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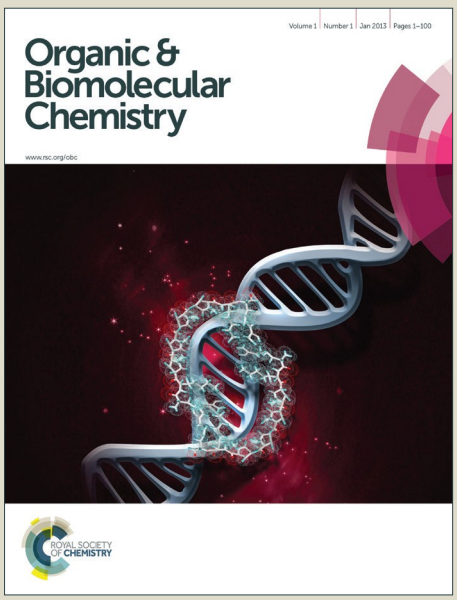

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ARTICLE

Elemental Step Thermodynamics of Various Analogues of Indazolium Alkaloids to Obtain Hydride in Acetonitrile

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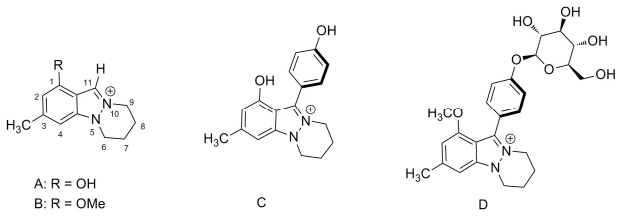
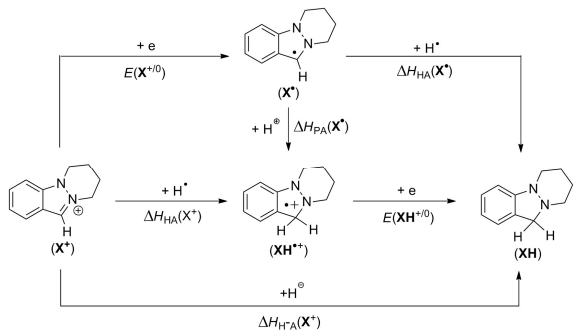
A series of analogues of indazolium alkaloids were designed and synthesized. The thermodynamic driving forces of the 6 elemental steps for the analogues of indazolium alkaloids to obtain hydride in acetonitrile were determined using isothermal titration calorimeter (ITC) and electrochemical methods, respectively. The effects of molecular structure and substituents on the thermodynamic driving forces of the 6 steps were examined. Meanwhile, the oxidation mechanism of NADH coenzyme by indazolium alkaloids was examined using chemical mimic method. The result shows that the oxidation of NADH coenzyme by indazolium alkaloids *in vivo* takes place by one-step concerted hydride transfer mechanism.

Introduction

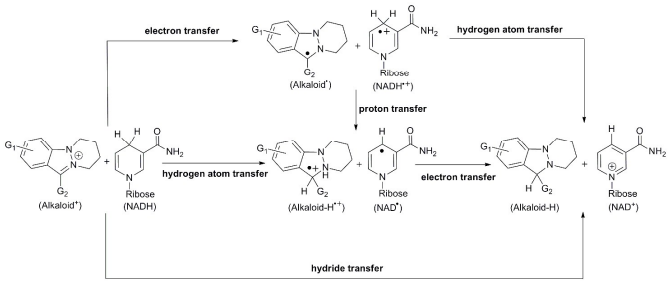
Well known as a traditional medicine, the seeds of *Nigella sativa* were extensively studied for their antidiabetic effect, but the actual bioactive compounds and the molecular mechanism responsible for this activity had not yet been well elaborated.[1-4] Until recently, several indazolium alkaloids were successfully isolated from the seeds of *Nigella sativa* (Scheme 1) and uncovered to show good antidiabetic effects, and an initiatory study concerning the molecular mechanism of their antidiabetic effects showed these indazolium alkaloids could increase the consumption of glucose by liver hepatocytes through the activation of AMP-activated protein kinase (AMPK).[5] However, deeper molecular mechanism about how these alkaloids activate AMPK was heretofore uninvestigated. It was revealed that the activity of mammalian AMPK could be biochemically regulated by the redox potential of $NADH/NAD^+$, which is directly related to the ratio of $NADH/NAD^+$, and it was further clarified that AMPK is activated by NAD^+ in a dose-dependent manner, whereas AMPK is inhibited by NADH.[6] A question might arise of whether the activation of AMPK by these alkaloids is due to that these indazolium alkaloids would act on (consume) NADH through chemical reactions. As a result, it should be of significance to investigate thoroughly the possibilities of reactions between these alkaloids and NADH. It was known that NADH plays a vital role in biochemical reactions through donating hydride, hydrogen atom, or electron to other substrates,[7-12] whilst the cationic motifs of these indazolium alkaloids tend to exhibit their good abilities to obtain hydride, hydrogen atom, electron (Scheme 2), etc.,[13-18] thus the possible reactions between these alkaloids and NADH should include hydride transfer, hydrogen atom transfer and electron transfer, all of which could be assigned to the possible elemental steps of hydride transfers between these alkaloids and NADH, as illustrated in Scheme 3. Since thermodynamics could offer intrinsic criterion in diagnosing the possibilities of reactions, it might be efficient and comprehensive if we could elucidate the possibilities of all the elemental steps of hydride transfer between these alkaloids and NADH from the perspective of thermodynamics. In fact, this issue might be addressed if we turn to a unique thermodynamic tool of "Molecular ID Card" developed in our group.[19-22]



Scheme 1. The Structures of Several Indazolium Alkaloids Derived from the Seeds of *Nigella Sativa* with Antidiabetic Effects.[5]



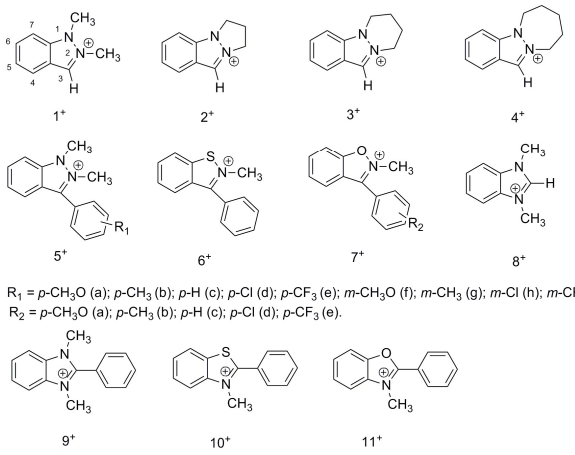
Scheme 2. Possible Elementary Steps and Corresponding Driving Forces for the Analogues of Indazolium Alkaloids (X^+) to Obtain Hydride Anion.



Scheme 3. Possible Elemental Steps of Hydride Transfer from NADH to Indazolium Alkaloids.

In this work, a series of analogues of indazolium alkaloids ($\mathbf{X}^+$) were designed and synthesized (Scheme 4) to mimic the naturally occurring antidiabetic alkaloids in Scheme 1. The thermodynamic driving forces of 6 possible elementary steps for $\mathbf{X}^+$ to obtain hydride (Scheme 2) in acetonitrile were determined, namely, the hydride affinities [$\Delta H_{\text{H-A}}(\mathbf{X}^+)$], hydrogen atom affinities [$\Delta H_{\text{HA}}(\mathbf{X}^+)$] and reduction potentials [$E(\mathbf{X}^{+/0})$] for $\mathbf{X}^+$ to obtain hydride, hydrogen atom and electron, respectively; the hydrogen atom affinities [$\Delta H_{\text{HA}}(\mathbf{X}^{\bullet})$] and the proton affinities [$\Delta H_{\text{PA}}(\mathbf{X}^{\bullet})$] for the neutral radicals of them ($\mathbf{X}^{\bullet}$) to obtain hydrogen atom and proton, respectively; and the reduction potentials [$E(\mathbf{XH}^{+/0})$] for the hydrogen adducts of them ($\mathbf{XH}^{\bullet+}$) to obtain electron. All these thermodynamic driving forces are very important and desired parameters for chemists not only to diagnose the reactivities of $\mathbf{X}^+$ and their various reaction intermediates, but to thoroughly elucidate mechanism of hydride transfer reactions to $\mathbf{X}^+$ *via* "Molecular ID Cards".[19-22] Based on structures designed in Scheme 4, we would figure out how these thermodynamic parameters were affected by structural isomerizations, variation of heteroatoms, and remote substituents. The possibilities of reactions between indazolium alkaloids and NADH were elucidated using the determined elemental step thermodynamic parameters.

In addition, since the most biochemical processes with the indazolium alkaloids and NADH coenzyme as a hydride acceptor or donor in living body all take place in the polar organic regions constructed with enzyme proteins rather than with pure water, the chemical information of the indazolium alkaloids as a hydride acceptor or donor in the polar organic regions constructed with enzyme proteins should be more important and valuable than that in the pure aqueous solution. In order to derive the characteristic chemical information of the indazolium alkaloids as a hydride acceptor in the polar organic regions constructed with enzyme proteins, in this work, acetonitrile was chosen as the solvent to imitate the polar organic regions constructed with enzyme proteins, because the polarity of acetonitrile ($\varepsilon = 37.5$) is quite close to that of peptide bond in proteins (ε = 37.0, 37.8 and 38.3 for $HCONMe_2$, $MeCONMe_2$ and N,N-dimethylbenzamide, respectively).[22]



Scheme 4. Structures of Analogues of Indazolium Alkaloids ($\mathbf{X}^+$) Examined in This Work (all of them are in the form of perchlorate salts).

Results

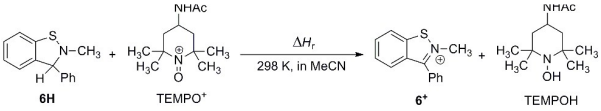
All these analogues of indazolium alkaloids ($\mathbf{X}^+$) in Scheme 4 were synthesized according to conventional procedures and the target molecules were identified by ^{1}HNMR and MS, and the detailed synthetic routes and procedures are provided in the Supporting Information. Hydride affinities of these analogues [$\Delta H_{\text{H-A}}(\mathbf{X}^+)$] in this work were defined as the enthalpy changes of reactions of $\mathbf{X}^+$ with a free hydride anion in acetonitrile to

$$\mathbf{X}^+ + \mathrm{H}^{\ominus} \xrightarrow[298\ \text{K, in MeCN}]{\Delta H_{\text{H-A}}(\mathbf{X}^+)} \mathbf{XH} \quad (1)$$

$$\Delta H_{\text{H-A}}(\mathbf{X}^+) = H_{\text{f}}(\mathbf{XH}) - [H_{\text{f}}(\mathbf{X}^+) + H_{\text{f}}(\mathrm{H}^-)] \quad (2)$$

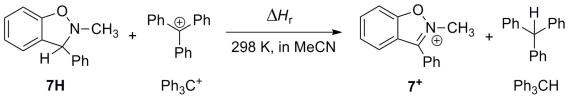
(3)

$$\Delta H_{\text{H-A}}(\mathbf{X}^+) = \Delta H_{\text{H-A}}(\text{TEMPO}^+) - \Delta H_{\text{r}} \quad (4)$$



(5)

$$\Delta H_{\text{H-A}}(\mathbf{X}^+) = \Delta H_{\text{H-A}}(\text{TEMPO}^+) - \Delta H_{\text{r}} \quad (6)$$



(7)

$$\Delta H_{\text{H-A}}(\mathbf{X}^+) = \Delta H_{\text{H-A}}(\text{Ph}_3\text{C}^+) - \Delta H_{\text{r}} \quad (8)$$

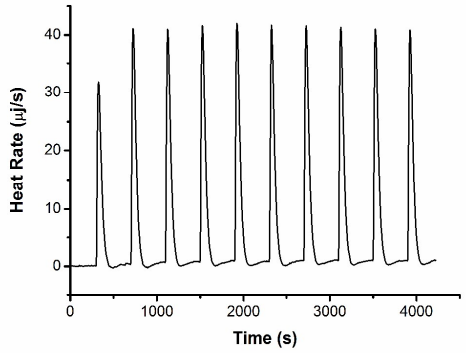
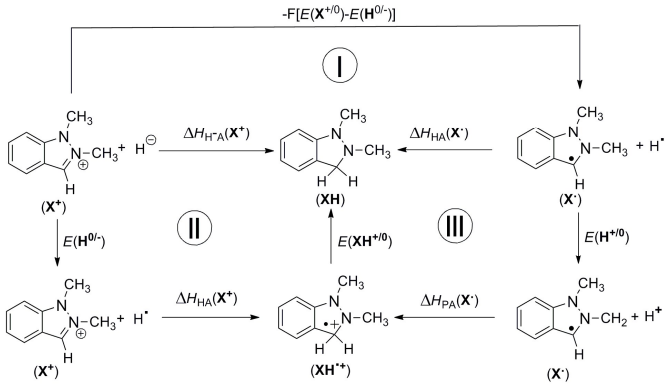


Figure 1. Isothermal titration calorimetry (ITC) graph of the reaction heat of **1H** with 4-acetamido-2,2,6,6-tetramethylpiperidine-1-oxo-ammonium perclorate ($TEMPO^+$) in acetonitrile at 298 K. Titration was conducted by adding 10 μL of $TEMPO^+$ (1.0 mM) every 300s into the acetonitrile solution containing **1H** (ca. 12.0 mM).

form their conjugated amines (**XH**) [eqn (1) and (2)] at 298 K in acetonitrile. The determinations of the enthalpy changes of $\mathbf{X}^+$ to gain hydride in acetonitrile were performed according to the following two strategies: (i) For $\mathbf{1}^+$-$\mathbf{7}^+$, the hydride affinities were obtained according to eqn (3)-(8), derived from the hydride exchange reactions between their conjugated amines (**XH**) and 4-acetamido-2,2,6,6-tetramethylpiperidine-1-oxoammonium perclorate ($TEMPO^+ClO_4^-$) or trityl perclorate ($Ph_3C^+ClO_4^-$) in acetonitrile, respectively. In eqn (3)-(8), ΔH_r were the reaction enthalpy changes, which could be determined via titration calorimetry (Figure 1); $\Delta H_{H^-A}(TEMPO^+)$ was the hydride affinity of $TEMPO^+ClO_4^-$, which was determined to be -105.6 kcal/mol,[23] and $\Delta H_{H^-A}(Ph_3C^+)$ was the hydride affinity of $Ph_3C^+ClO_4^-$, which was previously determined to be -104.6 kcal/mol.[24] (ii) For $\mathbf{8}^+$-$\mathbf{11}^+$, since the enthalpy changes [ΔH_{H^-D}(**XH**)] for their conjugated amines to release hydride had already been determined by our previous work, the enthalpy changes for $\mathbf{8}^+$-$\mathbf{11}^+$ to gain hydride in acetonitrile, could be derived by switching the sign of ΔH_{H^-D}(**XH**).[25] The enthalpy changes of these hydride exchange reactions were listed in Table 1, while the hydride affinities of these alkaloid analogues were summarized in Table 2.



Scheme 5. Three Thermodynamic Cycles Constructed on the Basis of the Reactions of the Analogues of Indazolium Alkaloids ($\mathbf{X}^+$) with Hydride Anion (H^-).

$$\Delta H_{HA}(\mathbf{X}^+) = \Delta H_{H^-A}(\mathbf{X}^+) - F[E(H^{0/-}) - E(\mathbf{XH}^{+/0})] \quad (9)$$

$$\Delta H_{HA}(\mathbf{X}^\bullet) = \Delta H_{H^-A}(\mathbf{X}^+) - F[E(H^{0/-}) - E(\mathbf{X}^{+/0})] \quad (10)$$

$$\Delta H_{PA}(\mathbf{X}^\bullet) = \Delta H_{HA}(\mathbf{X}^+) - F[E(H^{+/0}) - E(\mathbf{XH}^{+/0})] \quad (11)$$

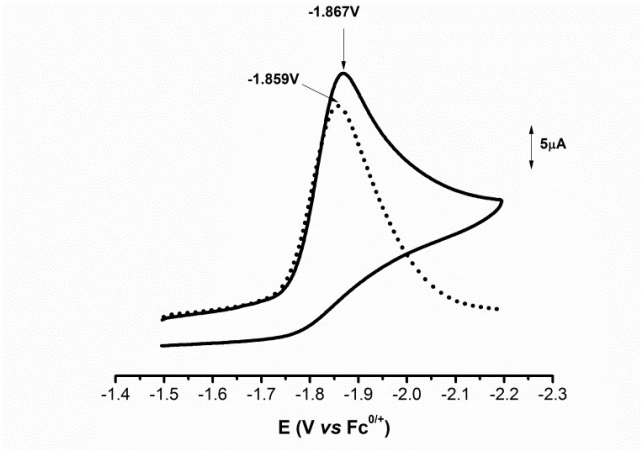


Figure 2. Cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) of $\mathbf{1}^+$ in deaerated acetonitrile containing 0.1 M n-Bu_4NBF_4 as supporting electrolyte. The full line: CV graph (sweep rate = 0.1 V/s), the dashed line: OSWV graph.

The hydrogen atom affinities of $\mathbf{X}^+$ were defined as the enthalpy changes for $\mathbf{X}^+$ to gain hydrogen atom [$\Delta H_{HA}(\mathbf{X}^+)$] in acetonitrile at 298 K; the hydrogen atom affinities and proton affinities of the neutral radicals intermediates ($\mathbf{X}^\bullet$) were also defined as the enthalpy changes for $\mathbf{X}^\bullet$ to gain hydrogen atom [$\Delta H_{HA}(\mathbf{X}^\bullet)$] and to gain proton [$\Delta H_{PA}(\mathbf{X}^\bullet)$] in acetonitrile at 298 K, respectively. These parameters were indicators of their hydrogen atom accepting abilities or proton accepting abilities. To derive these parameters, three thermodynamic cycles were constructed following the possible routes of hydride transfer to these indazolium analogues (Scheme 5). Through these cycles, eq.9-11 could be derived according to Hess's law, where $E(\mathbf{X}^{+/0})$, $E(\mathbf{XH}^{+/0})$, $E(\mathbf{H}^{0/-})$ and $E(\mathbf{H}^{+/0})$ are standard redox potentials of $\mathbf{X}^+$, $\mathbf{XH}^{\bullet+}$, H^- and H^+, respectively. Since $E(\mathbf{H}^{0/-})$ and $E(\mathbf{H}^{+/0})$ could be obtained from literatures,[26] $E(\mathbf{X}^{+/0})$, $E(\mathbf{XH}^{+/0})$ could be determined through electrochemical methods (Table 1, Figures 2, 3), plus that $\Delta H_{H^-A}(\mathbf{X}^+)$ could obtained from above works, the values of $\Delta H_{HA}(\mathbf{X}^+)$, $\Delta H_{HA}(\mathbf{X}^\bullet)$ and $\Delta H_{PA}(\mathbf{X}^\bullet)$ could easily calculated via eqn (9)-(11). These parameters, together with $\Delta H_{H^-A}(\mathbf{X}^+)$, were summarized in Table 2.[27,28]

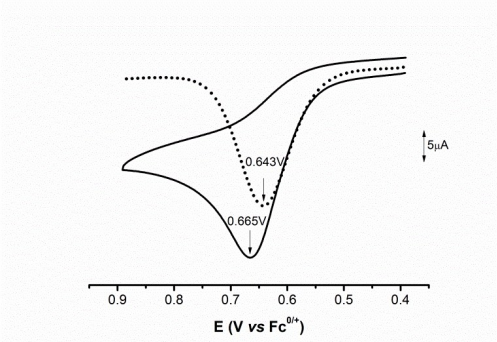


Figure 3. Cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) of **1H** in deaerated acetonitrile containing 0.1 M n-Bu_4NBF_4 as supporting electrolyte. The full line: CV graph (sweep rate = 0.1 V/s), the dashed line: OSWV graph.

Discussion

Hydride Affinities of the Analogues of Indazolium Alkaloids (X^+) in Acetonitrile.

In this study, the hydride affinities of $\mathbf{X}^+$ were defined as the standard state enthalpy changes for $\mathbf{X}^+$ to obtain hydride in acetonitrile at 298 K. From the second column in Table 2, it is clear that the scales of hydride affinities of $\mathbf{1}^+$-$\mathbf{11}^+$ covers almost 41 kcal/mol, ranging from -49.5 to -90.6 kcal/mol, offering us a library of analogues of indazolium alkaloids with diverse electrophiles to hydride. For an intuitive comparison, the hydride affinities of $\mathbf{X}^+$ were ranked in an decreasing order: $\mathbf{8}^+ > \mathbf{1}^+ > \mathbf{9}^+ > \mathbf{4}^+ > \mathbf{2}^+ > \mathbf{3}^+ > \mathbf{5c}^+ > \mathbf{6}^+ > \mathbf{10}^+ > \mathbf{7c}^+ > \mathbf{11}^+$, corresponding to an increasing order of electrophilicities of these alkaloid analogues to hydride: $\mathbf{8}^+ < \mathbf{1}^+ < \mathbf{9}^+ < \mathbf{4}^+ < \mathbf{2}^+ < \mathbf{3}^+ < \mathbf{5c}^+ < \mathbf{6}^+ < \mathbf{10}^+ < \mathbf{7c}^+ < \mathbf{11}^+$, as illustrated in Figure 4. To be specific, indazoliumions ($\mathbf{1}^+$-$\mathbf{5}^+$), benzo[*d*]imidazoliumions ($\mathbf{8}^+$, $\mathbf{9}^+$), and benzo[*d*]isothiazolium ion ($\mathbf{6}^+$) belong to weak electrophiles to hydride. When reducing them to their conjugated amines, some strong inorganic hydrides like boron hydrides or aluminum hydrides were recommended.[16,18,29] In contrast, benzo[*d*]thiazoliumions ($\mathbf{10}^+$), benzo[*d*]isoxazoliumion ($\mathbf{7}^+$) and benzo[*d*]oxazoliumions ($\mathbf{11}^+$) belong to the category of strong electrophiles to hydride, some well-known organic hydride donors such as dihydrobenzo[*d*]imidazoles (like **8H** and **9H**) and Hantzch esters are capable to reduce them to their conjugated amines.[25,30] Structurally, $\mathbf{5c}^+$ shows a slightly stronger electrophilicity to hydride than its isomeric $\mathbf{9}^+$, which is consistent with the fact that $\mathbf{5c}^+$ was able to slowly grab hydride from the conjugated amine of $\mathbf{9}^+$ under a harsh condition, illustrated as an evidence of the reliability of our data.[31] Variation of the heteroatom in the structures of these analogues could lead to a change of the hydride affinity up to around 37 kcal/mol, such as $\mathbf{9}^+$ (-54.1 kcal/mol) *vs* $\mathbf{11}^+$ (-91.2 kcal/mol), indicating that the electrophilicities of these analogues to hydride could be adjusted in a flexible scope to meet diverse demands.

If the hydride affinities of these analogues are compared with those of primary benzyl carbon cations (e.g. -106 kcal/mol for 4-MeOPhCH_2^+) in acetonitrile,[32] it is found the hydride affinities of these analogues are more positive than those of benzyl carbon cations by at least 14 kcal/mol. Unlike the reduction of a benzyl carbon cation in which only one new C-H bond formed, the reduction of an alkaloid analogue is accompanied by additional cleavage of one C=N bond and loss of aromatization of the heterocycle to consume energy, which would offset the energy released in the formation of the new C-H bond. In sharp contrast, if the hydride affinities of these indazolium analogues are compared with those of imines (e.g. -40.8 kcal/mol for N-benzylideneaniline)[19], it seems a paradox that the hydride affinities of these analogues are more negative than those of imines by over 12 kcal/mol, since at first glance, the hydride transfer to an alkaloid analogue is accompanied by one more energy-consuming loss of aromatization of heterocycle compared to the hydride transfer to an imine, apart from the common energy-releasing formation of one C-H bond and energy-consuming cleavage of C=N bond. Nevertheless, if the contribution from molecular charge is considered, it may conclude that it is more spontaneous for cationic alkaloid analogues to accept hydride than it for neutral imines to accept hydride, which is different from the case between indazolium alkaloid analogues and benzyl carbon cations mentioned above. This result might inspire us that the cationic indazolium analogues of alkaloids are more ready to obtain hydride than their neutral indazole analogues.

Hydrogen Atom Affinities of the Analogues of Indazolium Alkaloids (X^+) in Acetonitrile.

The hydrogen atom affinities of $\mathbf{X}^+$ were defined as the standard state enthalpy changes for $\mathbf{X}^+$ to obtain hydrogen atom in acetonitrile at 298 K. As shown in the third column of Table 2 and Figure 5, the hydrogen atom affinities of $\mathbf{X}^+$ decrease in an order: $\mathbf{2}^+ > \mathbf{1}^+ > \mathbf{3}^+ > \mathbf{4}^+ > \mathbf{8}^+ > \mathbf{6}^+ > \mathbf{9}^+ > \mathbf{5c}^+ > \mathbf{10}^+ > \mathbf{7c}^+ > \mathbf{11}^+$, corresponding to an increasing order of the electrophilicities of indazolium alkaloid analogues to hydrogen atom. Generally, these analogues are poor electrophiles to hydrogen atom, since they are not able to be reduced by some strong antioxidant reagents such as Vitamin E (80.9 kcal/mol to release hydrogen atom), the commercial available BHT (2,6-ditert-butyl-4-methylphenol, 81.6 kcal/mol to release hydrogen atom) and phenothiazine (79.7 kcal/mol to release hydrogen atom).[33-35] When comparing their hydrogen atom affinities with their hydride affinities (Table 2, column 3 *vs* column 2), it may conclude that these analogues are more ready to be reduced by hydride ion than by hydrogen atom, thus the hydride transfer

Table 1. Reaction Enthalpy Changes of Indazolium Alkaloid Analogues ($\mathbf{X}^+$), together with Reduction Potentials of $\mathbf{X}^+$ and Reduction Potentials of Their Hydrogen Adducts ($\mathbf{XH}^{\bullet+}$) in Acetonitrile.

Indazolium Analouges ($\mathbf{X}^+$)[a]		$E(\mathbf{X}^{+/0})$ [a,c,d]		$E(\mathbf{XH}^{+/0})$ [a,c,d]	
	ΔH_r[b]	CV	OSWV	CV	OSWV
$\mathbf{1}^+$	-51.9	-1.867	-1.859	0.665	0.643
$\mathbf{2}^+$	-49.9	-1.893	-1.871	0.766	0.751
$\mathbf{3}^+$	-48.0	-1.813	-1.797	0.720	0.703
$\mathbf{4}^+$	-50.5	-1.926	-1.909	0.511	0.491
$\mathbf{5}^+$ **(a-i)**					
p-MeO (**a**)	-47.8	-1.780	-1.759	-0.059	-0.089
p-Me (**b**)	-47.5	-1.778	-1.743	-0.056	-0.080
p-H (**c**)	-47.0	-1.776	-1.715	-0.040	-0.065
p-Cl(**d**)	-46.2	-1.727	-1.675	-0.017	-0.045
p-CF_3 (**e**)	-45.2	-1.699	-1.624	0.008	-0.020
m-MeO (**f**)	-46.5	-1.755	-1.694	-0.035	-0.055
m-Me (**g**)	-47.2	-1.763	-1.726	-0.051	-0.072
m-Cl (**h**)	-45.7	-1.720	-1.653	-0.010	-0.033
m- CF_3 (**i**)	-45.5	-1.695	-1.642	0.004	-0.028
$\mathbf{6}^+$	-55.0	-1.201	-1.162	0.244	0.198
$\mathbf{7}^+$ **(a-e)**					
p-MeO (**a**)	-19.6	-1.073	-1.055	0.703	0.667
p-Me (**b**)	-19.0	-1.051	-1.035	0.713	0.679
p-H (**c**)	-17.9	-1.024	-1.001	0.729	0.703
p-Cl(**d**)	-16.3	-0.983	-0.955	0.776	0.743
p-CF_3 (**e**)	-14.5	-0.938	-0.909	0.809	0.787
$\mathbf{8}^+$		-2.217	-2.173	-0.145	-0.179
$\mathbf{9}^+$		-2.055	-2.024	-0.068	-0.103
$\mathbf{10}^+$		-1.430	-1.400	0.365	0.332
$\mathbf{11}^+$		-1.141	-1.111	0.595	0.564

[a] $\mathbf{X}^+$ stood for the analogues of indazolium alkaloids in this work, while $\mathbf{X}^{\bullet}$ stood for their neutral radicals. [b] ΔH_r were the reaction enthalpy changes of eq.3, 5, and 7 measured by titration calorimetry in acetonitrile at 298 K, respectively. The data, given in kcal/mol, were average values of at least three independent runs. The reproducibility was estimated to be ±0.5 kcal/mol. [c] Measured by CV and OSWV methods in acetonitrile at 298 K, the unit in volt *vs* $Fc^{+/0}$ and reproducible to 5mv or better. The reduction potentials [$E(\mathbf{XH}^{+/0})$] of $\mathbf{1H}^{\bullet+}$ **-** $\mathbf{7H}^{\bullet+}$ were determined by the oxidation potentials [$E(\mathbf{XH}^{0/+})$] of **1H - 7H**, since they are equal in value.[27,28] [d] $E(\mathbf{X}^{+/0})$ and $E(\mathbf{XH}^{+/0})$ of $\mathbf{8}^+$, $\mathbf{9}^+$, $\mathbf{10}^+$, and $\mathbf{11}^+$ and their intermediates were derived from our previous work.[25]

Table 2. Enthalpy Changes of $\mathbf{X}^+$ to Accept Hydride and to Accept Hydrogen Atom, as well as the Enthalpy Changes of $\mathbf{X}^{\bullet}$ to Accept Proton and to Accept Hydrogen Atom in Acetonitrile (kcal/mol)[a,b]

Indazolium Analouges ($\mathbf{X}^+$)	$\Delta H_{H^-A}(\mathbf{X}^+)$[c]	$\Delta H_{HA}(\mathbf{X}^+)$[d]	$\Delta H_{PA}(\mathbf{X}^{\bullet})$[d]	$\Delta H_{HA}(\mathbf{X}^{\bullet})$[d]
$\mathbf{1}^+$	-53.7	-12.7	-2.3	-70.3
$\mathbf{2}^+$	-55.7	-12.2	-2.1	-72.6
$\mathbf{3}^+$	-57.6	-15.2	-3.4	-72.8
$\mathbf{4}^+$	-55.1	-17.6	-8.4	-72.9
$\mathbf{5}^+$ **(a-i)**				
p-MeO (**a**)	-57.8	-33.6	-21.0	-72.1
p-Me (**b**)	-58.1	-33.7	-20.7	-72.1
p-H (**c**)	-58.6	-33.9	-20.2	-71.9
p-Cl(**d**)	-59.4	-34.2	-19.6	-71.8
p-CF_3 (**e**)	-60.4	-34.6	-18.9	-71.6
m-MeO (**f**)	-59.1	-34.0	-20.0	-71.9
m-Me (**g**)	-58.4	-33.8	-20.5	-72.0
m-Cl (**h**)	-59.9	-34.4	-19.4	-71.8
m- CF_3 (**i**)	-60.1	-34.5	-19.1	-71.7
$\mathbf{6}^+$	-60.6	-29.8	-3.4	-61.2
$\mathbf{7}^+$ **(a-e)**				
p-MeO (**a**)	-84.7	-43.1	-14.2	-82.8
p-Me (**b**)	-85.3	-43.4	-14.1	-83.0
p-H (**c**)	-86.4	-44.0	-13.9	-83.3
p-Cl(**d**)	-88.0	-44.6	-13.5	-83.8
p-CF_3 (**e**)	-89.8	-45.4	-13.2	-84.5
$\mathbf{8}^{+\,e}$	-49.5	-27.2	-24.1	-73.4
$\mathbf{9}^{+\,e}$	-54.1	-30.2	-23.7	-74.6
$\mathbf{10}^{+\,e}$	-73.0	-39.1	-18.2	-79.1
$\mathbf{11}^{+\,e}$	-91.2	-51.9	-24.3	-90.6

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[a] Relative uncertainties were estimated to be smaller than or close to 1.0 kcal/mol in each case. [b] $\mathbf{X}^+$ stood for the analogues of indazolium alkaloids in this work, while $\mathbf{X}^\bullet$ Stood for their neutral radicals. [c] $\Delta H_{H^-A}(\mathbf{X}^+)$ were estimated from eqn 4, 6 and 8, respectively. [d] $\Delta H_{HA}(\mathbf{X}^+)$, $\Delta H_{PA}(\mathbf{X}^\bullet)$ and $\Delta H_{HA}(\mathbf{X}^\bullet)$ were estimated from eqn 9-11, respectively, with a unit of kcal/mol, taking $E(H^{+/0}) = -2.307$ (V *vs* $Fc^{+/0}$), $E(H^{0/-}) = -1.137$V (V *vs* $Fc^{+/0}$) (Fc = ferrocene), and choosing the redox potentials of $\mathbf{X}^+$ and $\mathbf{XH}^{\bullet+}$ measured by OSWV method (Table 1) as $E(\mathbf{X}^{+/0})$ and $E(\mathbf{XH}^{+/0})$, since the values by OSWV were proved to be closer to corresponding standard redox potentials than those by CV.[25] [e] Derived from our previous work.[25]

them are unlikely initiated by hydrogen atom transfer. Notably, the hydrogen atom affinities of $\mathbf{1}^+$-$\mathbf{4}^+$ are much more positive than that of $\mathbf{5c}^+$ by about over 16 kcal/mol, which is probably due to that the delocalization effect by phenyl group at C(3) position greatly stabilizes the yielding radical cation of $\mathbf{5c}^+$ after hydrogen atom transfer, which will be discussed infra.

Electron Affinities of the Analogues of Indazolium Alkaloids (X^+) in Acetonitrile.

The standard reduction potentials of these analogues of indazolium alkaloids were employed as the indicators of their electron affinities. From column 4 in Table 1, it is found that the one-electron reduction potentials of these analogues locate in a scale ranging from -2.173 to -1.001 V (*vs* $Fc^{+/0}$), or an energy scope of ca. 27 kcal/mol. Judging from the values of their standard reduction potentials, $\mathbf{6}^+$, $\mathbf{7}^+$, $\mathbf{10}^+$ and $\mathbf{11}^+$ should belong to the category of good electrophiles to electron, while $\mathbf{1}^+$, $\mathbf{2}^+$, $\mathbf{3}^+$, $\mathbf{4}^+$, $\mathbf{5}^+$, $\mathbf{8}$ and $\mathbf{9}^+$ are due to weak electrophiles to electron. In living bodies, it should be thermodynamically unfavorable for these alkaloid analogues to be reduced by electron from naturally existing reducing reagents like NADH or vitamin C, since the standard oxidation potentials of both NADH (0.280 V *vs* $Fc^{+/0}$ in neutral water) and of vitamin C (-0.276 V *vs* $Fc^{+/0}$ in neutral water) are more positive than those of them by over 1.281 V (equivalent to 29.5 kcal/mol) and 0.725 V (equivalent to 16.7 kcal/mol), respectively.[19]

As illustrated in Figure 6, the standard reduction potentials of these analogues are ranked in an increasing order: $\mathbf{8}^+ < \mathbf{9}^+ < \mathbf{4}^+ < \mathbf{2}^+ < \mathbf{1}^+ < \mathbf{3}^+ < \mathbf{5c}^+ < \mathbf{10}^+ < \mathbf{6}^+ < \mathbf{11}^+ < \mathbf{7c}^+$, corresponding to an increasing order of electron-obtaining abilities: $\mathbf{8}^+ < \mathbf{9}^+ < \mathbf{4}^+ < \mathbf{2}^+ < \mathbf{1}^+ < \mathbf{3}^+ < \mathbf{5c}^+ < \mathbf{10}^+ < \mathbf{6}^+ < \mathbf{11}^+ < \mathbf{7c}^+$. Considering the effects of structural isomerizations, the $E(\mathbf{X}^{+/0})$ of $\mathbf{1}^+$, $\mathbf{5c}^+$, $\mathbf{6}^+$ and $\mathbf{7c}^+$ are more positive than their corresponding isomers $\mathbf{8}^+$, $\mathbf{9}^+$, $\mathbf{10}^+$ and $\mathbf{11}^+$ by more than 0.1 V (equivalent to 2.3 kcal/mol), implying the electrophilicities of the former ones to electron are stronger than their isomers. When examining the effect of the variation of heteroatoms on the reduction potentials of these alkaloid analogues, it is found that altering heteroatoms could result in a change up to 0.9 V (equivalent to 20.7 kcal/mol), with a similar trend of N < S < O corresponding to the potentials.

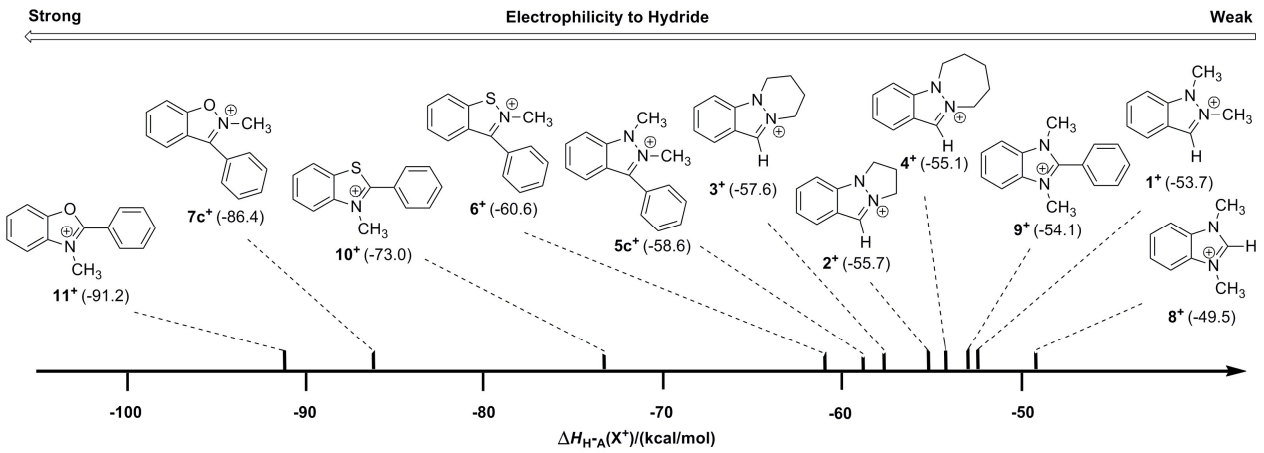


Figure 4. Comparison of the hydride affinities of the analogues of indazolium alkaloids ($\mathbf{X}^+$).

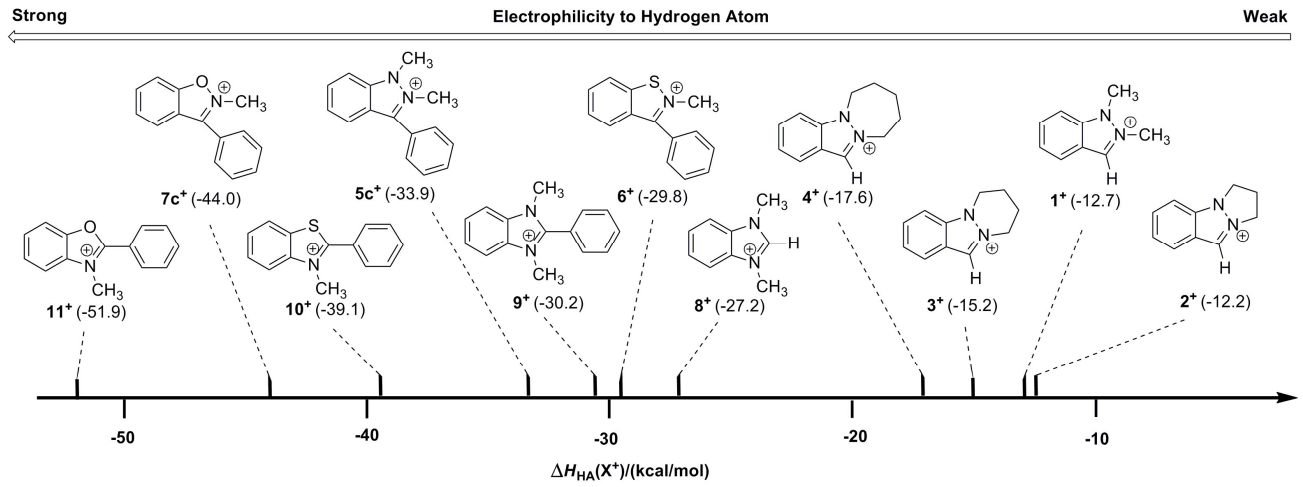


Figure 5. Comparison of the hydrogen atom affinities of the analogues of indazolium alkaloids ($\mathbf{X}^+$).

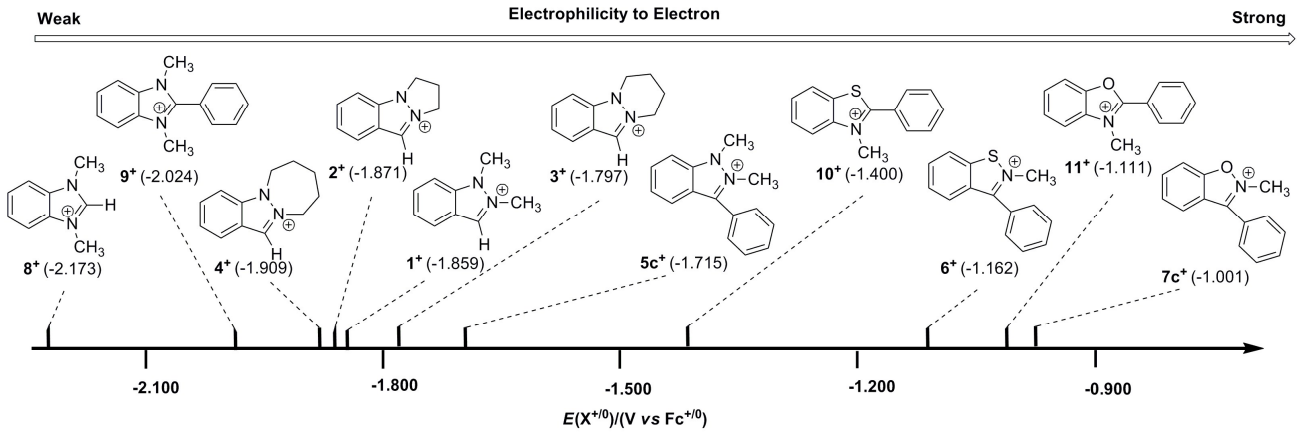


Figure 6. Comparison of the one electron reduction potentials of the analogues of indazolium alkaloids ($\mathbf{X}^+$).

Hydrogen Atom Affinities and Proton Affinities of Neutral Radical Intermediates ($\mathbf{X}^\bullet$) of the Analogues of Indazolium Alkaloids in Acetonitrile.

As stated above and shown in Scheme 2, if hydride transfer to these analogues of indazolium alkaloids is initiated by single electron transfer, neutral radicals intermediates ($\mathbf{X}^\bullet$) could formed as transient species, which could either grab a hydrogen atom to give their conjugated amines (**XH**), or accept a proton to generate corresponding radical cations of them ($\mathbf{XH}^{\bullet+}$). Consequently, the thermodynamic driving forces for the two possible elementary steps of hydride transfer, namely hydrogen atom affinities [$\Delta H_{HA}(\mathbf{X}^\bullet)$] for $\mathbf{X}^\bullet$ to obtain hydrogen atom and proton affinities [$\Delta H_{PA}(\mathbf{X}^\bullet)$] for $\mathbf{X}^\bullet$ to obtain proton in acetonitrile at 298 K, should be vital parameters not only in diagnosing the chemical activities of $\mathbf{X}^\bullet$ but in predicting whether hydrogen atom transfer or proton transfer to $\mathbf{X}^\bullet$ is thermodynamically more favorable. From column 4 and 5 in Table 2, it is found that, $\Delta H_{HA}(\mathbf{X}^\bullet)$ locates in a scale from -61.2 to -84.5 kcal/mol, while $\Delta H_{PA}(\mathbf{X}^\bullet)$ ranges from -2.1 to -20.5 kcal/mol. For each neutral radical, $\Delta H_{HA}(\mathbf{X}^\bullet)$ is much more negative than $\Delta H_{PA}(\mathbf{X}^\bullet)$ by at least 49 kcal/mol, implying that the attack to $\mathbf{X}^\bullet$ by hydrogen atom should be thermodynamically much more favorable than that by proton, that is, once the hydride transfer to an alkaloid analogue is initiated by the single electron transfer, the following step should be a hydrogen atom transfer rather than a proton transfer. When $\Delta H_{HA}(\mathbf{X}^\bullet)$ is compared with corresponding $\Delta H_{HA}(\mathbf{X}^+)$ (Table 2, column 5 *vs* column 3), it is found that $\Delta H_{HA}(\mathbf{X}^\bullet)$ is much more negative than $\Delta H_{HA}(\mathbf{X}^+)$ by up to 71 kcal/mol, which means the antioxidant abilities of these analogues might be greatly strengthened after obtaining one electron. As a result, after accepting electron, the analogues $\mathbf{7}^+$ and $\mathbf{11}^+$ will be scavenged by antioxidant reagents like Vitamin E, BHT, and phenothiazine. By comparing $\Delta H_{H\text{-}A}(\mathbf{X}^+)$ with corresponding $\Delta H_{PA}(\mathbf{X}^\bullet)$, it is evident that electron transfer could convert these analogues from Lewis acids to bases. Notably, $\Delta H_{PA}(\mathbf{5c}^\bullet)$ is smaller than $\Delta H_{PA}(\mathbf{1}^\bullet)$ by almost 20 kcal/mol, which is close to the difference (ca. 21 kcal/mol) between $\Delta H_{HA}(\mathbf{5c}^+)$ and $\Delta H_{HA}(\mathbf{1}^+)$, again this might attribute to that the yielding $\mathbf{5cH}^{\bullet+}$ is more stable than $\mathbf{1H}^{\bullet+}$, which will subject to further evidences infra. For an intuitive comparison, the hydrogen or proton obtaining abilities of $\mathbf{X}^\bullet$ were ranged in order and shown in Figure 7 and Figure 8. Apparently, both the hydrogen atom accepting abilities and the proton accepting abilities of the neutral radicals of these alkaloid analogues could be regulated in an energy scope of over 20 kcal/mol through structural variations.

Electron Affinities of Radical Cation Intermediates ($XH^{\bullet+}$) of the Analogues of Indazolium Alkaloids in Acetonitrile.

Electron affinities of radical cation intermediates ($\mathbf{XH}^{\bullet+}$) of these alkaloid analogues were defined as the one electron reduction potentials of $\mathbf{XH}^{\bullet+}$. From column 6 of Table 1, it is found that reduction potentials of $\mathbf{XH}^{\bullet+}$ range from 0.787 V to -0.179 V (*vs* $Fc^{+/0}$), and the one electron reduction potential of $\mathbf{XH}^{\bullet+}$ decreases in an order: $\mathbf{7cH}^{\bullet+} > \mathbf{2H}^{\bullet+} > \mathbf{3H}^{\bullet+} > \mathbf{1H}^{\bullet+} > \mathbf{11H}^{\bullet+} > \mathbf{4H}^{\bullet+} > \mathbf{10H}^{\bullet+} > \mathbf{6H}^{\bullet+} > \mathbf{5cH}^{\bullet+} > \mathbf{9H}^{\bullet+} > \mathbf{8H}^{\bullet+}$, corresponding to an decreasing order of the electrophilicity of $\mathbf{XH}^{\bullet+}$ to electron (Figure 9). For an intuitive comparison, the one electron reduction potentials of $\mathbf{XH}^{\bullet+}$ were ranged in order corresponding to their structures, as illustrated in Figure 9. From the perspective of effects of structural variations, it is evident that $E(\mathbf{XH}^{+/0})$ could be adjusted within a scale approximately to 1.0 V (equivalent to 23.06 kcal/mol) *via* isomerizations, variations of heteroatomsor remote substituents. Notably, the one electron reduction potential of $\mathbf{1H}^{\bullet+}$ (0.643 V) is much larger than that of $\mathbf{5cH}^{\bullet+}$ (-0.065 V), implying a much smaller stability of $\mathbf{1H}^{\bullet+}$ than $\mathbf{5cH}^{\bullet+}$, which is coincident with our deductions in proceeding paragraphs. For a further verification, cyclic voltametry (CV) technology was employed to test the relative stabilities of radical cations.[36,37] As an example, radical cation $\mathbf{1H}^{\bullet+}$ would generate during the sweeping of oxidant potential of **1H** in anaerobic acetonitrile by CV. If the newly generated transient specie $\mathbf{1H}^{\bullet+}$ is stable within the sweeping time, a reversible CV graph will be obtained and vice versa, so CV might be utilized to semi quantitatively compare the relative stability of radical cations. To compare the stabilities of $\mathbf{1H}^{\bullet+}$ and $\mathbf{5cH}^{\bullet+}$, CV sweepings were carried out for **1H** and **5cH**. As illustrated in Figure 10, the CV graphs of **1H** are found to be irreversible even at a high sweeping rate of 1 V/s, indicating $\mathbf{1H}^{\bullet+}$ could not stably exist in solution during the sweeping time. In contrast, the CV graphs for **5cH** are found to be nearly reversible at a wide range of sweeping rate, from 0.1 Volt per second (0.1V/s) to 1 V/s, indicating $\mathbf{5cH}^{\bullet+}$ could exist in solution for a while. As a result, it may conclude that $\mathbf{5cH}^{\bullet+}$ is more stable than $\mathbf{1H}^{\bullet+}$, which could respond to the differences of other thermodynamic parameters mentioned hereinbefore.

Effects of Remote Substituents on the Enthalpy Changes and Reduction Potentials.

From Table1 and Table 2, it is clear that all the enthalpy change values of $\mathbf{X}^+$ and $\mathbf{X}^{\bullet}$ and the reduction potentials of $\mathbf{X}^+$ and $\mathbf{XH}^{\bullet+}$ are strongly dependent on the nature of substituents on phenyl group at the position of C(3) in $\mathbf{5}^+$ and $\mathbf{7}^+$, as well as their reaction intermediates. To elucidate the relations of the substituents with the enthalpy changes and the reduction potentials, the effects of remote substituents at *para-* or *meta-*position are examined on the $\Delta H_{H\text{-}A}(\mathbf{X}^+)$ and $\Delta H_{HA}(\mathbf{X}^+)$, on

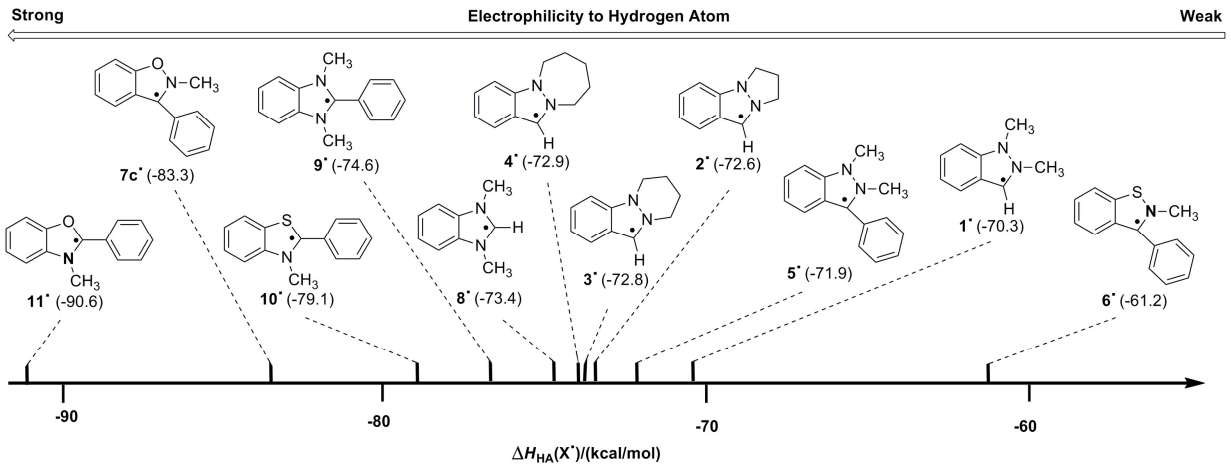


Figure 7. Comparison of the hydrogen atom affinities of neutral radical intermediates of indazolium alkaloid analogues ($\mathbf{X}^{\bullet}$).

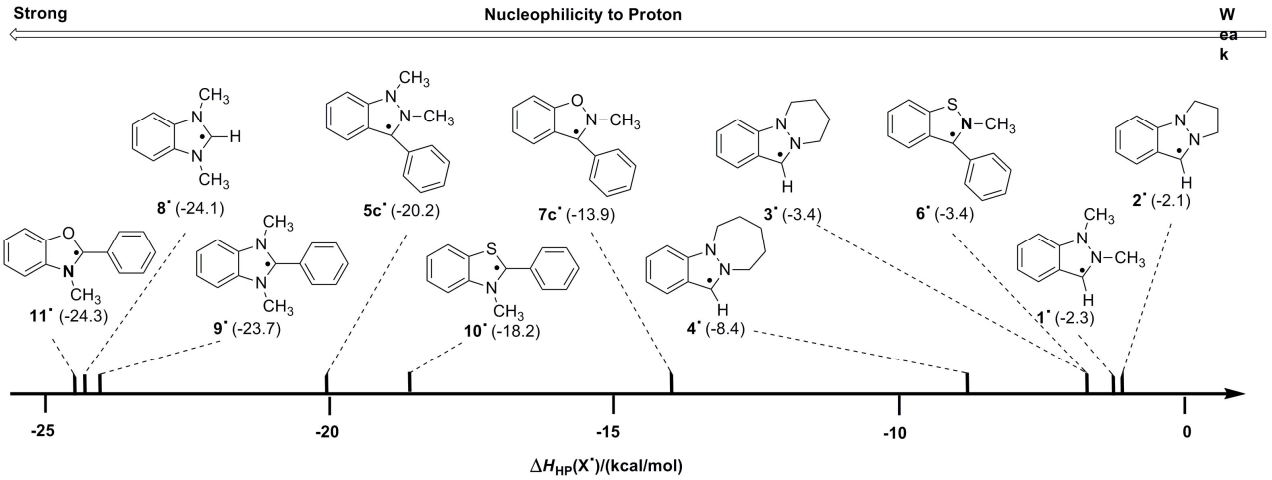


Figure 8. Comparison of the proton affinities of the neutral radical intermediates of indazolium alkaloid analogues (**X**$^{\bullet}$).

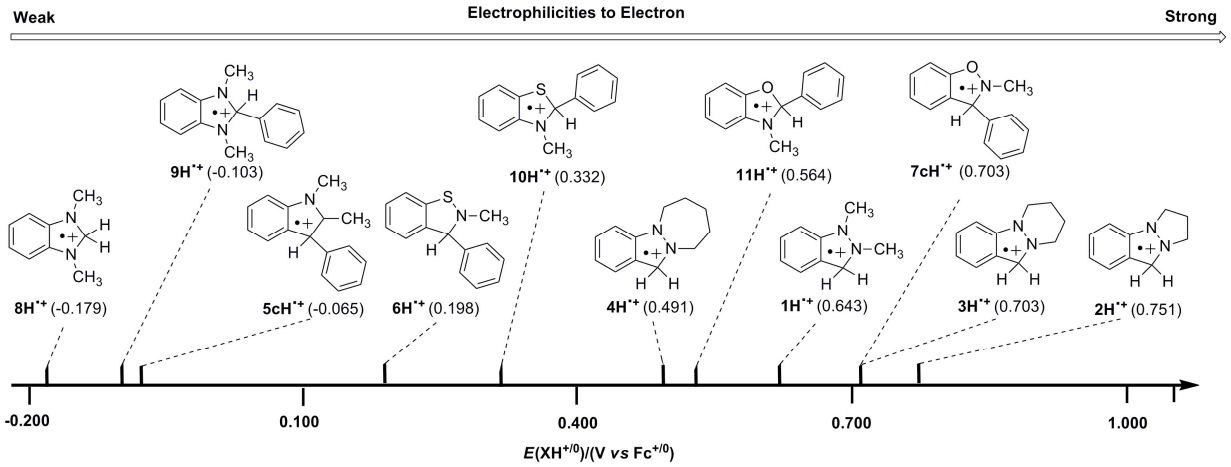


Figure 9. Comparison of one electron reduction potential of the radical cation intermediates of indazolium alkaloid analogues (**XH**$^{\bullet+}$).

the ΔH_{PA}(**X**$^{\bullet}$) and ΔH_{HA}(**X**$^{\bullet}$), as well as on the E(**X**$^{+/0}$) and E(**XH**$^{+/0}$), respectively; The results in Figure 11 and Figure S1-S5 (Supporting Information) show that all these values are linearly dependent on the Hammett substituent parameters σ_p or σ_m with very good correlation coefficients, implying that Hammett linear free energy relationship[38,39] hold in these chemical and electrochemical processes. From the slopes and the intercepts of these linear correlations, twelve mathematical formulas are derived [eqn (12)-(23)]. Evidently, for any *para*- or *meta*-position on phenyl ring at the position of C(3), it was not difficult to estimate the values of $\Delta H_{H\text{-}A}$(**X**$^{+}$), ΔH_{HA}(**X**$^{+}$), ΔH_{PA}(**X**$^{\bullet}$), ΔH_{HA}(**X**$^{\bullet}$), E(**X**$^{+/0}$) as well as E(**XH**$^{+/0}$) according to eqn (12)-(23), as long as the corresponding Hammett substituent parameters (σ_p or σ_m) are available and the standard deviation of these estimations is less than 0.25 kcal/mol/mol for enthalpies or less than 25 mV for reduction potentials. Also, these formulas might quantitatively guide us to select species of the alkaloid analogues with suitable substituents for special use.

Diagnosing Possible Reactions between Indazolium Alkaloids with NADH *via* Chemical Mimics.

As mentioned in the part of introduction, several indazolium alkaloids isolated from the seeds of *Nigella sativa* (Scheme 1) showed good effects against diabetes, but the molecular mechanism of their antidiabetic effects has not yet been well elaborated to date. A latest study showed that their antidiabetic effects originate from the activation of AMP-activated protein kinase (AMPK) by them, but deeper mechanism about how these alkaloids activate AMPK remains uninvestigated. It was revealed that the activity of AMPK could be regulated by the redox potential of NADH/NAD$^+$, which is directly related to the ratio of NADH/NAD$^+$, and it was further clarified that AMPK is activated by NAD$^+$ in a dose-dependent manner, whereas AMPK is inhibited by NADH. We then suspected that the activation of AMPK by these alkaloids might be due to these alkaloids would act on (consume) NADH through chemical reactions. Possible reactions between these alkaloids and NADH should include hydride transfer, hydrogen atom transfer, and electron transfer, which could be assigned to the possible elemental steps of hydride transfer reactions between these alkaloids and NADH (Scheme 3). Since thermodynamics could offer us intrinsic criterions in diagnosing the possibilities

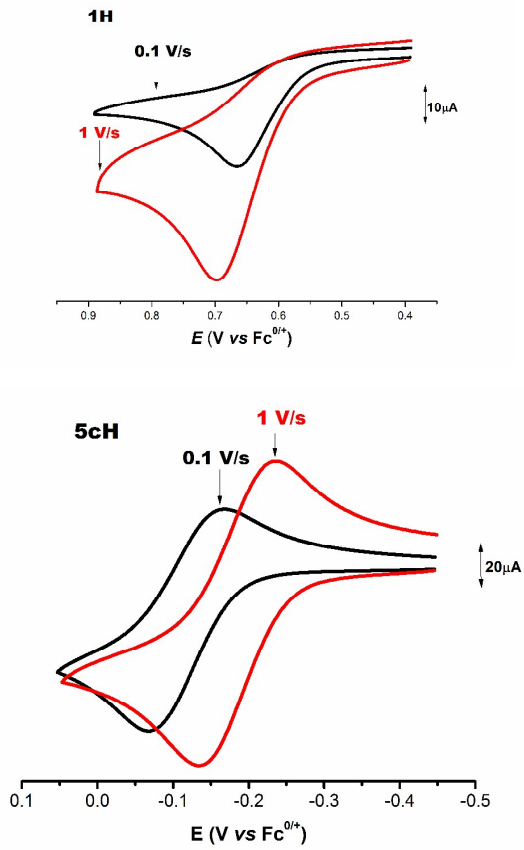


Figure 10. CV graphs at different sweeping rates in anaerobic MeCN containing 0.1 M n-Bu_4NBF_4 as supporting electrolyte. The upper graph is the CV graph of **1H**, while the lower one is the CV graph of **5cH**.

of reactions, it might be efficient and comprehensive to elucidate the possibilities of all the elemental steps of hydride transfer between these alkaloids and NADH from the perspective of thermodynamics. To tackle this problem, a mimic reaction of hydride transfer is employed, where **BPH** and $\mathbf{3}^+$ are chosen as the model compounds of NADH and of indazolium alkaloids, respectively.[40] As verified by our previous jobs, "Molecular ID Card" might be used as a unique thermodynamic tool to efficiently diagnose the possibilities of elemental steps of hydride transfer between $\mathbf{3}^+$ (hydride acceptor) and **BPH** (hydride donor), provided that the "Molecular ID Cards" of them are available. Fortunately, the "Molecular ID Card" for $\mathbf{3}^+$ to accept hydride could be obtained by this work, whist the "Molecular ID Card" for **BPH** to release hydride could be derived based on our previous work.[41] According to Hess's Law, it is easily accessible to the thermodynamic driving forces of all the possible elemental steps of hydride transfer from **BPH** to $\mathbf{3}^+$, as is shown in Scheme 6. Based on the driving forces of six possible elemental steps of hydride transfer from **BPH** to $\mathbf{3}^+$, we could make predictions as follows: (i) hydride transfer (step c) from **BPH** to $\mathbf{3}^+$ should be spontaneous, since this reaction is thermodynamically favorable by 4.6 kcal/mol, while both hydrogen atom transfer (step b) and electron transfer (step a) from **BPH** to $\mathbf{3}^+$ are thermodynamically forbidden by more than 41.8 kcal/mol. (ii) Even if hydrogen atom transfer or electron transfer is triggered, the yielding transient ion pairs of $\mathbf{BP}^{\bullet}$ and $\mathbf{3H}^{\bullet+}$ or $\mathbf{BPH}^{\bullet+}$ and $\mathbf{3H}^{\bullet}$, are not likely to exist long in solution, and would react with each other following possible routes (step b-step f, step a-step e, or step a-step d-step f) of stepwise hydride transfer, until they give the same pair of stable products of $\mathbf{BP}^+$ and **3H** as produced by the concerted hydride transfer (step c). Therefore, it might conclude that, when indazolium alkaloids encounter with NADH *in vivo*, hydride transfer reaction is likely to happen, rather than hydrogen atom transfer or electron transfer reaction, to give corresponding indazolines and $\mathbf{NAD}^+$ as products, and the way of hydride transfer tends to be concerted rather than stepwise.

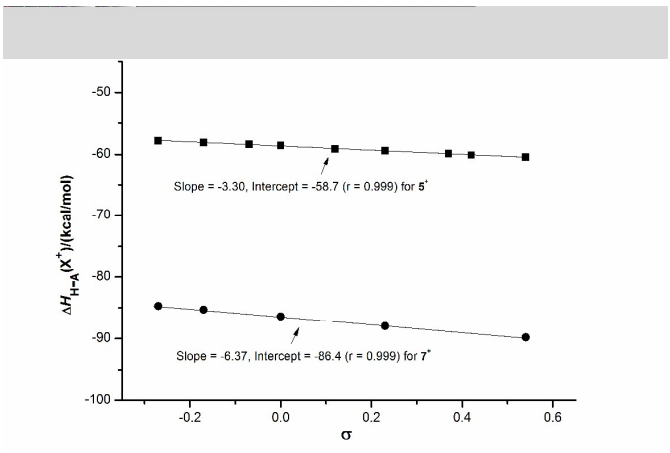


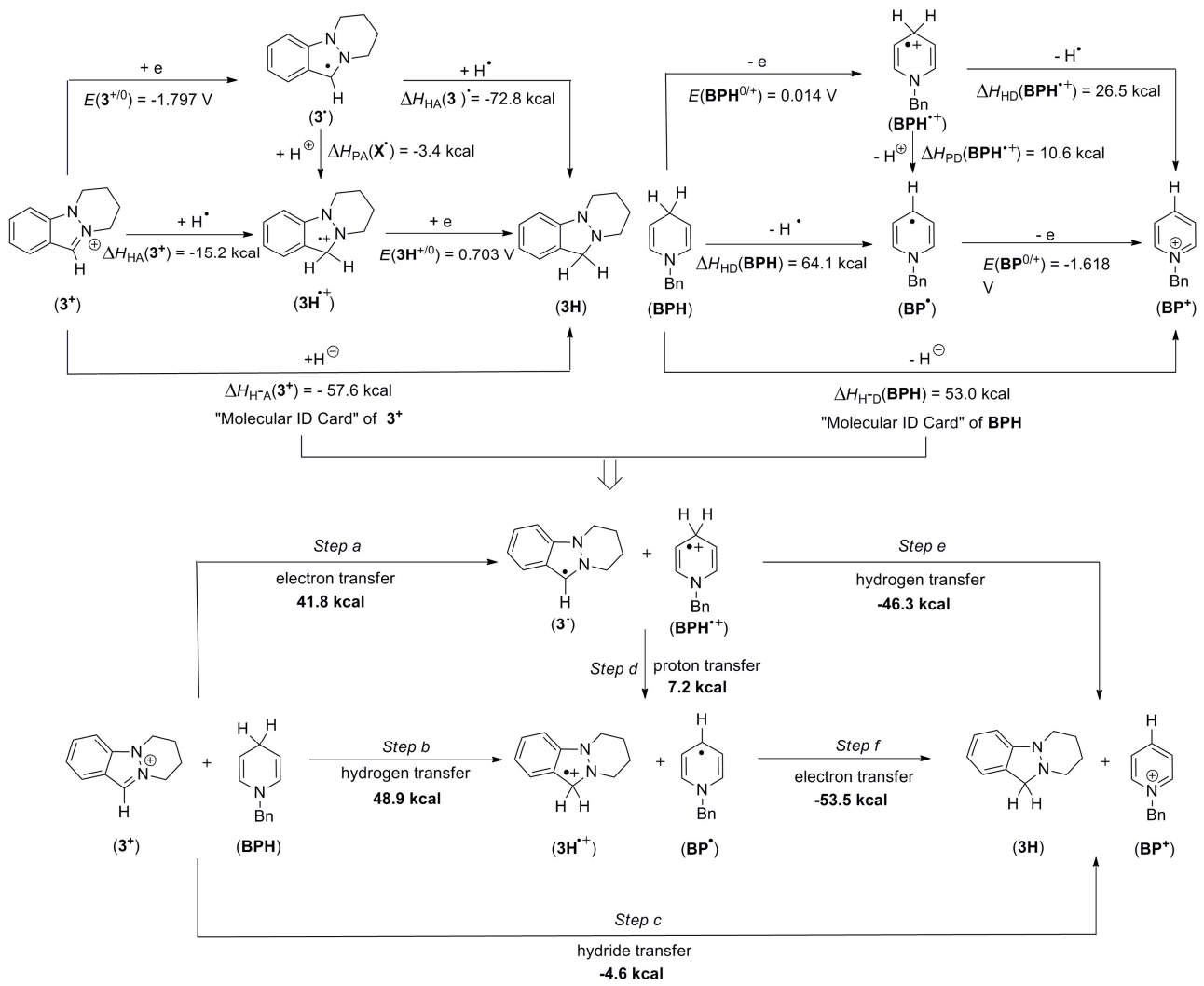
Figure 11. Plot of $\Delta H_{H\text{-}A}(\mathbf{X}^+)$ against Hammett substituent parameter (σ).

$\Delta H_{H\text{-}A}(\mathbf{X}^+) = -3.30\sigma - 58.7$ for $\mathbf{5}^+$ (12)
$\Delta H_{H\text{-}A}(\mathbf{X}^+) = -6.37\sigma - 86.4$ for $\mathbf{7}^+$ (13)
$\Delta H_{HA}(\mathbf{X}^+) = -1.29\sigma - 33.9$ for $\mathbf{5}^+$ (14)
$\Delta H_{HA}(\mathbf{X}^+) = -2.84\sigma - 43.9$ for $\mathbf{7}^+$ (15)
$\Delta H_{HA}(\mathbf{X}^{\bullet}) = 0.598\sigma - 72.0$ for $\mathbf{5}^{\bullet}$ (16)
$\Delta H_{HA}(\mathbf{X}^{\bullet}) = -2.10\sigma - 83.3$ for $\mathbf{7}^{\bullet}$ (17)
$\Delta H_{PA}(\mathbf{X}^{\bullet}) = 2.60\sigma - 20.3$ for $\mathbf{5}^{\bullet}$ (18)
$\Delta H_{PA}(\mathbf{X}^{\bullet}) = 1.28\sigma - 13.9$ for $\mathbf{7}^{\bullet}$ (19)
$E(\mathbf{X}^{+/0}) = 0.1681\sigma - 1.7142$ for $\mathbf{5}^+$ (20)
$E(\mathbf{X}^{+/0}) = 0.1821\sigma - 1.0030$ for $\mathbf{7}^+$ (21)
$E(\mathbf{XH}^{+/0}) = 0.0866\sigma - 0.0654$ for $\mathbf{5H}^{\bullet+}$ (22)
$E(\mathbf{XH}^{+/0}) = 0.1511\sigma + 0.7058$ for $\mathbf{7H}^{\bullet+}$ (23)

On the basis of the information disclosed above, we proposed a molecular mechanism to understand the deeper molecular mechanism of the antidiabetic effects of indazolium alkaloids as follows: when indazolium alkaloids encounter with NADH *in vivo*, indazolium alkaloids would oxidize NADH to NAD^+ through hydride transfer reactions, leading to a lower dose of NADH and a higher dose of NAD^+, both of which would contribute to the activation of AMPK, followed by an increasing consumption of glucose to cure hyperglycaemia. Additionally, since the driving forces of hydride transfer reactions between these alkaloids and NADH are not far from zero, these reactions should be reversible, i.e., their adjustments to the dose of NADH and NAD^+ and then to the activities of AMPK should not be violent, rendering their regulations of the metabolism of glucose be mild and not likely to slop over the safe level to avoid the side effect of hypoglycaemia.

If our proposal about the deeper molecular mechanism of antidiabetic effects of indazolium alkaloids is tenable, it should also be of importance for us to examine potential impacts of the structures of indazolium alkaloids on their antidiabetic effects. As illustrated in the Scheme 1, the most eye-catching feature about the structures of indazolium alkaloids is that they all bear a six membered nonaromatic ring bridged to the core of indazoles. Pharmaceutical chemists might ask whether the existence of the bridge ring and its size would impact on the antidiabetic effects of alkaloids by affecting hydride transfer reactions between these alkaloids and NADH. To address this issue, we examined the driving force of hydride transfer from **BPH** to $\mathbf{1}^+$-$\mathbf{4}^+$, where $\mathbf{1}^+$ was chosen as the model compound of alkaloids whose structure bears no bridge ring but two methyl substituents instead, and $\mathbf{2}^+$, $\mathbf{3}^+$ and $\mathbf{4}^+$ were chosen as the model compounds structurally bearing a five, six, and seven membered bridge ring, respectively. The thermodynamic driving forces of hydride transfer from **BPH** to these model compounds were calculated and listed in Table 3. As shown in Table 3, the driving forces of hydride transfer from **BPH** to $\mathbf{1}^+$ is smaller than that from **BPH** to $\mathbf{2}^+$, $\mathbf{3}^+$ or $\mathbf{4}^+$ by at least 2 kcal/mol, implying that the bridge ring in the structures of indazolium alkaloids would make hydride transfer from NADH to the alkaloids more spontaneous. In addition, when the enthalpy change of $\mathbf{3}^+$ to accept hydride from **BPH** was compared with that of $\mathbf{2}^+$ and of $\mathbf{4}^+$, it was found the enthalpy change decreases in an order: $\mathbf{4}^+$ (-2.1 kcal/mol) > $\mathbf{2}^+$ (-2.7 kcal/mol) > $\mathbf{3}^+$ (-4.6 kcal/mol), corresponding to an increasing driving force of hydride transfer with the change of the size of bridge ring as follows: seven membered < five membered < six membered, suggesting that the natural preference of the indazolium alkaloids to bear a six membered bridge ring happens to facilitate hydride transfer from NADH to them, which might make a positive contribution to the antidiabetic effects of these alkaloids *via* a superior activation of AMPK. Besides, when examining the substituents at C(11) position in the structures of these alkaloids, it was found that either a phenyl group or H might occupy at C(11) position of these

natural alkaloids (Scheme 1). Which substituent might be in favor of the antidiabetic effects of indazolium alkaloids? With **5c**$^+$ and **1**$^+$ as corresponding model compounds, it is demonstrated that the hydride transfer from **BPH** to **5c**$^+$ is thermodynamically more favorable than that to **1**$^+$ by almost 5 kcal/mol (Table 3), implying that those alkaloids structurally bearing a phenyl group at C(11) position are more



Scheme 6. Possible Elementary Steps of Hydride Transfer from **BPH** to **3**$^+$ and Thermodynamic Driving Forces of Each Step Derived from The "Molecular ID Cards" of **BPH** and **3**$^+$.

Table 3. Thermodynamic Driving Forces (ΔH_r(H$^-$T)) of Hydride Transfer from the Model of NADH (**BPH**) to the Models of Indazolium Alkaloids (**1**$^+$-**5c**$^+$) in Acetonitrile

Models of Alkaloids	**1**$^+$	**2**$^+$	**3**$^+$	**4**$^+$	**5c**$^+$
ΔH_r(H$^-$T) / (kcal/mol)	-0.7	-2.7	-4.6	-2.1	-5.6

ready to grab hydride from NADH and might show better antidiabetic activities than those bearing H at C(11) position, which might explain why the antidiabetic activities of alkaloids C and D were disclosed to be stronger than those of alkaloids A and B in Scheme 1.[5] Obviously, these thermodynamic implications might shed light on screening suitable indazolium alkaloids and their analogues for antidiabetic use.

Conclusions

In this study, a series of analogues of indazolium alkaloids (**X**$^+$) were designed and synthesized. The thermodynamic driving forces of 6 possible elementary steps for **X**$^+$ to obtain hydride in acetonitrile were determined. Based on these parameters, we diagnosed possible reactions between indazolium alkaloids and NADH, and proposed a molecular mechanism to understand the root of antidiabetic effects of these alkaloids. We further evaluated potential structural impacts on the antidiabetic effects of indazolium alkaloids. After detailed discussions, we arrived at conclusions as follows:

(1) These analogues of indazolium alkaloids are weak to strong electrophiles to hydride. **1**$^+$-**5**$^+$, **8**$^+$, **9**$^+$ and **6**$^+$ belong to weak electrophiles to hydride, and when reducing them to conjugated amines, some strong inorganic hydrides like boron hydrides or aluminum hydrides are recommended; **10**$^+$, **7**$^+$, and **11**$^+$ belong to strong electrophiles to hydride, and some organic hydrides like Hantzch ester and dihydrobenzo[*d*]imidazoles are capable to reduce them to their conjugated amines. The cationic

indazolium analogues of alkaloids are predicted to be more ready to obtain hydride than their neutral indazole analogues, mainly due to the contribution of molecular charge.
(2) These analogues are poor electrophiles to hydrogen atom, and are not likely to be reduced even by strong antioxidant reagents such as Vitamin E, commercial available BHT and phenothiazine. Since hydrogen atom affinities of these indazolium analogues are much more positive than their hydride affinities, these analouges are more ready to be reduced by hydride ion than by hydrogen atom, and hydride transfers to them are unlikely initiated by hydrogen atom transfers.
(3) These analogues are weak to good electrophiles to electron. $\mathbf{6}^+$, $\mathbf{7}^+$, $\mathbf{10}^+$ and $\mathbf{11}^+$ should belong to the category of good electrophiles to electron, while $\mathbf{1}^+$, $\mathbf{2}^+$, $\mathbf{3}^+$, $\mathbf{4}^+$, $\mathbf{5}^+$, $\mathbf{8}^+$ and $\mathbf{9}^+$ are due to weak electrophiles to electron. In living bodies, it should be thermodynamically unfavorable for these analogues to be reduced by electron from NADH or vitamin C.
(4) If hydride transfer to these analogues is initiated by single electron transfer, neutral radicals intermediates ($\mathbf{X}^{\bullet}$) could form, which could either grab a hydrogen atom or accept a proton. For each $\mathbf{X}^{\bullet}$, the attack to $\mathbf{X}^{\bullet}$ by hydrogen atom is thermodynamically much more favorable than that by proton, that is, if hydride attack to these indazolium analogues is initiated by single electron transfer, the following step tends to be hydrogen atom transfer rather than proton transfer.
(5) The reduction potentials of the radical cation intermediates of these indazolium analogues cover a scope from 0.787 to -0.179 V, implying these radical cations unstable and easy to accept electron. Electrochemical experiments verified that the stability of $\mathbf{5cH}^{\bullet+}$ is much larger than that of $\mathbf{1H}^{\bullet+}$, which could explain related differences of the thermodynamic parameters.
(6) All the thermodynamic parameters could be adjusted in a flexible scope through variation of the heteroatoms or structural isomerizations. Good Hammett linear free-energy relationship held between these thermodynamic parameters and the natures of remote substituents on phenyl ring at C(3) position, and 12 mathematical formulas were derived. These results might guide us select the analogues with suitable structures or remote substituents for applications.
(7) On the basis of the thermodynamic parameters determined, the oxidation mechanism of NADH coenzyme by indazolium alkaloids *in vivo* were predicted to take place by one-step concerted hydride transfer mechanism; a deeper molecular mechanism about antidiabetic effects of indazolium alkaloids was then proposed as follows: when indazolium alkaloids encounter with NADH *in vivo*, they would oxidize NADH to NAD^+ through hydride transfer reactions, leading to a lower dose of NADH and a higher dose of NAD^+, both of which would activate AMPK and then increase the consumption of glucose to cure hyperglycaemia, and their regulation of the metabolism of glucose should be mild and not likely to slop over the safe level to avoid hypoglycaemia. The natural preference for indazolium alkaloids to bear a six membered bridge ring happens to facilitate hydride transfer from NADH to them, which might make a positive contribution to the antidiabetic effects of these alkaloids; those alkaloids structurally bearing a phenyl group at C(11) position are more ready to grab hydride from NADH and accordingly might show better antidiabetic activities than those bearing H at C(11) position, which might reply why the antidiabetic activities of alkaloids C and D were found to be stronger than those of alkaloids A and B. Obviously, our results might provide vital implications towards the root of antidiabetic activities of indazolium alkaloids and shed light on screening suitable indazolium alkaloids or their analogues for antidiabetic use.

Experimental Section

Materials. All reagents were of commercial quality from freshly opened containers or were purified before use. Reagent grade acetonitrile was refluxed over $KMnO_4$ and K_2CO_3 for several hours and was doubly distilled over P_2O_5 under argon before use. The commercial tetrabutylammonium hexafluoro phosphate (n-Bu_4NPF_6, Aldrich) was recrystallized from CH_2Cl_2/ether and was vacuum-dried at 110 ℃ overnight before the preparation of supporting electrolyte solution. 4-acetamido-2,2,6,6-tetra-methylpiperidine-1-oxo-ammonium perclorate ($TEMPO^+ClO_4^-$) and trityl perclorate ($Ph_3C^+ClO_4^-$) were prepared following operations described in literatures.[42,43]
Syntheses of the Analogues of Indazolium Alkaloids (1^+-7^+). The analogues of indazolium alkaloids 1^+-4^+,[44,45] 5^+,[46] and 6^+-7^+[47] were synthesized according to the literatures, respectively, and the detailed synthetic routes were provided in Supporting Information. The analogues of indazolium alkaloids (1^+-7^+) are all known compounds.
Measurement of Redox Potentials. The electrochemical experiments were carried out by CV or OSWV using a BAS-100B electrochemical apparatus in deaerated acetonitrile under argon atmosphere at 298 K as described previously.[48] 0.1 M n-Bu_4NPF_6 in acetonitrile was employed as the supporting electrolyte. A standard three-electrode cell consists of a glassy carbon disk as work electrode, a platinum wire as a counter electrode, and 0.1 M $AgNO_3$/Ag (in 0.1 M n-Bu_4NPF_6-acetonitrile) as reference electrode. The ferrocenium/ferrocene redox couple (Fc^+/Fc) was taken as the internal standard. The reproducibilities of the potentials were usually ≤ 5 mV for ionic species and ≤ 10 mV mV for neutral species.
Isothermal Titration Calorimetry (ITC). The titration experiments were performed on a CSC4200 isothermal titration calorimeter in acetonitrile at 298 K as described previously.[49] The performance of the calorimeter was checked by measuring the standard heat of neutralization of an aqueous solution of sodium hydroxide with a standard aqueous HCl solution. Data points were collected every 2 s. The heat of reaction was determined following 10 automatic injections from a 250 μL injection syring containing a standard solution (2 mM) into the reaction cell (1.30 mL) containing 1 mL of other concentrated reactant (~12 mM). Injection volumes (10 μL) were delivered in 0.5 s time intervals with 300-450 s between every two injections. The reaction heat was obtained by integration of each peak except for the first one.

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Notes and references

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Electronic Supplementary Information (ESI) available: Detailed synthetic routes and preparation procedures of these analogues of indazolium alkaloids; the plots of thermodynamic parameters $\Delta H_{HA}(\mathbf{X}^+)$, $\Delta H_{HA}(\mathbf{X}^{\bullet})$, $\Delta H_{PA}(\mathbf{X}^{\bullet})$, $E(\mathbf{X}^{+/0})$ and $E(\mathbf{XH}^{+/0})$ against the Hammett substituent parameter σ. See DOI: 10.1039/b000000x/

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