Organic & Biomolecular Chemistry

PAPER

View Article Online
View Journal | View Issue

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

Cite this: *Org. Biomol. Chem.*, 2015, **13**, 11472

Elemental step thermodynamics of various analogues of indazolium alkaloids to obtaining hydride in acetonitrile†

Nan-Ping Lei,*‡ Yan-Hua Fu‡ and Xiao-Qing Zhu*

Received 14th August 2015,
Accepted 24th September 2015

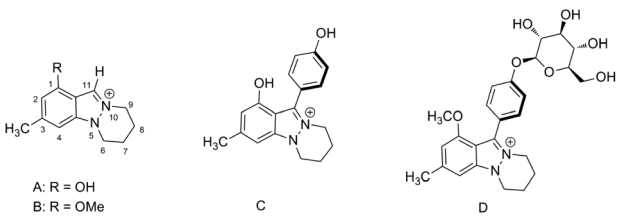
DOI: 10.1039/c5ob01715g

www.rsc.org/obc

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 an 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 the 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* have been 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] 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, the deeper molecular mechanism about how these alkaloids activate AMPK was heretofore not investigated. 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 whether the activation of AMPK by these alkaloids is due to the fact that these indazolium alkaloids would act upon (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 atoms, or electrons to other substrates,[7–12] whilst the cationic motifs of these indazolium alkaloids tend to exhibit their good abilities to obtain hydride, hydrogen atoms, electrons (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 criteria 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 the 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]

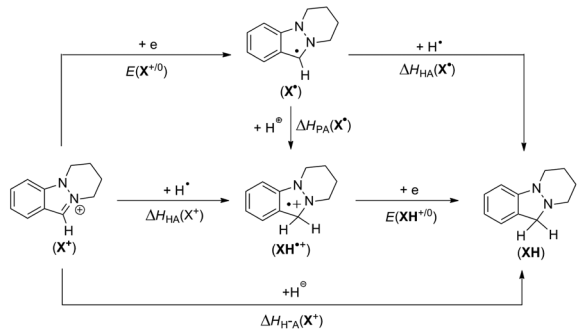
The State Key Laboratory of Elemento-Organic Chemistry, Department of Chemistry, Collaborative Innovation Center of Chemical Science and Engineering, Nankai University, Tianjin 300071, China. E-mail: leinp@mail.nankai.edu.cn, xqzhu@nankai.edu.cn

†Electronic supplementary information (ESI) available: Detailed synthetic routes and preparation procedures of the analogues of indazolium alkaloids; the plots of thermodynamic parameters $\Delta H_{HA}(X^+)$, $\Delta H_{HA}(X^\bullet)$, $\Delta H_{PA}(X^\bullet)$, $E(X^{+/0})$ and $E(XH^{+/0})$ against the Hammett substituent parameter σ. See DOI: 10.1039/c5ob01715g

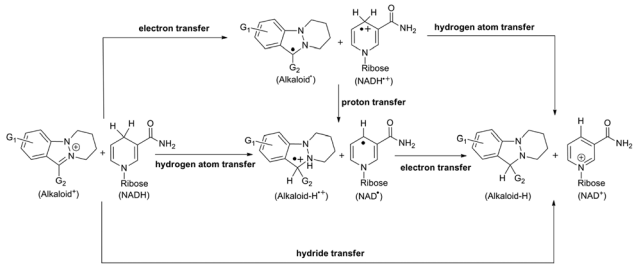
‡These two authors contributed equally to this paper.

This journal is © The Royal Society of Chemistry 2015

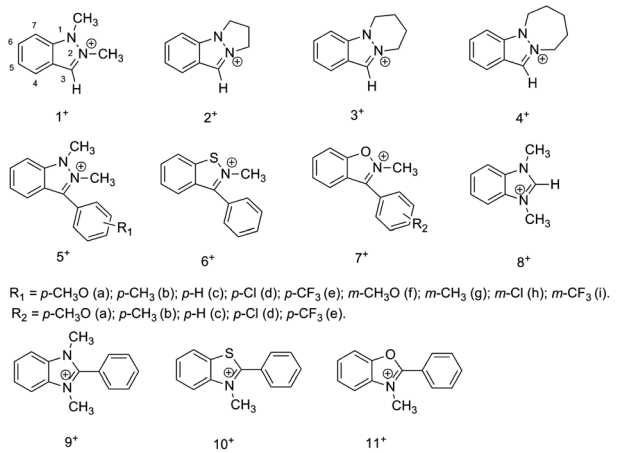
Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.



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.



Scheme 4 Structures of analogues of indazolium alkaloids (X^+) examined in this work (all of them are in the form of perchlorate salts).

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

In addition, since most biochemical processes with the indazolium alkaloids and NADH coenzyme as a hydride acceptor or donor in a 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 (ε = 37.5) is quite close to that of the peptide bond in proteins (ε = 37.0, 37.8 and 38.3 for $HCONMe_2$, $MeCONMe_2$ and *N*,*N*-dimethylbenzamide, respectively).[22]

Results

All the analogues of indazolium alkaloids (X^+) in Scheme 4 were synthesized according to conventional procedures and the target molecules were identified by ^{1}H NMR and MS, and the detailed synthetic routes and procedures are provided in the ESI.† Hydride affinities of these analogues [$\Delta H_{H\text{-}A}(X^+)$] in this work were defined as the enthalpy changes of the reactions of X^+ with a free hydride anion in acetonitrile to form their conjugated amines (**XH**) [eqn (1) and (2)] at 298 K in acetonitrile. The determinations of the enthalpy changes of X^+ to gain hydride in acetonitrile were performed according to the following two strategies: (i) for 1^+–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 perchlorate ($TEMPO^+ClO_4^-$) or trityl perchlorate ($Ph_3C^+ClO_4^-$) in acetonitrile, respectively. In eqn (3)–(8), ΔH_r were the reaction enthalpy changes, which could be determined *via* titration calorimetry (Fig. 1); $\Delta H_{H\text{-}A}(TEMPO^+)$ was the hydride affinity of $TEMPO^+ClO_4^-$, which was determined

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

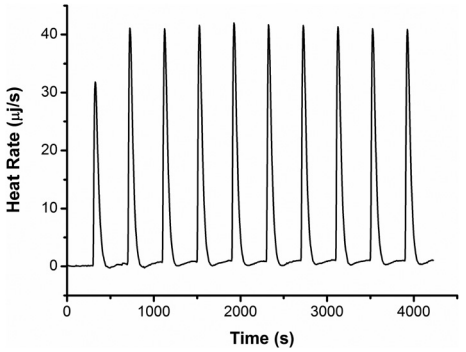


Fig. 1 Isothermal titration calorimetry (ITC) graph of the reaction heat of **1H** with 4-acetamido-2,2,6,6-tetramethylpiperidine-1-oxo-ammonium perchlorate ($TEMPO^+$) in acetonitrile at 298 K. Titration was conducted by adding 10 μL of $TEMPO^+$ (1.0 mM) every 300 s into the acetonitrile solution containing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

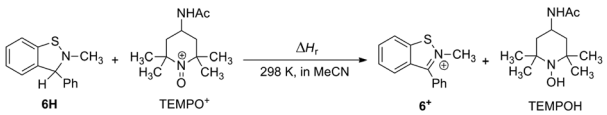
to be −105.6 kcal mol^{-1},[23] and $\Delta H_{H\text{-}A}(Ph_3C^+)$ was the hydride affinity of $Ph_3C^+ClO_4^-$, which was previously determined to be −104.6 kcal mol^{-1}.[24] (ii) For $\mathbf{8^+}$–$\mathbf{11^+}$, since the enthalpy changes [$\Delta H_{H\text{-}D}$ (**XH**)] for their conjugated 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 $\Delta H_{H\text{-}D}$(**XH**).[25] The enthalpy changes of these hydride exchange reactions are listed in Table 1, while the hydride affinities of these alkaloid analogues are summarized in Table 2.

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

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

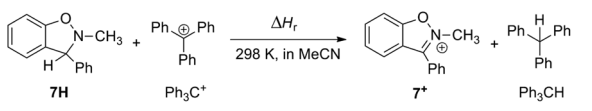
(3)

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



(5)

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



(7)

$$\Delta H_{H\text{-}A}(\mathbf{X}^+) = \Delta H_{H\text{-}A}(Ph_3C^+) - \Delta H_r \quad (8)$$

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

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 analogues ($\mathbf{X}^+$)[a]	ΔH_r[b]	$E(\mathbf{X}^{+/0})$[a,c,d] CV	$E(\mathbf{X}^{+/0})$[a,c,d] OSWV	$E(\mathbf{XH}^{+/0})$[a,c,d] CV	$E(\mathbf{XH}^{+/0})$[a,c,d] 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] $\mathbf{X}^+$ stands for the analogues of indazolium alkaloids in this work, while $\mathbf{X}^\bullet$ stands for their neutral radicals. [b] ΔH_r were the reaction enthalpy changes of eqn (3), (5), and (7) measured by titration calorimetry in acetonitrile at 298 K, respectively. The data, given in kcal mol^{-1}, were average values of at least three independent runs. The reproducibility was estimated to be ±0.5 kcal mol^{-1}. [c] Measured by CV and OSWV methods in acetonitrile at 298 K, the unit in volt *vs.* $Fc^{+/0}$ and reproducible to 5 mV 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]

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

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

The hydrogen atom affinities of $\mathbf{X}^+$ were defined as the enthalpy changes for $\mathbf{X}^+$ to gain a hydrogen atom [$\Delta H_{HA}(\mathbf{X}^+)$] in acetonitrile at 298 K; the hydrogen atom affinities and proton affinities of the neutral radical intermediates ($\mathbf{X}^\bullet$) were also defined as the enthalpy changes for $\mathbf{X}^\bullet$ to gain a hydrogen atom [$\Delta H_{HA}(\mathbf{X}^\bullet)$] and to gain a 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, eqn (9)–(11) could be derived according to Hess's

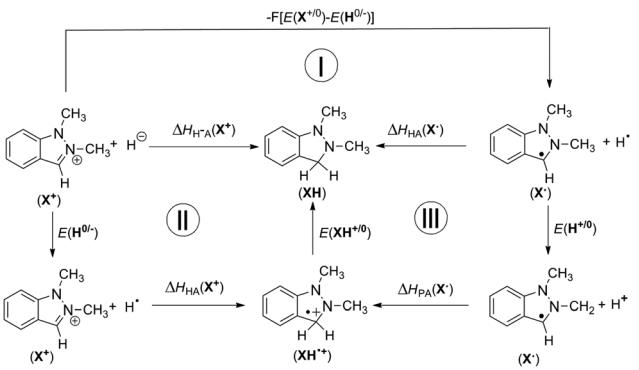
 This journal is © The Royal Society of Chemistry 2015

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

Table 2 Enthalpy changes of X^+ to accept hydride and to accept hydrogen atom, as well as the enthalpy changes of $X^{\bullet}$ to accept proton and to accept hydrogen atom in acetonitrile (kcal mol^{-1})[a,b]

Indazolium analogues (X^+)	$\Delta H_{H\text{-}A}(X^+)$[c]	$\Delta H_{HA}(X^+)$[d]	$\Delta H_{PA}(X^{\bullet})$[d]	$\Delta H_{HA}(X^{\bullet})$[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

[a] Relative uncertainties were estimated to be smaller than or close to 1.0 kcal mol^{-1} in each case. [b] X^+ stands for the analogues of indazolium alkaloids in this work, while $X^{\bullet}$ stands for their neutral radicals. [c] $\Delta H_{H\text{-}A}(X^+)$ were estimated from eqn (4), (6) and (8), respectively. [d] $\Delta H_{HA}(X^+)$, $\Delta H_{PA}(X^{\bullet})$ and $\Delta H_{HA}(X^{\bullet})$ were estimated from eqn (9)–(11), respectively, with a unit of kcal mol^{-1}, 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 X^+ and $XH^{\bullet+}$ measured by the OSWV method (Table 1) as $E(X^{+/0})$ and $E(XH^{+/0})$, 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]



Scheme 5 Three thermodynamic cycles constructed on the basis of the reactions of the analogues of indazolium alkaloids (X^+) with hydride anion (H^-).

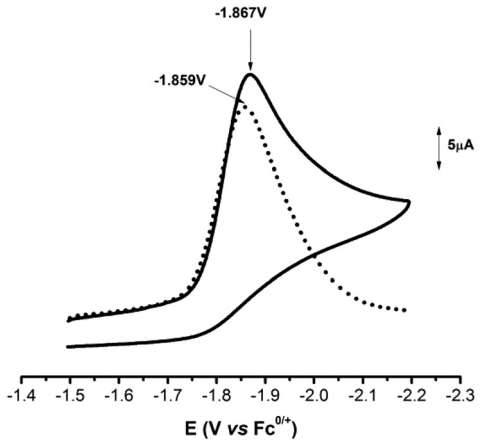


Fig. 2 Cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) of $\mathbf{1}^+$ in deaerated acetonitrile containing 0.1 M *n*-Bu_4NBF_4 as the supporting electrolyte. The full line: CV graph (sweep rate = 0.1 V s^{-1}), the dashed line: OSWV graph.

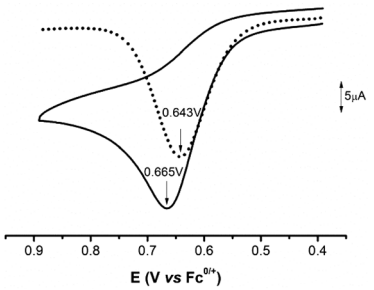


Fig. 3 Cyclic voltammetry (CV) and Osteryoung square wave voltammetry (OSWV) of **1H** in deaerated acetonitrile containing 0.1 M *n*-Bu_4NBF_4 as the supporting electrolyte. The full line: CV graph (sweep rate = 0.1 V s^{-1}), the dashed line: OSWV graph.

law, where $E(X^{+/0})$, $E(XH^{+/0})$, $E(H^{0/-})$ and $E(H^{+/0})$ are the standard redox potentials of X^+, $XH^{\bullet+}$, H^- and H^+, respectively. Since $E(H^{0/-})$ and $E(H^{+/0})$ could be obtained from the literature,[26] $E(X^{+/0})$, $E(XH^{+/0})$ could be determined through electrochemical methods (Table 1, Fig. 2 and 3), and $\Delta H_{H\text{-}A}(X^+)$ could be obtained from the above studies, and the values of $\Delta H_{HA}(X^+)$, $\Delta H_{HA}(X^{\bullet})$ and $\Delta H_{PA}(X^{\bullet})$ could easily be calculated *via* eqn (9)–(11). These parameters, together with $\Delta H_{H\text{-}A}(X^+)$, are summarized in Table 2.[27,28]

Discussion

Hydride affinities of the analogues of indazolium alkaloids (X^+) in acetonitrile

In this study, the hydride affinities of X^+ were defined as the standard state enthalpy changes for 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 $\mathbf{1}^+$–$\mathbf{11}^+$ scale almost 41 kcal mol^{-1}, ranging from −49.5 to −90.6 kcal mol^{-1},

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

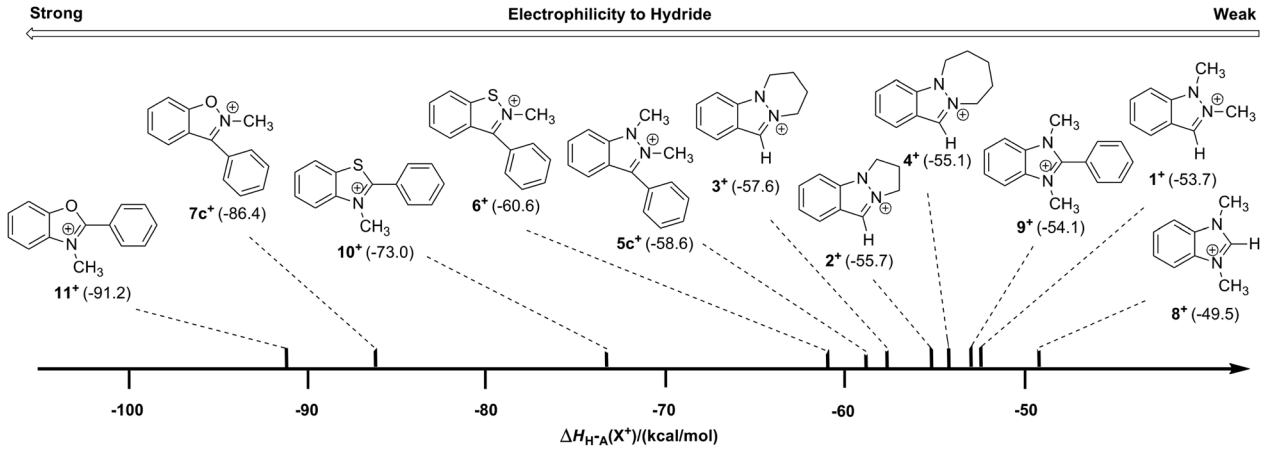


Fig. 4 Comparison of the hydride affinities of the analogues of indazolium alkaloids (X^+).

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 the 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 the 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 Fig. 4. To be specific, indazolium ions ($\mathbf{1}^+$–$\mathbf{5}^+$), benzo[*d*]imidazolium ions ($\mathbf{8}^+$, $\mathbf{9}^+$), and benzo[*d*]isothiazolium ions ($\mathbf{6}^+$) 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*]thiazolium ions ($\mathbf{10}^+$), benzo[*d*]isoxazolium ions ($\mathbf{7}^+$) and benzo[*d*]oxazolium ions ($\mathbf{11}^+$) 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 Hantzsch esters are capable of reducing them to their conjugated amines.[25,30] Structurally, $\mathbf{5c}^+$ shows a slightly stronger electrophilicity to the hydride than its isomeric $\mathbf{9}^+$, which is consistent with the fact that $\mathbf{5c}^+$ was able to slowly grab the hydride from the conjugated amine of $\mathbf{9}^+$ under harsh conditions, illustrated as evidence for 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^{-1}, such as for $\mathbf{9}^+$ (−54.1 kcal mol^{-1}) *vs.* $\mathbf{11}^+$ (−91.2 kcal mol^{-1}), indicating that the electrophilicities of these analogues to hydride could be adjusted in a flexible manner 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^{-1} for 4-MeOPhCH$_2^+$) in acetonitrile,[32] it is found the hydride affinities of these analogues are more positive than those of the benzyl carbon cations by at least 14 kcal mol^{-1}. 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 the additional cleavage of one C=N bond and the 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^{-1} for *N*-benzylideneaniline),[19] it seems a paradox that the hydride affinities of these analogues are more negative than those of the imines by over 12 kcal mol^{-1}, since at first glance, the hydride transfer to an alkaloid analogue is accompanied by one more energy-consuming loss of aromatization of a heterocycle compared to the hydride transfer to an imine, apart from the common energy-releasing formation of one C–H bond and an energy-consuming cleavage of the C=N bond. Nevertheless, if the contribution from the molecular charge is considered, it may be concluded that it is more spontaneous for cationic alkaloid analogues to accept the hydride than for neutral imines to accept the hydride, which is different from the case between indazolium alkaloid analogues and the benzyl carbon cations mentioned above. This result might suggest to us that the cationic indazolium analogues of alkaloids more readily 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 Fig. 5, the hydrogen atom affinities of $\mathbf{X}^+$ decrease in the 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 the increasing order of the electrophilicities of indazolium alkaloid analogues to the hydrogen atom. Generally, these analogues are poor electrophiles to hydrogen atoms, since they are not able to be reduced by some strong antioxidant reagents such as vitamin E (80.9 kcal mol^{-1} to release hydrogen atom), the commercially available BHT (2,6-di-*tert*-butyl-4-methylphenol, 81.6 kcal mol^{-1} to release hydrogen atom) and phenothiazine (79.7 kcal mol^{-1} to release hydrogen atoms).[33–35] When comparing their hydrogen atom

 This journal is © The Royal Society of Chemistry 2015

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

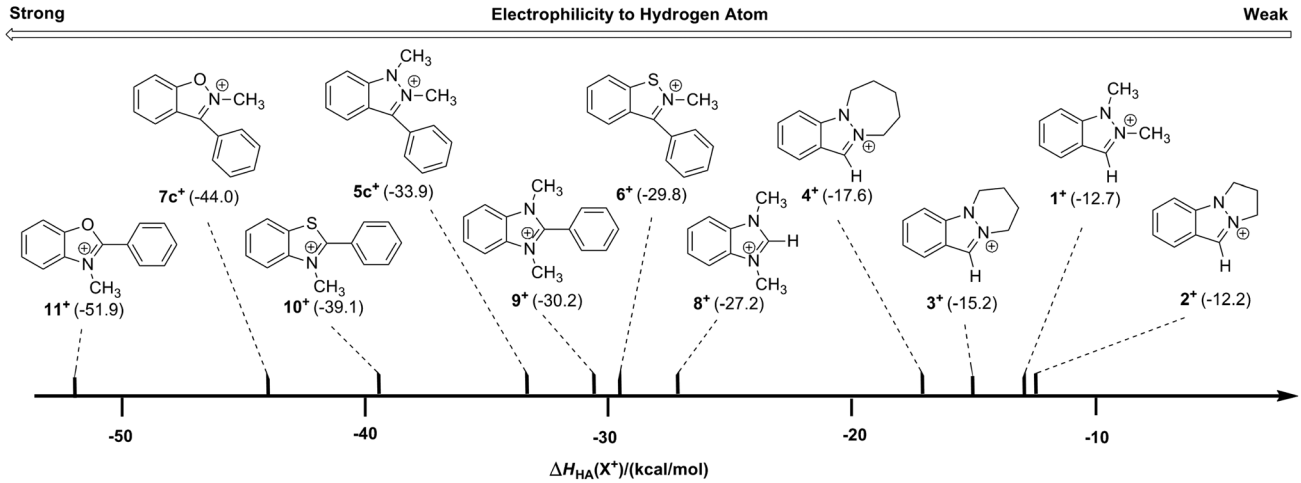


Fig. 5 Comparison of the hydrogen atom affinities of the analogues of indazolium alkaloids (X^+).

affinities with their hydride ion affinities (Table 2, column 3 *vs.* column 2), it may be concluded that these analogues are more readily reduced by the hydride ion than by the hydrogen atom, thus the hydride transfer is probably not 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^{-1}, which is probably due to the fact that the delocalization effect by the phenyl group at the C(3) position greatly stabilizes the yielded radical cation of $\mathbf{5c}^+$ after hydrogen atom transfer, which will be discussed *vide 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^{-1}. 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 electrons, while $\mathbf{1}^+$, $\mathbf{2}^+$, $\mathbf{3}^+$, $\mathbf{4}^+$, $\mathbf{5}^+$, **8** and $\mathbf{9}^+$ are due to weak electrophiles to electrons. In living bodies, it should be thermodynamically unfavorable for these alkaloid analogues to be reduced by electrons 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 that of the alkaloid analogues by over 1.281 V (equivalent to 29.5 kcal mol^{-1}) and 0.725 V (equivalent to 16.7 kcal mol^{-1}), respectively.[19]

As illustrated in Fig. 6, the standard reduction potentials of these analogues are ranked in the 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 the increasing order of electron-withdrawing 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 isomerization, the $E(X^{+/0})$ of $\mathbf{1}^+$, $\mathbf{5c}^+$, $\mathbf{6}^+$ and $\mathbf{7c}^+$ is 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^{-1}),

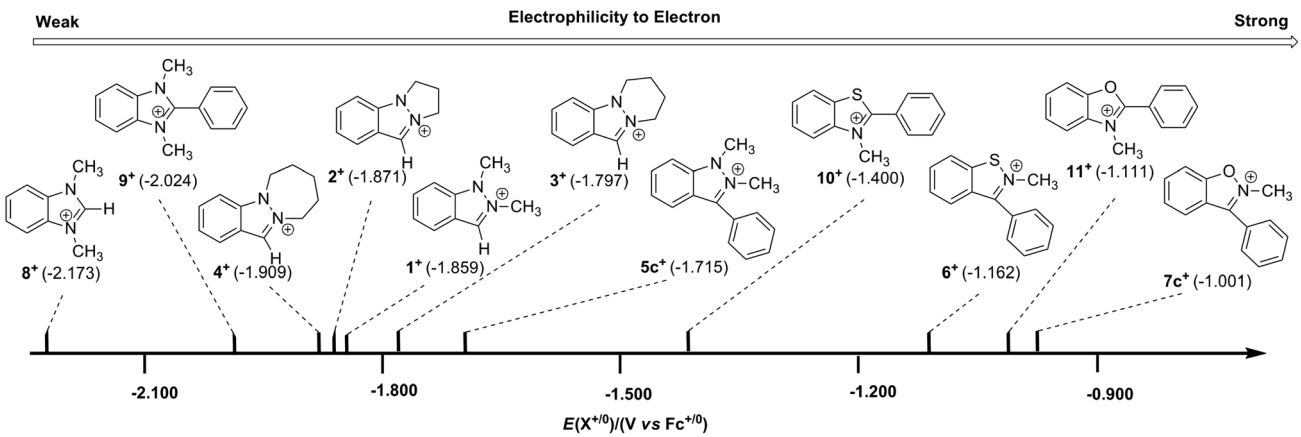


Fig. 6 Comparison of the one electron reduction potentials of the analogues of indazolium alkaloids (X^+).

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

implying the electrophilicities of the former ones to electrons 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^{-1}), with a similar trend of N < S < O corresponding to the potentials.

Hydrogen atom affinities and proton affinities of neutral radical intermediates ($X^{\bullet}$) 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 radical intermediates ($X^{\bullet}$) could be formed as transient species, which could either grab a hydrogen atom to give their conjugated amines (**XH**), or accept a proton to generate their corresponding radical cations ($XH^{\bullet+}$). Consequently, the thermodynamic driving forces for the two possible elementary steps of hydride transfer, namely hydrogen atom affinities [$\Delta H_{HA}(X^{\bullet})$] for $X^{\bullet}$ to obtain hydrogen atoms and proton affinities [$\Delta H_{PA}(X^{\bullet})$] for $X^{\bullet}$ to obtain protons in acetonitrile at 298 K, should be vital parameters not only in diagnosing the chemical activities of $X^{\bullet}$ but in predicting whether hydrogen atom transfer or proton transfer to $X^{\bullet}$ is thermodynamically more favorable. From columns 4 and 5 in Table 2, it is found that, $\Delta H_{HA}(X^{\bullet})$ locates on a scale from −61.2 to −84.5 kcal mol^{-1}, while $\Delta H_{PA}(X^{\bullet})$ ranges from −2.1 to −20.5 kcal mol^{-1}. For each neutral radical, $\Delta H_{HA}(X^{\bullet})$ is much more negative than $\Delta H_{PA}(X^{\bullet})$ by at least 49 kcal mol^{-1}, implying that the attack on $X^{\bullet}$ by the 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 $\Delta H_{HA}(X^{\bullet})$ is compared with the corresponding $\Delta H_{HA}(X^{+})$ (Table 2, column 5 *vs.* column 3), it is found that $\Delta H_{HA}(X^{\bullet})$ is much more negative than $\Delta H_{HA}(X^{+})$ by up to 71 kcal mol^{-1}, which means the antioxidant abilities of these analogues might be greatly strengthened after obtaining one electron. As a result, after accepting the electron, the analogues 7^{+} and 11^{+} will be scavenged by antioxidant reagents like vitamin E, BHT, and phenothiazine. By comparing $\Delta H_{H\text{-}A}(X^{+})$ with the corresponding $\Delta H_{PA}(X^{\bullet})$, 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^{-1}, which is close to the difference (*ca.* 21 kcal mol^{-1}) between $\Delta H_{HA}(\mathbf{5c}^{+})$ and $\Delta H_{HA}(\mathbf{1}^{+})$, again this might be attributed to the fact that the yielded $\mathbf{5cH}^{\bullet+}$ is more stable than $\mathbf{1H}^{\bullet+}$, which will be provided further evidence *vide infra*. For an intuitive comparison, the hydrogen or proton obtaining abilities of $X^{\bullet}$ were ranged in order and are shown in Fig. 7 and 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^{-1} 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 ($XH^{\bullet+}$) of these alkaloid analogues were defined as the one electron reduction potentials of $XH^{\bullet+}$. From column 6 of Table 1, it is found that reduction potentials of $XH^{\bullet+}$ range from 0.787 V to −0.179 V (*vs.* $Fc^{+/0}$), and the one electron reduction potential of $XH^{\bullet+}$ decreases in the 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 the decreasing order of the electrophilicity of $XH^{\bullet+}$ to electron (Fig. 9). For an intuitive comparison, the one electron reduction potentials of $XH^{\bullet+}$ were ranged in the order corresponding to their structures, as illustrated in Fig. 9. From the perspective of effects of structural variations, it is evident that $E(XH^{+/0})$ could be adjusted within a scale approximating 1.0 V (equivalent to 23.06 kcal mol^{-1}) *via* isomerizations, variations of heteroatoms or remote substituents. Notably, the one

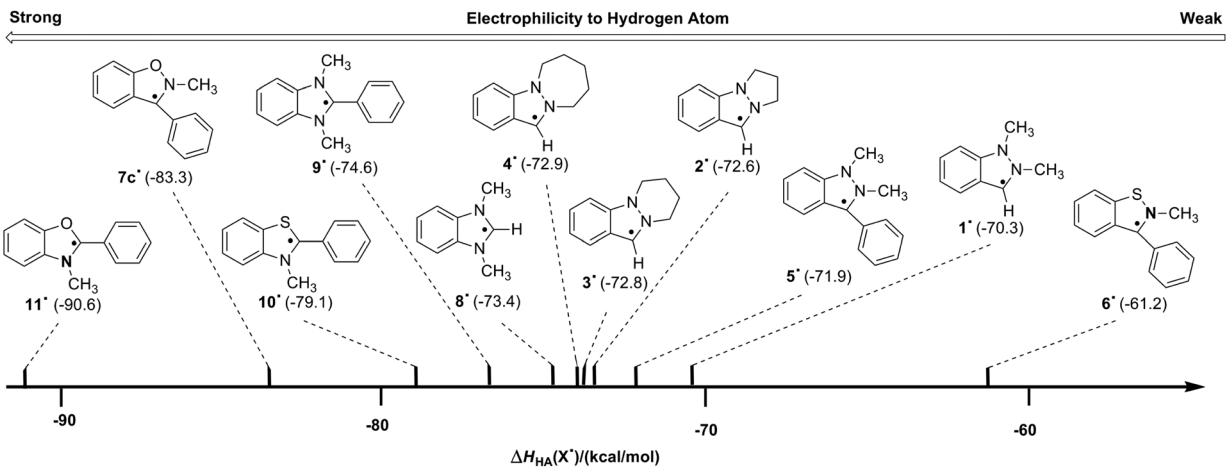


Fig. 7 Comparison of the hydrogen atom affinities of neutral radical intermediates of indazolium alkaloid analogues ($X^{\bullet}$).

 This journal is © The Royal Society of Chemistry 2015

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

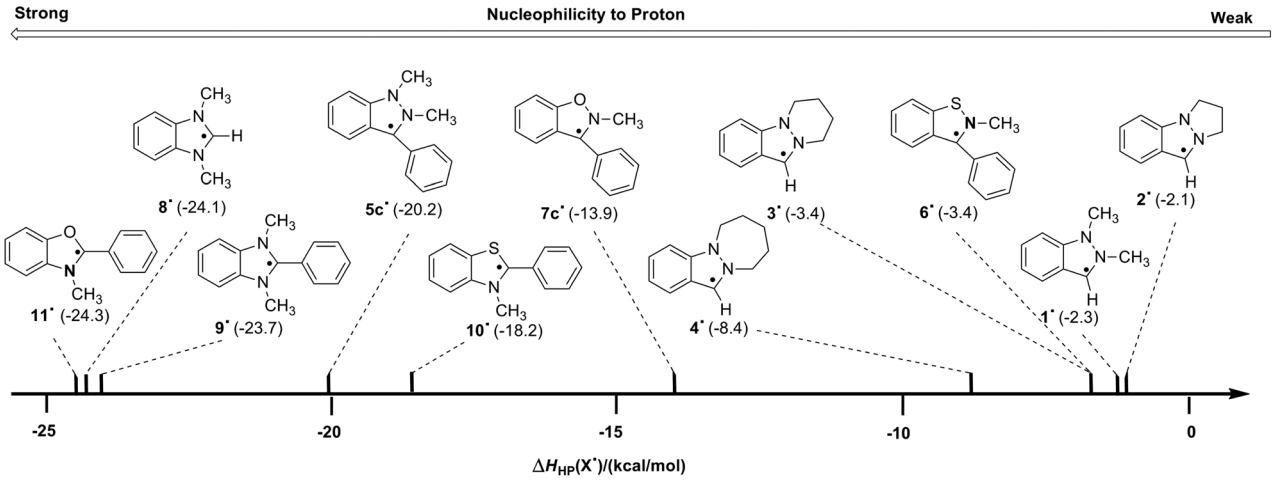


Fig. 8 Comparison of the proton affinities of the neutral radical intermediates of indazolium alkaloid analogues ($\mathbf{X}^{\bullet}$).

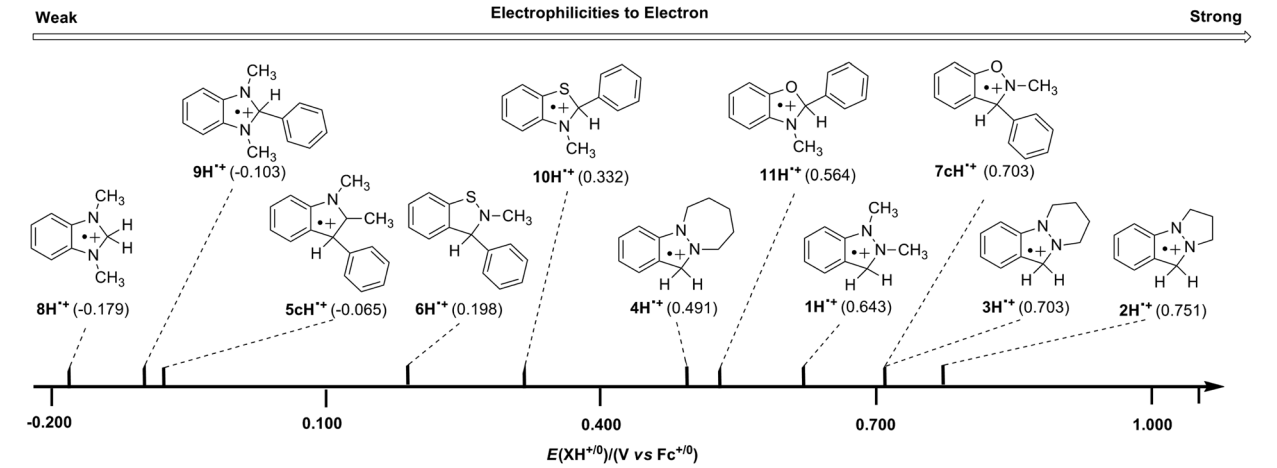


Fig. 9 Comparison of one electron reduction potentials of the radical cation intermediates of indazolium alkaloid analogues ($\mathbf{XH}^{\bullet+}$).

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 the preceding paragraphs. For further verification, cyclic voltammetry (CV) technology was employed to test the relative stabilities of radical cations.[36,37] For example, the radical cation $\mathbf{1H}^{\bullet+}$ would be generated during the sweeping of oxidant potential of **1H** in anaerobic acetonitrile by CV. If the newly generated transient species $\mathbf{1H}^{\bullet+}$ is stable within the sweeping time, a reversible CV graph will be obtained and *vice versa*, so CV can 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 Fig. 10, the CV graphs of **1H** are found to be irreversible even at a high sweeping rate of 1 V s^{-1}, 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 in a wide range of sweeping rate, from 0.1 volt per second (0.1 V s^{-1}) to 1 V s^{-1}, indicating $\mathbf{5cH}^{\bullet+}$ could exist in solution for a while. As a result, it may be concluded that $\mathbf{5cH}^{\bullet+}$ is more stable than $\mathbf{1H}^{\bullet+}$, which could respond to the differences of the other thermodynamic parameters mentioned hereinbefore.

Effects of remote substituents on the enthalpy changes and reduction potentials

From Tables 1 and 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 the phenyl group at the position of C(3) in $\mathbf{5}^{+}$ and $\mathbf{7}^{+}$, as well as their reaction intermediates. To elucidate the relationship 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 $\Delta H_{\text{H-A}}(\mathbf{X}^{+})$ and $\Delta H_{\text{HA}}(\mathbf{X}^{+})$, on the $\Delta H_{\text{PA}}(\mathbf{X}^{\bullet})$ and $\Delta H_{\text{HA}}(\mathbf{X}^{\bullet})$, as well as on the $E(\mathbf{X}^{+/0})$ and $E(\mathbf{XH}^{+/0})$, respectively. The results in Fig. 11 and S1–S5 (ESI†) show that

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

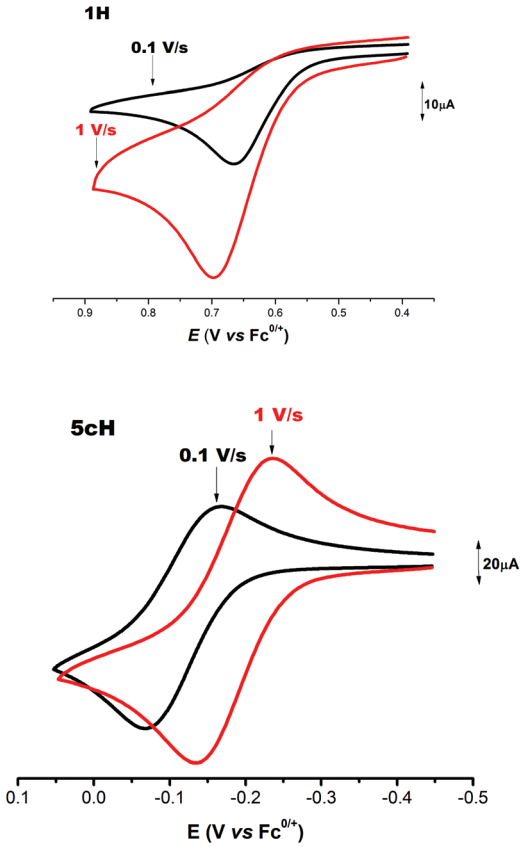


Fig. 10 CV graphs at different sweeping rates in anaerobic MeCN containing 0.1 M *n*-Bu_4NBF_4 as the supporting electrolyte. The upper graph is the CV graph of **1H**, while the lower one is the CV graph of **5cH**.

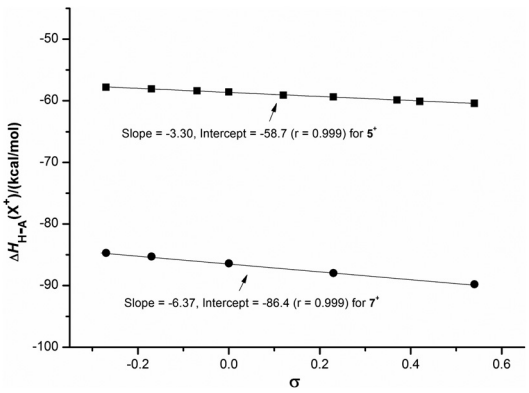


Fig. 11 Plot of $\Delta H_{H\text{-}A}(X^+)$ against Hammett substituent parameter (σ).

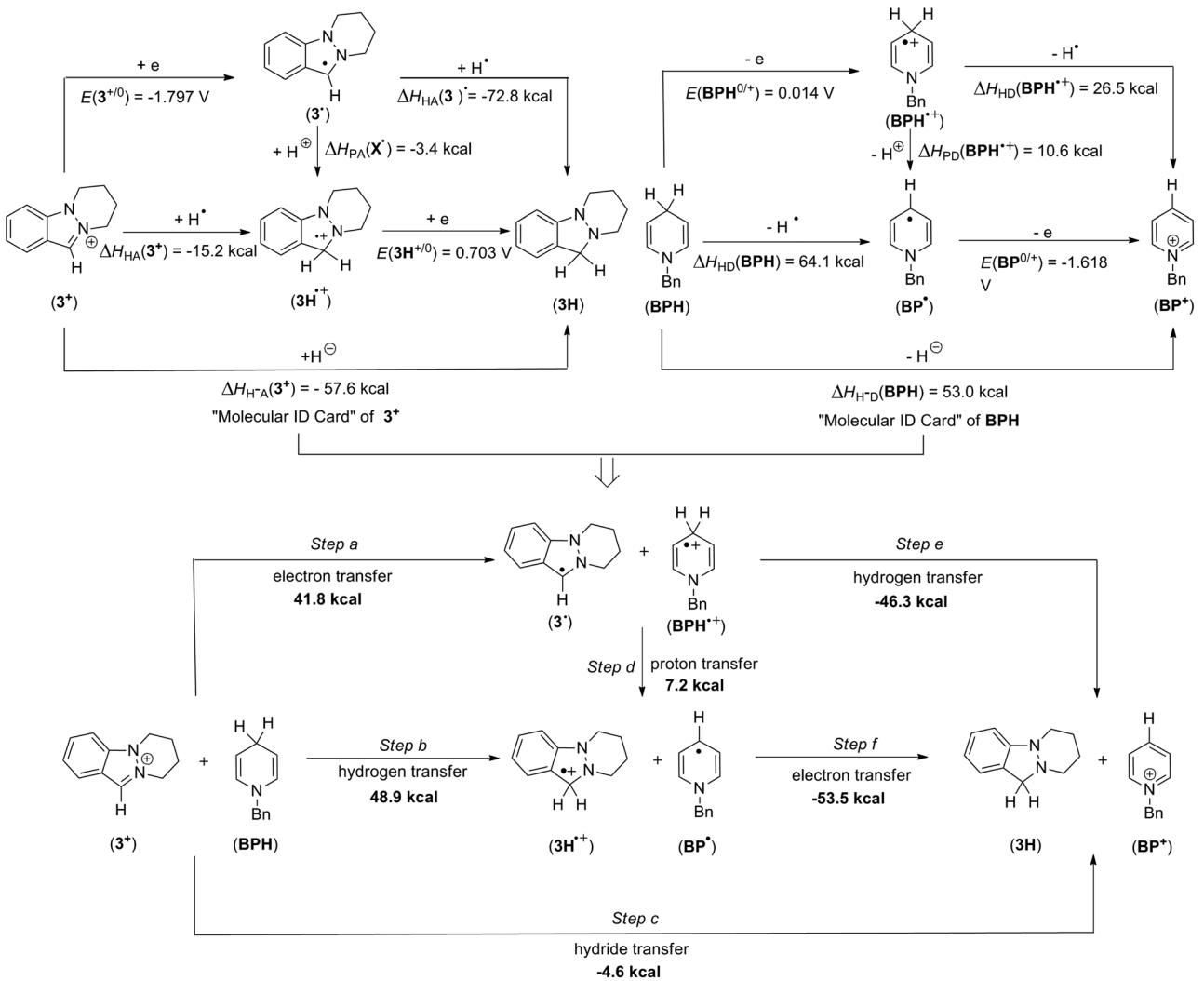
all these values are linearly dependent on the Hammett substituent parameters σ_p or σ_m with very good correlation coefficients, implying that the Hammett linear free energy relationship[38,39] holds in these chemical and electrochemical processes. From the slopes and the intercepts of these linear correlations, twelve mathematical formulae are derived [eqn (12)–(23)]. Evidently, for any *para*- or *meta*-position on the phenyl ring at the position of the C(3), it was not difficult to estimate the values of $\Delta H_{H\text{-}A}(\mathbf{X}^+)$, $\Delta H_{HA}(\mathbf{X}^+)$, $\Delta H_{PA}(\mathbf{X}^\bullet)$, $\Delta H_{HA}(\mathbf{X}^\bullet)$, $E(\mathbf{X}^{+/0})$ as well as $E(\mathbf{XH}^{+/0})$ 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^{-1} for enthalpies or less than 25 mV for reduction potentials. Also, these formulae 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 Introduction section, 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. The latest study showed that their antidiabetic effects originate from the activation of AMP-activated protein kinase (AMPK) by the alkaloids, but the in depth mechanism of the activation of AMPK by the alkaloids 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 the fact that these alkaloids would act upon (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 criteria in diagnosing the possibilities 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 studies, a "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 their "Molecular ID Cards" are available. Fortunately, the "Molecular ID Card" for $\mathbf{3}^+$ to accept hydride could be obtained by this work, whilst 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 easy to access the thermodynamic driving forces of all possible elemental steps of hydride transfer from **BPH** to $\mathbf{3}^+$, as shown in Scheme 6. Based on the driving forces of six possible elemental steps of hydride transfer from **BPH** to $\mathbf{3}^+$, we can make the following predictions: (i) hydride transfer (step c) from **BPH** to $\mathbf{3}^+$ should be spontaneous, since this reaction is thermo-

 This journal is © The Royal Society of Chemistry 2015

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.



Scheme 6 Possible elementary steps of hydride transfer from **BPH** to $\mathbf{3}^+$ and thermodynamic driving forces of each step derived from the "Molecular ID Cards" of **BPH** and $\mathbf{3}^+$.

dynamically favorable by 4.6 kcal mol^{-1}, 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^{-1}. (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 for long in solution, and would react with each other following the 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 be concluded that, when indazolium alkaloids encounter NADH *in vivo*, the hydride transfer reaction is likely to happen, rather than hydrogen atom transfer or electron transfer reaction, to give the corresponding indazoles and $\mathbf{NAD}^+$ as products, and the route of hydride transfer tends to be concerted rather than stepwise.

$$\Delta H_{\text{H-A}}(\mathbf{X}^+) = -3.30\sigma - 58.7 \text{ for } \mathbf{5}^+ \quad (12)$$

$$\Delta H_{\text{H-A}}(\mathbf{X}^+) = -6.37\sigma - 86.4 \text{ for } \mathbf{7}^+ \quad (13)$$

$$\Delta H_{\text{HA}}(\mathbf{X}^+) = -1.29\sigma - 33.9 \text{ for } \mathbf{5}^+ \quad (14)$$

$$\Delta H_{\text{HA}}(\mathbf{X}^+) = -2.84\sigma - 43.9 \text{ for } \mathbf{7}^+ \quad (15)$$

$$\Delta H_{\text{HA}}(\mathbf{X}^\bullet) = 0.598\sigma - 72.0 \text{ for } \mathbf{5}^\bullet \quad (16)$$

$$\Delta H_{\text{HA}}(\mathbf{X}^\bullet) = -2.10\sigma - 83.3 \text{ for } \mathbf{7}^\bullet \quad (17)$$

$$\Delta H_{\text{PA}}(\mathbf{X}^\bullet) = 2.60\sigma - 20.3 \text{ for } \mathbf{5}^\bullet \quad (18)$$

$$\Delta H_{\text{PA}}(\mathbf{X}^\bullet) = 1.28\sigma - 13.9 \text{ for } \mathbf{7}^\bullet \quad (19)$$

$$E(\mathbf{X}^{+/\mathbf{0}}) = 0.1681\sigma - 1.7142 \text{ for } \mathbf{5}^+ \quad (20)$$

$$E(\mathbf{X}^{+/\mathbf{0}}) = 0.1821\sigma - 1.0030 \text{ for } \mathbf{7}^+ \quad (21)$$

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

$$E(\mathbf{XH}^{+/0}) = 0.0866\sigma - 0.0654 \text{ for } \mathbf{5H}^{\bullet+} \quad (22)$$

$$E(\mathbf{XH}^{+/0}) = 0.1511\sigma + 0.7058 \text{ for } \mathbf{7H}^{\bullet+} \quad (23)$$

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 NADH *in vivo*, indazolium alkaloids would oxidize NADH to NAD^+ through hydride transfer reactions, leading to a lower concentration of NADH and a higher concentration of NAD^+, both of which would contribute to the activation of AMPK, followed by 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 for the concentrations of NADH, NAD^+ and the activity of AMPK should not be violent, rendering their regulations of the metabolism of glucose 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 the potential impacts of the structures of indazolium alkaloids on their antidiabetic effects. As illustrated in 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 force 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^{-1}, implying that the bridge ring in the structures of indazolium alkaloids would make the 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 that the enthalpy change decreases in the order: $\mathbf{4}^+$ (−2.1 kcal mol^{-1}) > $\mathbf{2}^+$ (−2.7 kcal mol^{-1}) > $\mathbf{3}^+$ (−4.6 kcal mol^{-1}), 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 the C(11) position in the structures of these alkaloids, it was found that either a phenyl group or H might occupy the C(11) position of these natural alkaloids (Scheme 1). Which substituent might be favourable for the antidiabetic effects of indazolium alkaloids? With $\mathbf{5c}^+$ and $\mathbf{1}^+$ as the corresponding model compounds, it is demonstrated that the hydride transfer from **BPH** to $\mathbf{5c}^+$ is thermodynamically more favorable than that to $\mathbf{1}^+$ by almost 5 kcal mol^{-1} (Table 3), implying that those alkaloids structurally bearing a phenyl group at the C(11) position are more ready to grab the hydride from NADH and might show better antidiabetic activities than those bearing H at the 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 the suitable indazolium alkaloids and their analogues for antidiabetic use.

Table 3 Thermodynamic driving forces (ΔH_r(H–T)) of hydride transfer from the model of NADH (**BPH**) to the models of indazolium alkaloids ($\mathbf{1}^+$–$\mathbf{5c}^+$) in acetonitrile

Models of alkaloids	$\mathbf{1}^+$	$\mathbf{2}^+$	$\mathbf{3}^+$	$\mathbf{4}^+$	$\mathbf{5c}^+$
ΔH_r(H–T) (kcal mol^{-1})	−0.7	−2.7	−4.6	−2.1	−5.6

Conclusions

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

(1) These analogues of indazolium alkaloids are weak to strong electrophiles to hydride. $\mathbf{1}^+$–$\mathbf{5}^+$, $\mathbf{8}^+$, $\mathbf{9}^+$ and $\mathbf{6}^+$ 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; $\mathbf{10}^+$, $\mathbf{7}^+$, and $\mathbf{11}^+$ belong to strong electrophiles to hydride, and some organic hydrides like Hantzsch ester and dihydrobenzo[*d*]imidazoles are capable of reducing them to their conjugated amines. The cationic indazolium analogues of alkaloids are predicted to be more capable of obtaining hydride than their neutral indazole analogues, mainly due to the contribution of molecular charge.

(2) These analogues are poor electrophiles to hydrogen atoms, and are not likely to be reduced even by strong antioxidant reagents such as vitamin E, commercially available BHT

 This journal is © The Royal Society of Chemistry 2015

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

and phenothiazine. Since the hydrogen atom affinities of these indazolium analogues are much more positive than their hydride affinities, these analogues are more readily reduced by hydride ions than by hydrogen atoms, and hydride transfers are probably not initiated by hydrogen atom transfers.

(3) These analogues are weak to good electrophiles to electrons. $\mathbf{6}^+$, $\mathbf{7}^+$, $\mathbf{10}^+$ and $\mathbf{11}^+$ should belong to the category of good electrophiles to electrons, while $\mathbf{1}^+$, $\mathbf{2}^+$, $\mathbf{3}^+$, $\mathbf{4}^+$, $\mathbf{5}^+$, $\mathbf{8}^+$ and $\mathbf{9}^+$ are due to weak electrophiles to electrons. In living bodies, it should be thermodynamically unfavorable for these analogues to be reduced by an electron from NADH or vitamin C.

(4) If hydride transfer to these analogues is initiated by single electron transfer, neutral radical intermediates ($\mathbf{X}^{\bullet}$) could form, which could either grab a hydrogen atom or accept a proton. For each $\mathbf{X}^{\bullet}$, the attack on $\mathbf{X}^{\bullet}$ by a hydrogen atom is thermodynamically much more favorable than that by a proton, that is, if hydride attack on 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 range from 0.787 to −0.179 V, implying these radical cations are unstable and easily accept the electron. Electrochemical experiments verified that the stability of $\mathbf{5cH}^{\bullet+}$ is much larger than that of $\mathbf{1H}^{\bullet+}$, which could explain the related differences of the thermodynamic parameters.

(6) All the thermodynamic parameters could be adjusted in a flexible manner through variation of the heteroatoms or structural isomerizations. The good Hammett linear free-energy relationship held between these thermodynamic parameters and the nature of the remote substituents on the phenyl ring at the C(3) position, and 12 mathematical formulae were derived. These results might guide us to 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 a one-step concerted hydride transfer mechanism; a deeper molecular mechanism of the antidiabetic effects of indazolium alkaloids was then proposed as follows: when indazolium alkaloids encounter NADH *in vivo*, they would oxidize NADH to NAD^+ through hydride transfer reactions, leading to a lower concentration of NADH and a higher concentration 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 exceed 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; these alkaloids structurally bearing a phenyl group at the C(11) position are more ready to grab the hydride from NADH and accordingly might show better antidiabetic activities than those bearing H at the C(11) position, which might answer the question 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 on 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 hexafluorophosphate (n-Bu_4NPF_6, Aldrich) was recrystallized from CH_2Cl_2/ether and was vacuum-dried at 110 °C overnight before the preparation of the supporting electrolyte solution. 4-Acetamido-2,2,6,6-tetra-methylpiperidine-1-oxo-ammonium perchlorate ($TEMPO^+ClO_4^-$) and trityl perchlorate ($Ph_3C^+ClO_4^-$) were prepared following operations described in the literature.[42,43]

Syntheses of the analogues of indazolium alkaloids ($\mathbf{1}^+$–$\mathbf{7}^+$)

The analogues of indazolium alkaloids $\mathbf{1}^+$–$\mathbf{4}^+$,[44,45] $\mathbf{5}^+$,[46] and $\mathbf{6}^+$–$\mathbf{7}^+$ (ref. 47) were synthesized according to the literature, respectively, and the detailed synthetic routes are provided in the ESI.† The analogues of indazolium alkaloids ($\mathbf{1}^+$–$\mathbf{7}^+$) 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 an 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 the working electrode, a platinum wire as the counter electrode, and 0.1 M $AgNO_3$/Ag (in 0.1 M n-Bu_4NPF_6–acetonitrile) as the 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 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 syringe containing a standard solution (2 mM) into the reaction cell (1.30 mL) containing 1 mL of other concentrated reactants (~12 mM). Injection

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

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 the integration of each peak except for the first one.

Acknowledgements

Financial support from the National Natural Science Foundation of China (Grant No. 21472099, 21390400 and 21102074) and the 111 Project (B06005) is gratefully acknowledged.

Notes and references

1 K. M. Fararh, Y. Atoji, Y. Shimizu, T. Shiina, H. Nikami and T. Takewaki, *Res. Vet. Sci.*, 2004, **77**, 123.
2 H. Rchid, H. Chevassus, N. Rachid, C. Guiral, P. Petit, M. Chokairi and Y. Sauvaire, *Fundam. Clin. Pharmacol.*, 2004, **18**, 525.
3 B. Meddah, R. Ducroc, M. E. A. Faouzi, B. Eto, L. Mahraoui, A. Benhaddou-Andaloussi, L. C. Martineau, Y. Cherrah and P. S. Haddad, *J. Ethnopharmacol.*, 2009, **121**, 419.
4 Y.-M. Liu, Y.-H. Jiang, Q.-H. Liu and B.-Q. Chen, *Evid Based Complement Alternat Med.*, 2011, **2011**, 538671.
5 T. Yuan, P. Nahar, M. Sharma, K. Liu, A. Slitt, H. A. Asia and N. P. Seeram, *J. Nat. Prod.*, 2014, **77**, 2316.
6 R. Rafaeloff-Phail, L.-Y. Ding, L. Conner, W.-K. Yeh, D. McClure, H.-H. Guo, K. Emerson and H. Brooks, *J. Biol. Chem.*, 2004, **279**, 52934, and related references cited therein.
7 C. A. Coleman, J. G. Rose and C. J. Murray, *J. Am. Chem. Soc.*, 1992, **114**, 9755.
8 J.-P. Cheng, Y. Lu, X.-Q. Zhu and L.-J. Mu, *J. Org. Chem.*, 1998, **63**, 6108.
9 J. Yuasa, S. Yamada and S. Fukuzumi, *J. Am. Chem. Soc.*, 2006, **128**, 14938.
10 S. Fukuzumi, H. Kotani, Y.-M. Lee and W. Nam, *J. Am. Chem. Soc.*, 2008, **130**, 15134.
11 X.-Q. Zhu, Y.-Y. Mu and X.-T. Li, *J. Phys. Chem. B*, 2011, **115**, 14794.
12 J. A. Birrell and J. Hirst, *Biochemistry*, 2013, **52**, 4048.
13 The motifs of these alkaloids belong to iminium ions, which are likely to accept hydride, hydrogen atoms and electrons as supported by: X.-M. Zhang, J. W. Bruno and E. Enyinaya, *J. Org. Chem.*, 1998, **63**, 4671, and nucleophilic addition reactions in ref. 14–18.
14 J. Elguero, E. G. Gutierrez-Puebla, A. Monge, C. Parco and M. Ramos, *Tetrahedron*, 1993, **49**, 11305.
15 A. Gonzalez-Ortega, *Eur. J. Org. Chem.*, 2005, 4663.
16 A. Schmidt, T. Habeck, B. Snovydovych and W. Eisfeld, *Org. Lett.*, 2007, **9**, 3515.
17 A. M. Gonzalez-Nogal, M. Calle and P. Cuadrado, *Eur. J. Org. Chem.*, 2007, 6089.
18 A. Schmidt, A. Beutler and B. Snovydovych, *Eur. J. Org. Chem.*, 2008, 4073.
19 X.-Q. Zhu, Q.-Y. Liu, Q. Chen and L.-R. Mei, *J. Org. Chem.*, 2010, **75**, 789.
20 Y. Cao, S.-C. Zhang, M. Zhang, G.-B. Shen and X.-Q. Zhu, *J. Org. Chem.*, 2013, **78**, 7154.
21 S.-M. Si, Y.-H. Fu and X.-Q. Zhu, *J. Phys. Chem. C*, 2015, **119**, 62.
22 K. Xia, G.-B. Shen and X.-Q. Zhu, *Org. Biomol. Chem.*, 2015, **13**, 6255.
23 X.-Q. Zhu, X. Chen and L.-R. Mei, *Org. Lett.*, 2011, **13**, 2456.
24 The hydride affinity of Ph_3C^+ cation in acetonitrile (-104.6 kcal mol^{-1}) is derived from the reaction heat of Ph_3C^+ with BNAH in acetonitrile at 298 K (40.4 kcal mol^{-1}) and the molar enthalpy change of BNAH to release hydride anions in acetonitrile at 298 K (64.2 kcal mol^{-1}). The former is measured using ITC and the latter is a known number (see ref. 25).
25 X.-Q. Zhu, M.-T. Zhang, A. Yu, C.-H. Wang and J.-P. Cheng, *J. Am. Chem. Soc.*, 2008, **130**, 2501.
26 D. D. M. Wayner and V. D. Parker, *Acc. Chem. Res.*, 1993, **26**, 287.
27 Eqn (9)–(11) were constructed from the thermodynamic cycles (I), (II), and (III) in Scheme 5, respectively. Note that we used the term free energy change ΔG_{et} to replace the enthalpy change ΔH_{et} in eqn (9)–(11) for the electron transfer process. The justification of this treatment was that entropies associated with electron transfer were negligible, as verified by Arnett *et al.*: E. M. Arnett, K. Amarnath, N. G. Harvey and J.-P. Cheng, *J. Am. Chem. Soc.*, 1990, **112**, 344.
28 The energetic data herewith was suggested to be used in a relative sense for the purpose of comparison within a family. This was because the electrode potentials were measured under irreversible conditions and some uncertainties in the absolute data were involved (possibly by a few tens of millivolts). For a method to correct an irreversible potential to a reversible one, please see ref. 26.
29 I. H. Lee, E. H. Jeoung and M. M. Kreevoy, *J. Am. Chem. Soc.*, 1997, **119**, 2722.
30 X.-Q. Zhu, F.-H. Deng, J.-D. Yang, X.-T. Li, Q. Chen, N.-P. Lei, F.-K. Meng, X.-P. Zhao, S.-H. Han, E.-J. Hao and Y.-Y. Mu, *Org. Biomol. Chem.*, 2013, **11**, 6071.
31 A. V. El'tsov and M. Z. Girshovich, *Zh. Org. Khim.*, 1967, **3**, 1332.
32 J.-P. Cheng, K. L. Handoo and V. D. Parker, *J. Am. Chem. Soc.*, 1993, **115**, 2655.
33 F. G. Bordwell and W.-Z. Liu, *J. Am. Chem. Soc.*, 1996, **118**, 10819.
34 D. D. M. Wayner, E. Lusztyk, K. U. Ingold and P. Mulder, *J. Org. Chem.*, 1996, **61**, 6430.
35 F. G. Bordwell, X.-M. Zhang and J.-P. Cheng, *J. Org. Chem.*, 1993, **58**, 6410.
36 B. Gollas and B. Speiser, *Angew. Chem., Int. Ed. Engl.*, 1992, **31**, 332.
37 Y. Li, K. C. Mondal, P. P. Samuel, H.-P. Zhu, C. M. Orben, S. Pannerselvam, B. Dittrich, B. Schwederski, W. Kaim,

 This journal is © The Royal Society of Chemistry 2015

Published on 29 September 2015. Downloaded by Gazi Universitesi on 09/03/2016 15:00:01.

T. Mondal, D. Koley and H. W. Roesky, *Angew. Chem., Int. Ed.*, 2014, **126**, 4252.
38 P. R. Wells, *Chem. Rev.*, 1963, **63**, 171.
39 C. D. Johnson, *Chem. Rev.*, 1975, **75**, 755.
40 **BPH** was chosen as the model compound of NADH, as **BPH** and NADH share the same core structure of 1,4-dihydropyridine, and the hydride donating ability of **BPH** (53.0 kcal mol^{-1} to release hydride in acetonitrile)[41] is close to that of NADH (53.6 kcal mol^{-1} to release hydride in neutral water); $\mathbf{3}^{+}$ was selected as the model compound of indazolium alkaloids, since $\mathbf{3}^{+}$ is structurally quite close to the indazolium alkaloids **A** and **B** in Scheme 1.
41 X.-Q. Zhu, Y. Tan and C.-T. Cao, *J. Phys. Chem. B*, 2010, **114**, 2058.
42 J. M. Bobbit, *J. Org. Chem.*, 1998, **63**, 9367.
43 H. Dauben, J. L. Honnen and K. J. Harmon, *Org. Chem.*, 1960, **25**, 1442.
44 A. Schmidt, B. Snovydovych, T. Habeck, P. Dröttboom, M. Gjikaj and A. Adam, *Eur. J. Org. Chem.*, 2007, 4909–4916.
45 J. Yang and P. Gharagozloo, *Synth. Commun.*, 2005, **35**(3), 409–415.
46 Z. N. Timofeeva, M. V. Petrova, M. Z. Girshovich and A. V. El'tsov, *Zh. Obshch. Khim.*, 1969, **39**, 54–59.
47 T. Kitamura, M. Todaka, I. Shin-machi and Y. Fujiwara, *Heterocycl. Commun.*, 1998, **4**(3), 205–208.
48 X.-Q. Zhu, Q. Li, W.-F. Hao and J.-P. Cheng, *J. Am. Chem. Soc.*, 2002, **124**, 9887.
49 X.-Q. Zhu, W.-F. Hao, C.-H. Tang and J.-P. Cheng, *J. Am. Chem. Soc.*, 2005, **127**, 2696.