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Kinetic and mechanism of the addition of piperidine and benzylamine to the aroylacrilic acid phenylamides

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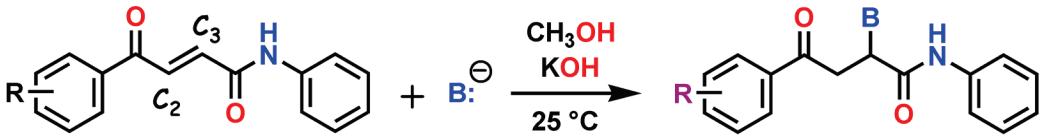
Introduction

The nucleophilic 1,4- Michael addition of amines or thiols to the activated double bonds of the xenobiotics has been suggested as a primary outcome responsible for their biological activity.[1] Knowledge on the mechanism of aza Michael addition to such compounds is important contribution for understanding their biological effects.

Results and discussion

Spectrophotometrically, under the pseudo-first order conditions, second order rate constants (k_2) for the Michael addition of piperidine and benzylamine to compounds **1**-**13**, in methanol, were obtained (**Table 1**).

*Table 1. Addition of deprotonated amines (piperidine and benzylamine, **B:**$^-$) to compounds **1-13**. Structures, reaction sheme, calculated charges and the Hammmett substituent constants.*



Comp N°	R-	k_2 ($M^{-1}s^{-1}$) (piperidine)	k_2 ($M^{-1}s^{-1}$) (benzylamine)	SP charge* (vacuum)	SP charge* (MeOH)	σ^2
1	tetralinil	0.64 (±0.05)	0.45 (±0.06)	– 0.15233	– 0.15499	– 0.48
2	3,4-di-Cl	3.87 (±0.27)	1.84 (±0.22)	– 0.14684	– 0.14939	0.52
3	3,4-di-Me	0.74 (±0.13)	0.25 (±0.01)	– 0.15228	– 0.15515	– 0.14
4	2,4-di-*i*-Pr	0.57 (±0.01)	0.53 (±0.03)	– 0.14291	– 0.14037	/
5	4-H	0.79 (±0.09)	0.50 (±0.01)	– 0.15054	– 0.15347	0
6	4-OMe	0.35 (±0.03)	0.23 (±0.03)	– 0.15281	– 0.15567	– 0.27
7	2,5-di-Me	1.58 (±0.12)	0.84 (±0.04)	– 0.14233	– 0.13922	/
8	4-F	1.52 (±0.12)	0.76 (±0.08)	– 0.14952	– 0.15274	0.06
9	4-Br	1.84 (±0.15)	1.31 (±0.05)	– 0.14884	– 0.15026	0.23
10	4-*n*-Bu	0.43 (±0.05)	0.33 (±0.03)	– 0.15189	– 0.15452	– 0.16
11	4-*t*-Bu	0.62 (±0.06)	0.45 (±0.02)	– 0.15168	– 0.15286	– 0.20
12	4-*i*-Pr	0.47 (±0.05)	0.39 (±0.01)	– 0.15165	– 0.15279	– 0.15
13	4-Cl	2.05 (±0.19)	1.25 (±0.13)	– 0.14890	– 0.15056	0.23

* SP- Mulliken charges, as obtained by single-point calculations in vacuum, or implicit solvent model for methanol.

Addition of amines proceeds exclusively to the activated C_3 atom, not to the C_2 (Table 1), as confirmed by the HMBC spectrum of the addition product of piperidine to compound **13**. The 3D depiction of the addition products of piperidine and benzylamine to compound **5**, are given in Figure 1.

Rate constants were determined by pseudo-first order kinetics,[3] following the decrease of absorbance on ~ 310 nm, using the 20-fold excess of amine (Figure 1).

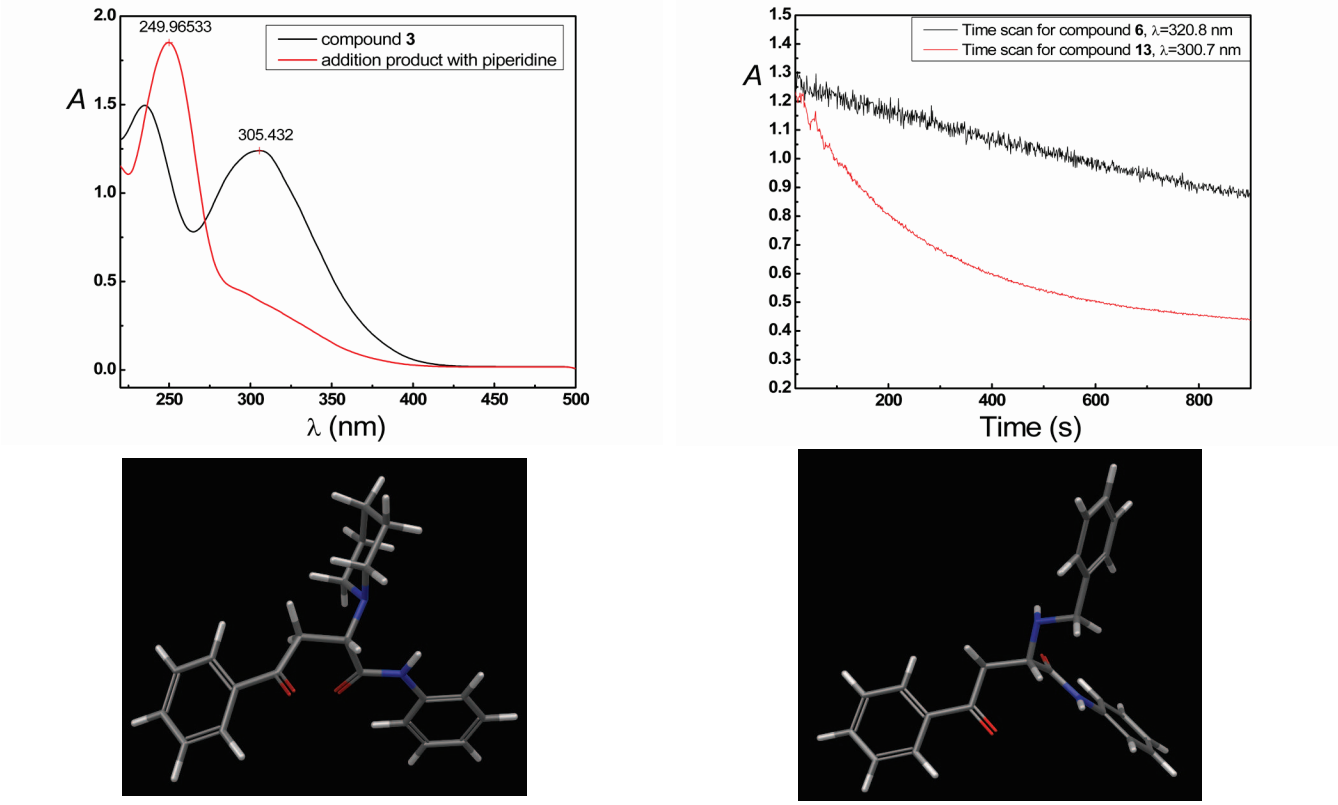


*Figure 1. The UV-VIS spectra of **3**, and its addition product with piperidine (upper left);*
*The difference in time scan curves obtained after the addition of piperidine to compounds **6** and **13** (upper right).*
*The 3D depiction of the addition products of piperidine (lower left) and benyzlamine (lower right) to **5**.*

Amines were deprotonated with the stoichiometric amount of stronger base (KOH) prior to addition, in order to generate the more nucleophilic amine anion. All measurements were done using Cintra 40 UV/VIS spectrophotometer, on the temperature of 25 ± 2 °C.

Rate constants for the addition of piperidine are higher than those for addition of benzylamine, as is expected, concerning basicity of amines used. Rate constants depend on substituent changes on the aroyl ring of the **1**-**13**. The substituent effects were quantified by using the Hammett substituent parameters (σ_x), as given by Equation 1:

$$\log \frac{k_x}{k_0} = \rho \ \sigma_x \qquad (1)$$

Where k_x is the rate constant of the *meta* or *para* X-substituted phenyl derivative, and k_0 is the rate constant for the unsubstituted derivative.

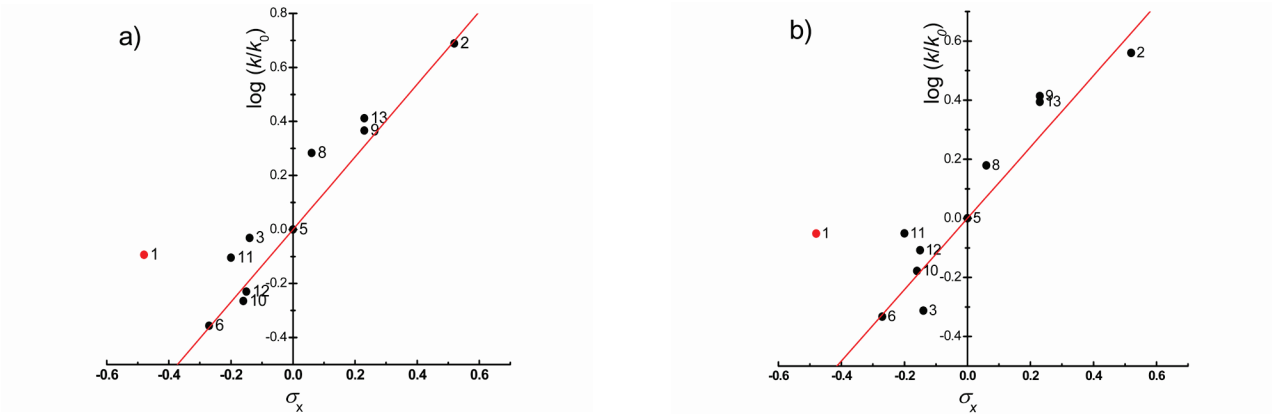


*Figure 2. Hammett plot for the second order rate constants of the addition of piperidine (a) and benzylamine (b) to compounds **1**-**13**.*

The least squares line fitting through zero gave the following equations:

Piperidine:

$$\log\left(\frac{k_x}{k_0}\right) = 1.35(\pm 0.15)\cdot\sigma_x$$

$$r = 0.96, s \quad 0.11, N \quad 10, P \quad 0.00001$$

Benzylamine:

$$\log\left(\frac{k_x}{k_0}\right) = 1.21(\pm 0.15)\cdot\sigma_x$$

$$r = 0.95, s \quad 0.11, N \quad 10, P \quad 0.00001$$

Reaction constant can be calculated as the slope of Hammett plots (Figure 2a and 2b). Reaction constant, ρ, represents the relative sensitivity of reaction under consideration upon the change of substituents on the aromatic ring. The sign and the magnitude of the reaction constants depend upon the nature of the reactants and the mechanism of reaction. Positive signs of the reaction constants for the addition of piperidine and benzylamine (ρ = 1.35, and ρ = 1.21, respectively) to **1**-**13** indicate that electron-withdrawing substituents increase the rate of addition. Numerical values of rate constants indicate the formation of the charged species in the rate-limiting step, which is in accordance with proposed mechanism of the formation of carbanionic intermediate, and subsequent rapid protonation of it.[4] Polar solvents are capable to stabilize charged species in the transition state, and lead to the formation of amino adduct. Reaction product was not observed in CCl_4 after 24^h, confirming the importance of polar/charged intermediates in reaction mechanism.

The geometries of the **1**-**13**, and the addition products of **5** with piperidine and benzylamine were optimized on a semiempirical MO level, using PM6 method,[5] as implemented in MOPAC 2009.[6] In this way obtained geometries were used for the single-point calculations at the DFT level[7] (B3LYP/6-311G**), with and without implicit solvent model (CH_3OH). DFT Calculations were performed by Gaussian03. Mulliken atomic charges, obtained from DFT calculations, were used to examine correlation between k_2 and atomic charges on an electrophilic center. Results are shown on Figure 3.

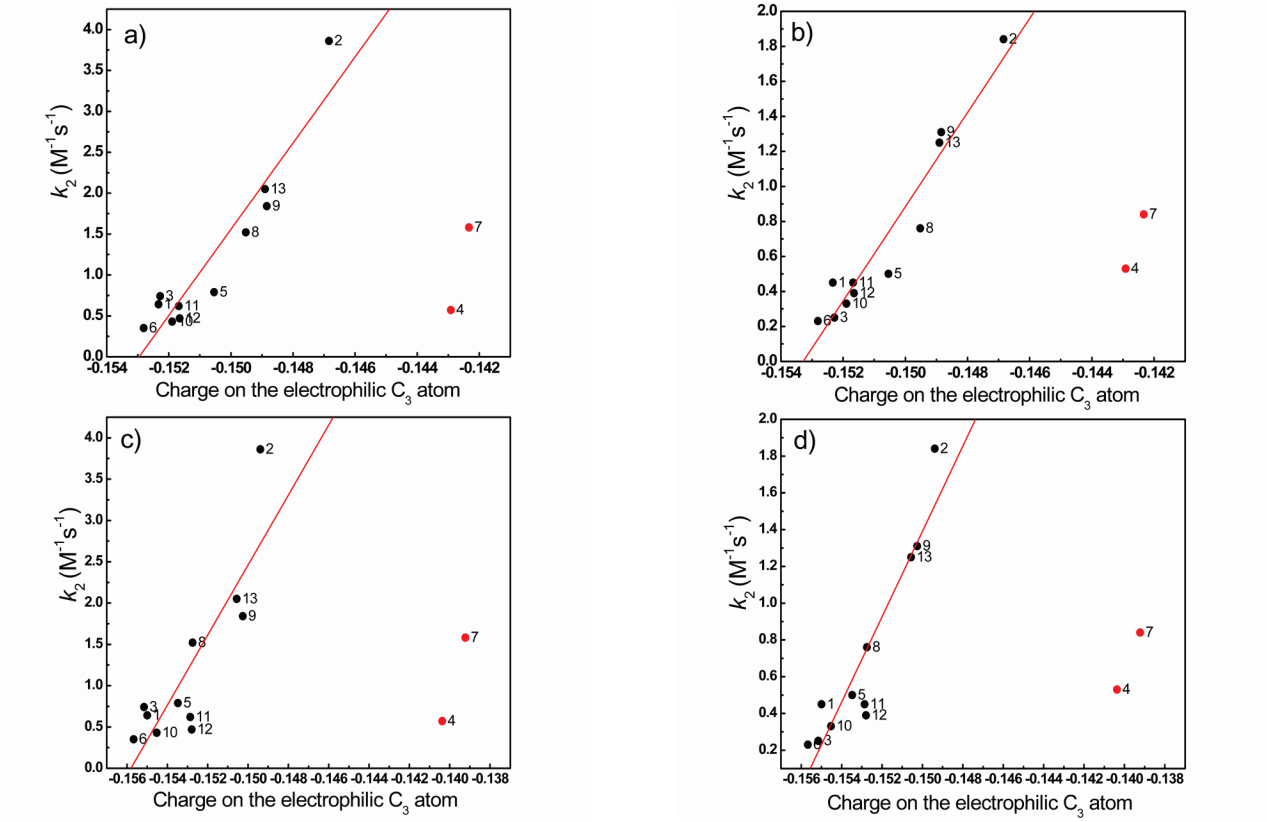


*Figure 3. Correlation of the rate constants for the addition of piperidine and benzylamine to compounds **1**-**13** with the calculated Mulliken atomic charges; a) Charges obtained without implicit solvent model and the rate constant for piperidine (r = 0.94) and b) benzylamine (r = 0.96); c) Charges obtained with the implicit solvent model (CH_3OH) in correlation with the rate constant for piperidine (r = 0.85) and d) benzylamine (r = 0.93).*

Statistically superrior correlations were found for the Mulliken charges obtained in vacuum, compared to implicit solvent model. Compounds **4** and **7** having *ortho*- substituents were omitted from Hammett corelations, because corresponding σ_o values could not be defined. Simillarly, the same compounds were outliers in correlations with atomic charges.

Molecular electrostatic potential (MEP) maps, with charges obtained on DFT level, show difference in the charge distribution around electrophylic center among **2**, **4** and **6** (**Figure 4**). Pictures are created by MOLEKEL software,[8] and Maestro.[9]

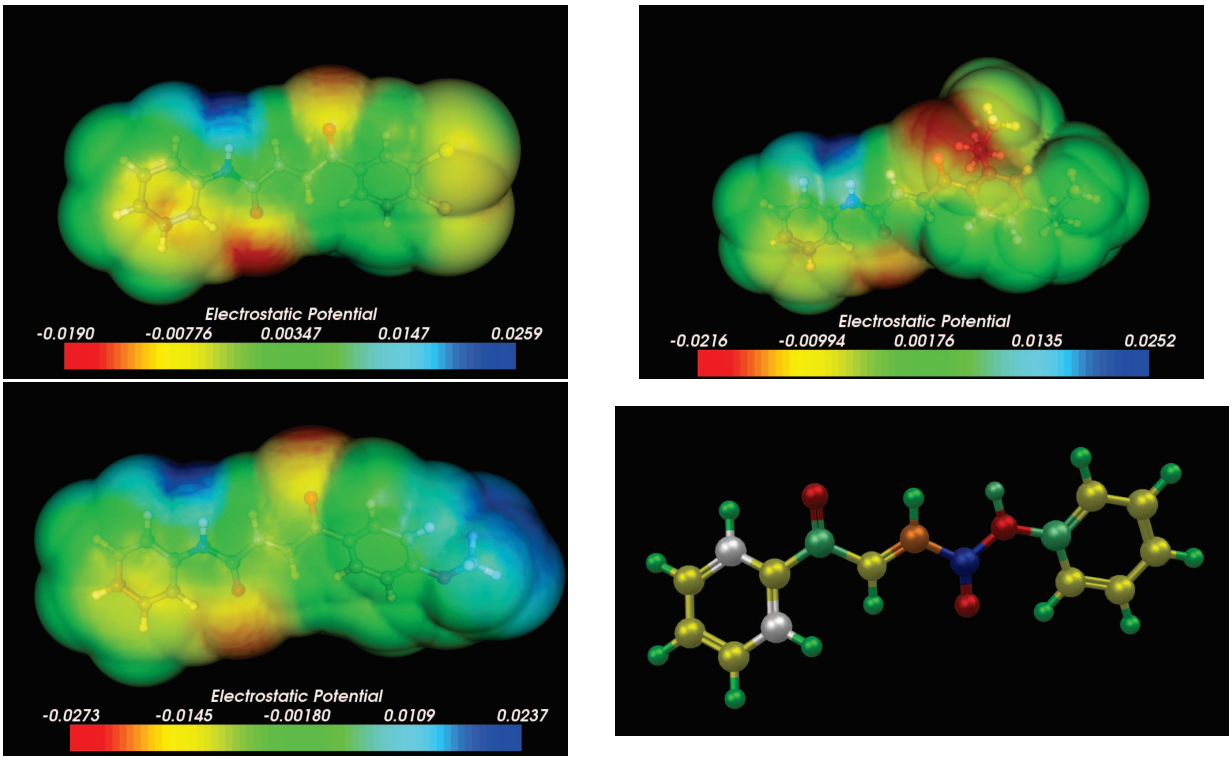


*Figure 4. Molecular electrostatic potential (MEP) mapped on solvent accessible area (1.4 Å probe - water) of **2** (upper left), **4** (upper right) and **6** (lower left). Lower right picture represents molecule **5** colored by Mulliken atomic charge; blue color- charge ≥ 0.25, red color- charge ≤ -0.25.*

Conclusion

Rate constants of the addition of piperidine and benzylamine to compounds **1**-**13** were determined spectrophotometrically. The addition product of **13** with piperidine was isolated and characterized. Rate constants were well correlated with the Hammett substituent constants and calculated charges on C_3 atom. MEP Maps provided insight on the differences in electron density around reaction center. Hammett reaction constants, ρ, gave an indication of the possible mechanism of conjugated 1,4- addition of amines to the aroylacrilic acid phenylamides. The further experimental and theoretical investigation of this biologically and synthetically important reaction will be conducted.

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Кинетика и механизам адиције пиперидина и бензиламина на фениламиде ароилакрилних киселина

Константе брзине реакције адиције депротонованог пиперидина и бензиламина на конгенерну серију фениламида ароилакрилних киселина у метанолу одређене су помоћу UV-VIS спектрофотометрије, под условима псеудо-првог реда. Утицај супституената на константе брзина реакције квантификован је помоћу Хаметових константи супституената и наелектрисања израчунатих за електрофилни угљеников атом (са и без модела имплицитног растварача), употребом DFT метода. Вредности Хаметових реакционих константи и испитивање реакције у различитим растварачима дају индикације о вероватном механизму ове реакције.

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Keto-enol Tautomerism of Aryldiketo Acids in Aqueous Solution: NMR Spectroscopy and Cyclic Voltammetry Study

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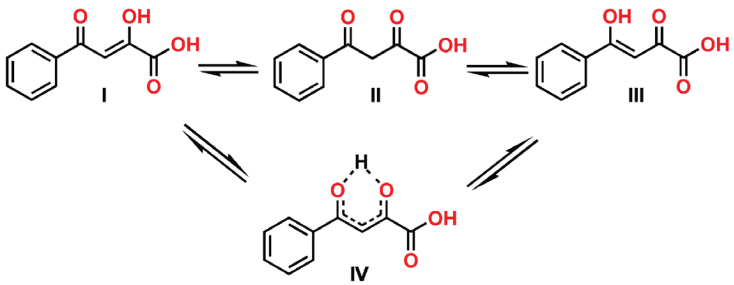
Introduction

β-Diketo acids and derivatives are the first, and so far the most successful group of compounds that selectively inhibit the integration of HIV-1 viral DNA in the host genome.[1-4] These compounds simultaneously exist in two enolate forms (**I** and **III**), conformationally locked by the pseudo-ring; and one diketo form (**II**) having two rotatable bonds responsible for the conformational flexibility (***Scheme 1***).[5-7]

Aryldiketo acids (ADK) act by functional sequestration of Mg^{2+} ion, an integral part of the active center of HIV-1 integrase (IN). This enzyme is responsible for integration of viral DNA in host genome. It was shown that ADK complexation ability depends on tautomeric form that is dominant in solution,[8] and that Mg^{2+} preferentially reacts with enolate form **I** (***Scheme 1***).[9] Furthermore, hydrolytic C–C bond cleavage of *β*-diketones by *β*-ketolases (mammals liver enzyme) is sensitive to tautomeric form in which a *β*-diketone is present in solution.[5,9,10]



Scheme 1. Tautomerization of 4-phenyl-2,4-dioxobutanoic acid (H- ADK) in aqueous solution.

Detailed study of keto-enol tautomerism in the set of eleven 4-alkyl- or 4-aryl-2,4-diketo acids revealed that in aprotic solvent ($CDCl_3$) enolate **I** is the predominant form (98%).[5] In aqueous media, tautomerization of aliphatic 2,4-diketo acids was discussed in the pH range 1.5–10. As aromatic 2,4-diketo acids (ADK) are much less soluble, their tautomerization was studied in solutions with pH ≥ 5.5, but three tautomeric forms (**I–III**) could not be distinguished due to the formation of pseudodienolate (**IV**) and fast interconversion between two enolate forms. No data on keto-enol tautomerism of ADK in aqueous solution with pH ≤ 5.5 was published so far.

Congeneric set of 3-, 4-, 3,4- and 2,5-phenyl substituted ADK was previously synthesized and studied within our research group.[11] During routine characterization, mass spectra of 3-alkyl substituted compounds showed significantly more intensive 2(M–1)+Na than 2M–1 peaks, opposite to other derivatives. UV-Vis and ^{1}H NMR spectroscopy revealed that of all studied ADK, just unsubstituted derivative (H- ADK, ***Scheme 1***), 3,4-di-Me-, 2,5-di-Me-, and *β*-naphtyl- ADK showed significant spectral changes upon the addition of Mg^{2+}, indicating their better complexation ability with Mg^{2+} ion.

The primary aim of this work was to study tautomeric equilibria of H-, 2,5-di-Me-, 3,4-di-Me-, and 4-Me- ADK (***Table 2***) in the aqueous solution within pH range 1–10. *β*-Naphtyl-ADK was omitted due to its insufficient solubility, and 4-Me- derivative was used as congener that showed no complexation ability with Mg^{2+} ion.[11]

Results and Discussion

ADK are diprotic acids weakly soluble in water, especially in acidic media where they are present in molecular (H_2A) form. Usable NMR spectra were obtained only for H- ADK after overnight signal acquisition (***Fig. 1***). Full structure-spectra assignments were achieved using COSY, HMQC, and HMBC spectra of H- ADK in CF_3COOD (***Table 1***), where H- ADK is present in its molecular (H_2A) form.

When dissolved in highly acidic media (CF_3COOD), H- ADK is in molecular (H_2A) form and the enolate **I** is predominant (***Fig. 1***). The singlet at 7.23 *ppm* in ^{1}H NMR spectrum is the signal of the vinyl group atom H_3 (***Table 1***). As the exchange rate between H_3 and D atom in CF_3COOD is fast, the integral of this signal is smaller than expected. Furthermore, the lack of a singlet around 4.5 *ppm* in ^{1}H NMR spectrum (not shown), as well as

a signal around 50 *ppm* in ^{13}C NMR spectrum which would correspond to $>CH_2$ group in diketo form **II,** confirm that diketo form does not exist. Signals at 7.33 *ppm* (*s*, 1H), 7.80 *ppm* (*t*, 1H), and 8.12 *ppm* (*d*, 2H), although very weak, confirm the existence of enolate **III**, but its concentration is negligible.

Table 1. ^{1}H and ^{13}C NMR chemical shifts of H- ADK in CF_3COOD (atom numeration as given in Table 2).

Atom	C_1	C_2	C_3	H_3	C_4	C_5	C_6	H_6	C_7	H_7	C_8	H_8
δ (*ppm*)	163.41	161.59	95.06	7.23[a)]	189.46	129.85	124.05	7.96[b)]	125.05	7.50[c)]	131.13	7.64[d)]

[a)](*s*, 1H); [b)](*d*, *J* = 7.37 Hz, 2H); [c)](*t*, *J* = 7.90 Hz, 2H); [d)](*t*, *J* = 7.47 Hz, 1H)

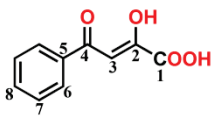
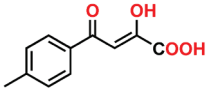
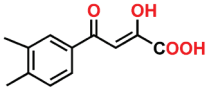
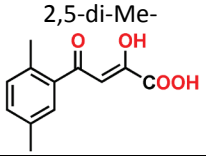
Figure 1. Characteristic parts of NMR spectra of H- ADK: a) ^{1}H NMR in CF_3COOD, b) ^{13}C NMR in CF_3COOD, c) ^{1}H NMR in D-acetate buffer pD 4.81, d) ^{1}H NMR in H-carbonate buffer pH 9.60.

When dissolved in highly acidic media (CF_3COOD), H- ADK is in molecular (H_2A) form and the enolate **I** is predominant (***Fig. 1***). The singlet at 7.23 *ppm* in ^{1}H NMR spectrum is the signal of the vinyl group atom H_3 (***Table 1***). As the exchange rate between H_3 and D atom in CF_3COOD is fast, the integral of this signal is smaller than expected. Furthermore, the lack of a singlet around 4.5 *ppm* in ^{1}H NMR spectrum (not shown), as well as a signal around 50 *ppm* in ^{13}C NMR spectrum which would correspond to $>CH_2$ group in diketo form **II,** confirm that diketo form does not exist. Signals at 7.33 *ppm* (*s*, 1H), 7.80 *ppm* (*t*, 1H), and 8.12 *ppm* (*d*, 2H), although very weak, confirm the existence of enolate **III**, but its concentration is negligible.

Due to long signal acquisition time, for all other solutions with different pH values, just ^{1}H NMR spectra were recorded. According to previously determined acidity constants (***Table 2***),[14] pD 4.8 (HA^- form) and pH 9.6 (A^{2-}) were chosen as solutions where just one form of ADK exists.

No signals of enolate **III,** and diketo form **II** were observed in ^{1}H NMR spectra in acetate (HA^- form) and carbonate (A^{2-}) buffers (***Fig. 1c*** and ***1d***). The reason for rather complicated structure of ^{1}H NMR spectrum of HA^- (***Fig. 1c***) is possible rotation around C_3–C_4 single bond and the presence of *Z* and *E* isomers of the –CH=CH(OH)– bond. In A^{2-} form *E* isomer is predominant, due to electrostatic repulsion between $–COO^-$ and $–CH=C–O^-$. Since *π*-electron delocalization occurs in keto-enol part of the molecule, the

Table 2. Spectrophotometrically determined pK_a values.[14]

Compound (ADK)	pK_{a1} ± SD pK_{a2} ± SD
H- 	2.06 ± 0.03 7.56 ± 0.02
4-Me- 	2.22 ± 0.05 7.99 ± 0.02
3,4-di-Me- 	2.09 ± 0.04 7.92 ± 0.04
2,5-di-Me- 	2.39 ± 0.04 7.23 ± 0.04

distinction between tautomers is not possible. Thus, the origin of signals at 7.82 *ppm* (*d*) and 7.57 *ppm* (*t*) is still unsolved (***Fig. 1d***).

As no data about 4-Me-, 3,4-di-Me-, and 2,5-di-Me- ADK were obtained by NMR, cyclic voltammetry was used as a method with much lower detection limit than NMR spectroscopy. Behavior of all four studied ADK was monitored in solution where these ADK are present in H_2A (pH 1), HA^- (pH 5), or A^{2-} (pH 10) form (***Fig. 2***).

Under the electrochemical conditions used, H-, 3,4-di-Me-, and 4-Me- ADK in H_2A form (***Fig. 2a***) show sharp anodic peak, and no reversible cathodic peak. As pH value is raised, the –COOH group dissociate and, as expected, the peak is lowered and moved to lower oxidation potential (***Fig. 2b***). With the dissociation of –OH group (A^{2-} form) and π-electron delocalization, peaks are completely lost (***Fig. 2c***).

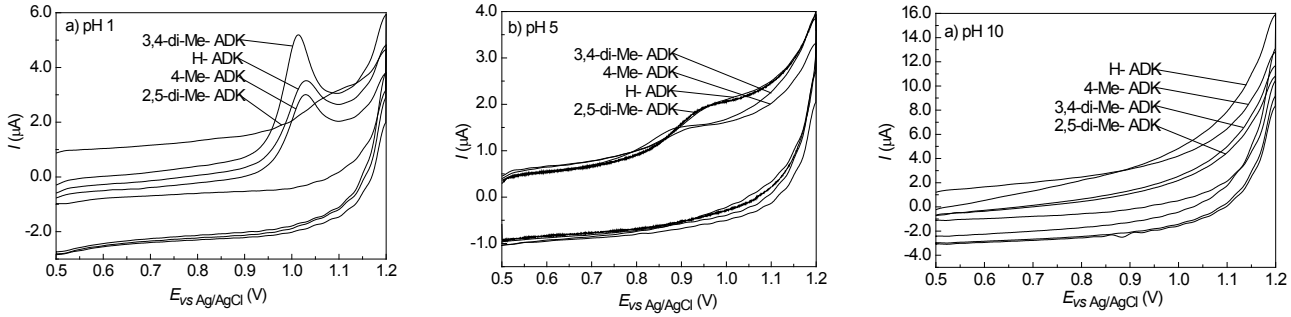


Figure 2. Cyclic voltammograms of studied ADK in aqueous solutions of different pH values.

The cyclic voltammogram (CV) of 2,5-di-Me- in H_2A form (***Fig. 2a***) is different than corresponding CVs of other ADK: characteristic peak is moved toward higher potentials and is not as sharp as peaks of other compounds. Still, the areas under all four observed peaks are the same, which implies that the electron exchange reaction is the same for all four ADK. Derivatization of CV at pH 1 shows that this wide peak consists of two overlapped peaks. This was confirmed in solutions with pH 2 and pH 3 (data not shown), where the mixture of H_2A and HA^- forms exists. If two different tautomers were present in solution, peaks in CV would be wide and overlapped. So, we may offer a possible explanation: in H_2A form, 2,5-di-Me- exists simultaneously in two tautomeric forms, probably as enolates **I** and **III** (***Scheme 1***). As the shape of all other peaks is the same, and as we have confirmed that the dominant form of H- ADK in solutions within pH range 1-10 is enolate **I**, we may conclude that, when in H_2A form, 3,4-di-Me- and 4-Me- ADK are also present as enolate **I**.

The conformation and electronic properties of studied ADK in their H_2A form, calculated using semiempirical molecular-orbital method, suggests possible reasons for different behaviour of 2,5-di-Me- ADK.

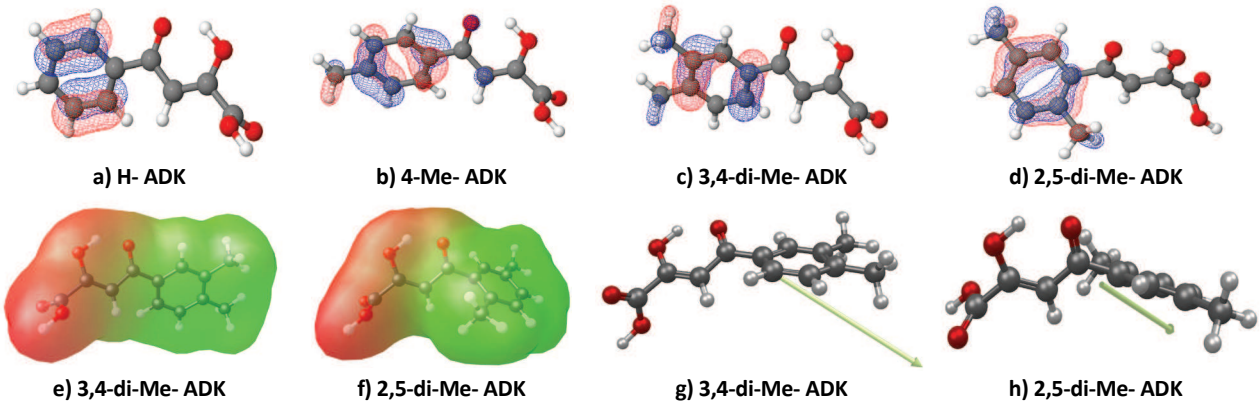


Figure 3. (a-d) HOMO of studied ADK; (e and *f) polar and apolar surface area for 3,4-di-Me- and 2,5-di-Me- derivatives;(g* and *h) dipole moments for 3,4-di-Me- and 2,5-di-Me- derivatives.*

Presence of *ortho*-alkyl substituent in 2,5-di-Me- ADK causes large torsion between aryl- and dioxo-carboxyl moiety. HOMO-s are located on phenyls in all studied congeners (***Fig. 3a - d***). Approach to electrode of the dioxo-carboxyl moiety, one most prone to electrochemical oxidation in 2,5-di-Me- derivative, is hindered in comparison to other compounds studied (shown by spatial arrangement of polar and apolar surface areas for 3,4-di-Me- and 2,5-di-Me- derivatives, ***Fig. 3e*** and ***3f***). Calculated heats of formation of all compounds in their molecular and radical cation forms show that 2,5-di-Me- derivative is the most stable one. Along with this, due

to its geometry, 2,5-di-Me- derivative has a lowest dipole in the set (***Fig. 3g*** and ***3h***), and consequently, can experience the attractive force in lesser extent than other derivatives when approaches the electrode.

Conclusion

Aryldiketo acids (ADK) complexation ability with Mg^{2+} ion (present in HIV-1 IN) and the hydrolytic C–C bond cleavage by *β*-ketolases depend on predominant tautomeric form in which they are present at the reaction site. Thus, the keto-enol tautomerism of H-, 4-Me-, 2,5-di-Me-, and 3,4-di-Me- ADK in the aqueous solution within pH range 1–10 was studied by NMR spectroscopy and cyclic voltammetry. The NMR results for H- ADK showed that the predominant tautomeric form in the studied pH range is the enolate **I** (***Scheme 1***). The cyclic voltammetry data in acidic media (pH 1) suggest that the ratio of possible tautomeric forms for 4-Me- and 3,4-di-Me- ADK is similar to H- ADK, *i.e.* that enolate **I** is the dominant form in the solution, whilst two tautomeric forms of 2,5-di-Me- ADK (enolates **I** and **III**) may be present in solution. The higher oxidation potential observed for H_2A form of 2,5-di-Me- ADK at pH 1 may be due to non-planarity of molecule and steric hindrance imposed by *ortho* substituents.

Experimental

^1H, ^{13}C, COSY, HMQC, and HMBC NMR spectra were acquired using Bruker Avance 500/125 MHz NMR spectrometer at $t = 25 \pm 1$ ºC, and constant ionic strength $I = 0.1$ M ($NaNO_3$ was used to adjust the ionic strength). TSP was used as the internal standard for spectra calibration; chemical shifts (δ) are given in *ppm*. pH Values were measured using Corning 120 pH-meter equipped with Corning Ag/AgCl microelectrode and converted to pD according to relation: $pD = pH_{measured} + 0{,}4$.[12,13]

Cyclic voltammograms were recorded using CHI760B instrument (CH Instruments, USA). The cell was equipped with glassy carbon electrode and an accessory Pt electrode of larger surface (Model CHI221, cell top including Pt wire counter electrode) and Ag/AgCl reference electrode (Model CHI111). Scan speed 100 mV/s.

Initial 3D structures of compounds were generated by OMEGA,[15] and optimized on semiempirical molecular-orbital level using PM6 method,[16] implemented in MOPAC2009,[17] to root mean square gradient bellow 0.001 kcal/mol Å. Implicit solvation in water was used. Molecular orbitals were visualized by Jmol.[18]

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Proučavanje keto-enolne tautomerije arildiketo kiselina u vodenoj sredini upotrebom NMR spektroskopije i ciklične voltametrije

*β-Diketo kiseline i njihovi derivati su prva, i do sada najuspešnija, grupa jedinjenja koja selektivno inhibira proces integracije HIV-1 provirusne DNK u DNK ćelije domaćina. Poznato je da β-diketoni u rastvoru podležu reakciji tautomerizacije i da afinitet ka kompleksiranju sa Mg^{2+} jonom (nalazi se u aktivnom centru HIV-1 integraze), kao i reakcija hidrolitičkog raskidanja C–C veze u diketo delu molekula β-ketolazama (enzim jetre sisara) zavise od oblika u kom se ova jedinjenja nalaze na mestu dejstva. U ovom radu je, NMR spektroskopijom i cikličnom voltametrijom, proučavana keto-enolna tautomerija četiri jedinjenja iz grupe aril diketo kiselina (H-, 4-Me-, 2,5-di-Me- i 3,4-di-Me- ADK) u vodenom rastvoru u pH oblasti 1–10. Rezultati dobijeni NMR spektroskopijom pokazuju da se, u ispitivanoj pH oblasti, nesupstituisana ADK (H-) nalazi u obliku enola **I**, enolni oblik **III** je prisutan u tragovima, dok signali koji bi poticali od diketo oblika nisu vidljivi u spektrima. Prema cikličnim voltamogramima izveden je zaključak da se, kada su u molekulskom (H_2A) obliku, 4-Me i 3,4-di-Me- ADK u rastvoru nalaze takođe u obliku enola **I**, dok je u rastvoru 2,5-di-Me ADK prisutna smeša tautomera i to najverovatnije enola **I** i **III**. Izračunavanja dobijena semiempirijskim molekulsko-orbitalnim PM6 metodom nude moguće objašnjenje: veći torzioni ugao između aril grupe i ostatka molekula kod 2,5-di-Me ADK može biti razlog višeg potencijala na kome se ova ADK oksiduje, u poređenju sa ostalima.*

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