each other in space structure and binding site during transition state. Therefore, the concerted two hydrogen ions (atoms) transfer (Step f) process is reasonably ruled out. It may be that in complex environments within the body, the hydride-acceptor/amino-acid residue pairs, two radicals, or benzoquinones could oxidize dipines through a concerted two hydrogen ions (atoms) transfer step.

In summary, the oxidation of 3H_2 by iAsc may experience three possible pathways, that is, $\text{H}^- + \text{H}^+$ (Step a–step g), $\text{H}^{\bullet} + \text{e} + \text{H}^+$ (Step c–Step j–Step g), and $\text{H}^{\bullet} + \text{H}^{\bullet}$ (Step c–Step k). But which one pathway or pathways are the real oxidation mechanism needs further experimental verification in the lab. Beyond a doubt, the thermodynamic analyses of the redox process between 3H_2 and iAsc provide us with a unique perspective into the dipines' aromatization by quinones in vivo.

3. Materials and Methods

Prediction Methods. The pK_a values of DH^+ and YH^+ in acetonitrile were predicted by the method developed by Luo and coworkers in 2020 at <http://pka.luoszgroup.com/prediction> (accessed on 9 January 2024). Prediction methods: XGBoost with RMSE = 1.79 and $r^2 = 0.918$ (80:20 train test split).

4. Conclusions

For dipines, their core skeleton is the 1,4-dihydropyridine structure, which is a key structural factor for biological activity. During the metabolism of dipines in vivo, 1,4-dihydropyridine rings are oxidized to release formal two hydrogen ions (atoms) and generate the aromatic pyridines, and six possible intermediates and 21 potential elementary steps are involved in the process. In this work, 4-substituted-phenyl-2,6-dimethyl-3,5-diethyl-formate-1,4-dihydropyridines (DH₂) are refined as the structurally closest dipine models, and the thermodynamic cards of these dipine models' aromatization on 21 potential elementary steps in acetonitrile have been established.

Based on the thermodynamic cards, the thermodynamic properties of dipine models and related intermediates acting as electrons, hydrides, hydrogen atoms, protons, and two hydrogen ions (atoms) donors are discussed. Several valuable conclusions are made as follows.

- (1) Electron-donating properties. The electron-donating abilities increase in the order of $\text{DH}_2 < \text{DH}^- < \text{DH}^\bullet < \text{D}^{\bullet-}$. DH₂, DH[−], DH[•], and D^{•−} are recognized as the weak electron donors, medium–strong electron donors, strong electron donors, and very strong electron donors, respectively.
- (2) Hydride-donating properties. DH₂ and DH[−] belong to thermodynamically medium–strong and strong hydride donors, respectively. It can be inferred that dipines may be oxidated by heme enzyme, cytochrome P450, flavin coenzyme, coenzyme Q, and H⁺ under suitable oxidoreductase by hydride oxidation in vivo.
- (3) Hydrogen-atom-donating properties. The thermodynamic hydrogen-atom-donating abilities increase in the order of $\text{DH}_2 (\text{N}_1\text{-H}) < \text{DH}^- < \text{DH}_2 (\text{C}_4\text{-H}) < \text{DH}^\bullet < \text{DH}_2^{\bullet+} < \text{DH}^{\bullet+}$. Dipines may be oxidated by heme enzyme, cytochrome P450, coenzyme Q (Co Q), and H[•] under suitable oxidoreductase, by hydrogen atom oxidation in vivo.
- (4) Proton-donating properties. DH₂ belong to weak proton donors, DH₂^{•+} (N₁-H), DH[•], and DH⁺ belong to medium–strong proton donors, and DH₂^{•+} (C₄-H) belong to strong proton donors. After DH₂ are oxidized by hydride acceptors, the acidities of resulting DH⁺ should be considered when the organic bases are involved in the reaction system.
- (5) Two hydrogen ions (atoms) donating properties. DH₂ belong to medium–strong two hydrogen ion donors. H₂ could hydrogenate D to regenerate DH₂ in solution under suitable catalysts. DH₂ maybe oxidated by Co Q via two hydrogen ions (atoms) transfer in vivo.

Moreover, the thermodynamic cards were applied to evaluate the redox properties and judge the possible oxidative mechanism of dipine models. The important conclusions are drawn as follows.

- (1) Application in evaluating the redox properties of DH₂: DH₂ generally release hydrogen atoms from the C₄-H bond instead of the N₁-H bond in the radical oxidation process. Generally, DH₂ undergoing hydride or radical oxidations is thermodynamically favorable. Since DH₂ are thermodynamically very weak proton donors, the concerted or successive proton and hydride (H⁺ + H[−]) transfer mechanism is thermodynamically practicable. The single-electron oxidation of DH₂ is thermodynamically disadvantageous, and a high single-electron oxidant is needed.
- (2) Application in judging the possible oxidative mechanism for important intermediates: DH₂^{•+} may act as proton donors or hydrogen atom donors, which depends on the properties of substrates. If the substrates are radicals, DH₂^{•+}, act as hydrogen atom donors. If the substrates are bases, DH₂^{•+}, act as proton donors. Most importantly, with a combination of the oxidation potentials of DH₂ and the properties of resulting DH₂^{•+} releasing hydrogen ions (atoms), the initial slight energy barrier (10–20 kcal/mol) of electron oxidation could be overcome by the subsequent protons transfer or hydrogen atoms transfer owing extreme thermodynamic driving forces ($\Delta G \ll 0$).

- (3) The application of the thermodynamic data of DH₂ to oxidative mechanism diagnosis between DH₂ and iAsc: Because of the ortho dicarbonyl feature, Asc has the property of *o*-benzoquinone. Therefore, the oxidative mechanism between 3H₂ and iAsc is taken as an example to represent the application of the thermodynamic cards of DH₂ to oxidative mechanism diagnosis. Based on thermodynamic analyses, the oxidation of 3H₂ by iAsc may experience three possible pathways, that is, H[−] + H⁺, H[•] + e + H⁺, and H[•] + H[•].

The thermodynamic data of dipine models' aromatization suggests that the oxidative metabolism of dipine drugs is unexpectedly complex, and may involve many active intermediates and potential elementary steps under the conditions of various oxidoreductases in vivo. Therefore, the effects and levels of electrons and hydrogen atoms, as well as hydride oxidoreductases may need sustained attention or detection during the treatment period. Without a doubt, the thermodynamic cards of dipine models could help us to understand the redox properties of DH₂ and related intermediates, and diagnose the possible mechanism and pathways of dipine metabolism in vivo.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/molecules29153706/s1>. The prediction method of pK_a, original data of pK_a prediction, and thermodynamic cards of DH₂ and YH₂ are available in Supporting Information. Reference [40] are cited in the supplementary materials.

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