XLIX savetovanje
SRPSKOG HEMIJSKOG DRUŠTVA

KNJIGA RADOVA

49th Meeting of the Serbian Chemical Society

Proceedings

Kragujevac, May 13-14, 2011

KNJIGA RADOVA
49th Meeting of the Serbian Chemical Society, Kragujevac, Serbia, May 13-14, 2011

PROCEEDINGS

Izdaje / Published by
Srpsko hemijsko društvo / Serbian Chemical Society
Karnegijeva 4/III, Beograd, Srbija, tel./fax: 011 3370 467; www.shd.org.rs, E-mail: Office@shd.org.rs

Za izdavača / For Publisher
Ivanka POPOVIĆ, predsednik Društva

Urednici / Editors
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Dizajn, slog i kompjuterska obrada teksta / Design, Page Making and Computer Layout
Aleksandar DEKANSKI

Tiraž / Circulation
200 primeraka / 200 Copy

Umnožavanje / Copying
Razvojno-istraživački centar grafičkog inženjerstva TMF - Karnegijeva 4/III, Beograd, Srbija

ISBN 978-86-7132-046-7
Kinetic and mechanism of the addition of piperidine and benzylamine to the arylacrylic 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. 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 scheme, calculated charges and the Hammett substituent constants.

<table>
<thead>
<tr>
<th>Comp N°</th>
<th>R-</th>
<th>$k_2$ (M⁻¹s⁻¹) (piperidine)</th>
<th>$k_2$ (M⁻¹s⁻¹) (benzylamine)</th>
<th>SP charge* (vacuum)</th>
<th>SP charge* (MeOH)</th>
<th>$\sigma^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>tetralinil</td>
<td>0.64 (±0.05)</td>
<td>0.45 (±0.06)</td>
<td>−0.1523</td>
<td>−0.15499</td>
<td>−0.48</td>
</tr>
<tr>
<td>2</td>
<td>3,4-di-Cl</td>
<td>3.87 (±0.27)</td>
<td>1.84 (±0.22)</td>
<td>−0.14684</td>
<td>−0.14939</td>
<td>0.52</td>
</tr>
<tr>
<td>3</td>
<td>3,4-di-Me</td>
<td>0.74 (±0.13)</td>
<td>0.25 (±0.01)</td>
<td>−0.15228</td>
<td>−0.15515</td>
<td>−0.14</td>
</tr>
<tr>
<td>4</td>
<td>2,4-di-i-Pr</td>
<td>0.57 (±0.01)</td>
<td>0.53 (±0.03)</td>
<td>−0.14291</td>
<td>−0.14037</td>
<td>/</td>
</tr>
<tr>
<td>5</td>
<td>4-H</td>
<td>0.79 (±0.09)</td>
<td>0.50 (±0.01)</td>
<td>−0.15054</td>
<td>−0.15347</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>4-OMe</td>
<td>0.35 (±0.03)</td>
<td>0.23 (±0.03)</td>
<td>−0.15281</td>
<td>−0.15567</td>
<td>−0.27</td>
</tr>
<tr>
<td>7</td>
<td>2,5-di-Me</td>
<td>1.58 (±0.12)</td>
<td>0.84 (±0.04)</td>
<td>−0.14233</td>
<td>−0.13922</td>
<td>/</td>
</tr>
<tr>
<td>8</td>
<td>4-F</td>
<td>1.52 (±0.12)</td>
<td>0.76 (±0.08)</td>
<td>−0.14952</td>
<td>−0.15274</td>
<td>0.06</td>
</tr>
<tr>
<td>9</td>
<td>4-Br</td>
<td>1.84 (±0.15)</td>
<td>1.31 (±0.05)</td>
<td>−0.14884</td>
<td>−0.15026</td>
<td>0.23</td>
</tr>
<tr>
<td>10</td>
<td>4-n-Bu</td>
<td>0.43 (±0.05)</td>
<td>0.33 (±0.03)</td>
<td>−0.15189</td>
<td>−0.15452</td>
<td>−0.16</td>
</tr>
<tr>
<td>11</td>
<td>4-t-Bu</td>
<td>0.62 (±0.06)</td>
<td>0.45 (±0.02)</td>
<td>−0.15168</td>
<td>−0.15286</td>
<td>−0.20</td>
</tr>
<tr>
<td>12</td>
<td>4-i-Pr</td>
<td>0.47 (±0.05)</td>
<td>0.39 (±0.01)</td>
<td>−0.15165</td>
<td>−0.15279</td>
<td>−0.15</td>
</tr>
<tr>
<td>13</td>
<td>4-Cl</td>
<td>2.05 (±0.19)</td>
<td>1.25 (±0.13)</td>
<td>−0.14890</td>
<td>−0.15056</td>
<td>0.23</td>
</tr>
</tbody>
</table>

* 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 C3 atom, not to the C2 (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, following the decrease of absorbance on ~310 nm, using the 20-fold excess of amine (Figure 1).
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 ($\sigma_s$), as given by Equation 1:

$$\log \frac{k}{k_0} = \rho \sigma_s$$

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

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

\[
\log \left( \frac{k}{k_0} \right) = 1.35(\pm0.15) \cdot \sigma_X
\]

\[ r = 0.96, s \quad 0.11 \leq N \quad 10, P \quad 0.0001 \]

Benzyamine:

\[
\log \left( \frac{k}{k_0} \right) = 1.21(\pm0.15) \cdot \sigma_X
\]

\[ r = 0.95, s \quad 0.11 \leq N \quad 10, P \quad 0.0001 \]

Reaction constant can be calculated as the slope of Hammett plots (Figure 2a and 2b). Reaction constant, \( \rho \), 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 benzyamine (\( \rho = 1.35 \), and \( \rho = 1.21 \), 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.\(^6\) 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 benzyamine 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\(_3\)OH). 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.

Statistically superior 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 correlations, because corresponding \( \sigma \) values could not be defined. Similarly, 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 electrophilic center among 2, 4 and 6 (Figure 4). Pictures are created by MOLEKEL software, and Maestro.

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 C3 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 arylacrylic acid phenylamides. The further experimental and theoretical investigation of this biologically and synthetically important reaction will be conducted.

Acknowledgement: The Ministry of Science and Technological Development of Serbia supports this work. Grant 172035.

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

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**Innovation Center of the Faculty of Chemistry, Univ. of Belgrade, Studentski Trg 16, Belgrade, Serbia.

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. 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). Aryldiketo acids (ADK) act by functional sequestration of Mg2+ 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, and that Mg2+ preferentially reacts with enolate form I (Scheme 1). 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. 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 (CDCl3) enolate I is the predominant form (98%). In aqueous media, tautomeration of all phatic 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 tautomeration 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. 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 1H 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 Mg2+, indicating their better complexation ability with Mg2+ ion.

Results and Discussion

ADK are diprotic acids weakly soluble in water, especially in acidic media where they are present in molecular (H2A) 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 CF3COOD (Table 1), where H- ADK is present in its molecular (H2A) form. When dissolved in highly acidic media (CF3COOD), H- ADK is in molecular (H2A) form and the enolate I is predominant (Fig. 1). The singlet at 7.23 ppm in 1H NMR spectrum is the signal of the vinyl group atom H3 (Table 1). As the exchange rate between H3 and D atom in CF3COOD is fast, the integral of this signal is smaller than expected. Furthermore, the lack of a singlet around 4.5 ppm in 1H 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$_3$COOD (atom numeration as given in Table 2).

<table>
<thead>
<tr>
<th>Atom</th>
<th>$^1$H (ppm)</th>
<th>$^{13}$C (ppm)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>C1</td>
<td>C2</td>
</tr>
<tr>
<td>δ</td>
<td>163.41</td>
<td>161.59</td>
</tr>
</tbody>
</table>

$^{a}$[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$_3$COOD, b) $^{13}$C NMR in CF$_3$COOD, c) $^1$H NMR in D-acetate buffer pH 4.81, d) $^1$H NMR in H-carbonate buffer pH 9.60.

When dissolved in highly acidic media (CF$_3$COOD), H- ADK is in molecular (H$_2$A) 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$_3$COOD 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),$^{14}$ pH 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$_2$-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>
<thead>
<tr>
<th>Compound (ADK)</th>
<th>$pK_{a1}$ ± SD</th>
<th>$pK_{a2}$ ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-ADK</td>
<td>2.06 ± 0.03</td>
<td>7.56 ± 0.02</td>
</tr>
<tr>
<td>4-Me-ADK</td>
<td>2.22 ± 0.05</td>
<td>7.99 ± 0.02</td>
</tr>
<tr>
<td>3,4-di-Me-ADK</td>
<td>2.09 ± 0.04</td>
<td>7.92 ± 0.04</td>
</tr>
<tr>
<td>2,5-di-Me-ADK</td>
<td>2.39 ± 0.04</td>
<td>7.23 ± 0.04</td>
</tr>
</tbody>
</table>
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 H₂A form (Fig. 2). Under the electrochemical conditions used, H-, 3,4-di-Me-, and 4-Me- ADK in H₂A form (Fig. 2a) show sharp anodic peak, and no reversible cathodic peak. As pH value is raised, the –COOH group dissociates, and, as expected, the peak is lowered and moved to lower oxidation potential (Fig. 2b). With the dissociation of –OH group (A²⁻ form) and π-electron delocalization, peaks are completely lost (Fig. 2c).

The cyclic voltammogram (CV) of 2,5-di-Me- in H₂A 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 forms. If two different tautomers were present in solution, peaks in CV would be wide and overlapped. So, we may offer a possible explanation: in H₂A 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 H₂A 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 H₂A form, calculated using semiempirical molecular-orbital method, suggests possible reasons for different behaviour of 2,5-di-Me- ADK.

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

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

**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.
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 6-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 voltammograms 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 H2A 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

\(^{1}\)H, \(^{13}\)C, COSY, HMQC, and HMBC NMR spectra were acquired using Bruker Avance 500/125 MHz NMR spectrometer at \(T = 25 \pm 1 \, ^{\circ}\)C, and constant ionic strength \(I = 0.1 \, \text{M} \) (NaNO\(_2\) was used to adjust the ionic strength). TSP was used as the internal standard for spectra calibration; chemical shifts (\(\delta\)) are given in ppm. pH Values were measured using Corning 120 pH-meter equipped with Corning Ag/AgCl microelectrode and converted to pH according to relation: \(pD = pH_{\text{measured}} + 0.4\). 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, and optimized on semiempirical molecular-orbital level using PM6 method, implemented in MOPAC2009, to root mean square gradient bellow 0.001 kcal/mol Å. Implicit solvation in water was used. Molecular orbitals were visualized by Jmol.

Acknowledgment: The authors highly appreciate the help and comments provided by MSc Branko J. Drakulić, ICHTM. The Ministry of Education and Science of Serbia supports this work. Grants 172035 and 172030.

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

6-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 6-diketoni u rastvoru podležu reakciji tautomerizacije i da afinitet ka kompleksiranju sa Mg\(^{2+}\) jonom (nalazi se u aktivnom centru HIV-1 integraza), kao i reakcija hidrolitičkog raskidanja C–C veze u diketo delu molekule 6-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 vodenoj 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 trigovima, dok signali koji bi poticali od diketo oblika nisu vidljivi u spektrom. Prema cikličnim voltamogramima izveden je zaključak da se, kada su u molekulskom (H\(_2\)A) 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 tautomerija i to najverovatnije enola I i III. Izračunavanja dobijena semipiemijskim 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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