The Effect of Phenyl Substituents on ^{13}C NMR Shifts and Metal Ions Binding to 4-Phenyl-2,4-Dioxobutanoic Acid Derivatives

Tatjana Ž. Verbić[a], Branko J. Drakulić*[,b], Mire Zloh[c] and Ivan O. Juranić[a]

[a]*Faculty of Chemistry, University of Belgrade, Studentski Trg 12-16, 11000 Belgrade, Serbia;* [b]*Department of Chemistry - Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia;* [c]*The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX, UK*

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Abstract: Butanoic moiety of 4-aryl-2,4-dioxobutanoic acids is involved in interactions with metal ions within HIV-1 integrase active site. Sixteen congeneric 4-phenyl-2,4-dioxobutanoic acid derivatives with different substitution on the phenyl ring were prepared. Effects of substitution were studied by spectrometric methods (NMR, MS, UV/VIS) and linear free energy relationships. Better metal complexation ability of *meta*-alkyl substituted compounds, was observed. This observation might have pharmacological implications.

Keywords: 4-Aryl-2,4-dioxobutanoic acids, Linear free energy relationships, NMR, MS, UV/VIS spectroscopy, metal complexation ability.

INTRODUCTION

Recently, antiretroviral activity of 4-aryl/heteroaryl-2,4-dioxobutanoic acids (Ar–C(O)–CH_2–C(O)–COOH) has been reported. Targeting HIV-1 integrase [1-7], the enzyme responsible for integration of viral DNA in the host genome [8], as well as the Hepatitis C virus NS5b RNA-dependent RNA polymerase [9], are among the most important ones. The number of pharmacophore models concerning aforementioned type of activity is suggested [10-14].

Compounds having core structure Ph–C(O)–CH_2–C(O)–COOH can exist in two enolic forms (probably locked by intramolecular H-bonding) and one flexible diketo form (due to presence of two rotatable bonds) [15]. It is proposed that β-diketo moiety functionally sequestrate divalent metal ions, critical cofactors at the enzyme catalytic core, while the enolic forms act by ligation of one metal ion and simultaneous H-bonding to catalytic core amino acid residues [8]. Additionally, the keto-enol tautomerism of β-diketones attracts attention persistently [16-18]. Computational study on density functional theory level of two actual 4-aryl-2,4-dioxobutanoic acid based HIV-1 integrase inhibitors, was recently reported [19]. It was shown that enolic forms of studied compounds have lower relative energy than diketo forms.

Due to the importance of butanoic moiety for the inhibitory activity against HIV-1 integrase, congeneric set of 4-, 3,4- and 2,5-phenyl substituted 4-phenyl-2,4-dioxobutanoic acids, was synthesized and an influence of phenyl substitution on the butanoic moiety was examined. 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 studied derivatives. This indicates significantly better complexation ability of 3-alkyl substituted derivatives and might have pharmacological implications.

*Address correspondence to this author at the Department of Chemistry - Institute of Chemistry, Technology and Metallurgy, University of Belgrade, Njegoševa 12, 11000 Belgrade, Serbia; Tel: +381 11 3336 738; Fax: +381 11 2636 061; E-mail: bdrakuli@chem.bg.ac.yu

EXPERIMENTAL

The NMR spectra were acquired using Bruker Avance 500/125 MHz NMR instrument. Samples for NMR studies were dissolved in DMSO-d_6 (c = 0.04 mol/L) and spectra calibrated by using residual solvent signals (^{1}H: δ 2.50 *ppm*, ^{13}C NMR: δ 39.7 *ppm*). These data were used in deriving subsequent correlations. Mass spectra (LC ESI-MS) were recorded on ThermoQuest Navigator in negative mode, using MeOH as a solvent. The infrared (IR) spectra were recorded on FT Perkin-Elmer 1725X spectrometer, KBr disc. UV/VIS Spectra were recorded on GBC Cintra 6 spectrophotometer.

Synthesis and characterization of majority of studied compounds (**1**-**13**) were reported previously [20]. Additional congeners (**14**-**16**) were prepared and characterized by using same procedures:

(**14**) 4-(3,4-Dimethylphenyl)-2,4-dioxobutanoic acid M.p. 175-177 ºC, dec. white powder (6.30 g, 57 %, PhMe); M_w 220.22, $C_{12}H_{12}O_4$; M^+ 219 (100 %); 147 (64.3 %); IR ν (cm^{-1}): 1705.0; 1612.0; 1261.0. ^{1}H NMR (500 MHz, DMSO-d_6) δ 7.77 (*s*, 1H); 7.71 (*d*, J = 7.86 Hz, 1H); 7.28 (*d*, J = 7.86 Hz, 1H); 6.64 (*b*); 4.40 (*b*). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.42; 142.81; 137.34; 133.28; 130.30; 128.70; 97.93; 19.84; 19.55. ESI-MS, m/z: 219 [M-1], 100 %.

(**15**) 4-(2,5-Dimethylphenyl)-2,4-dioxobutanoic acid: M.p. 150-152 ºC, dec. white powder (7.93 g, 72 %, AcO-Et/PhH), $C_{12}H_{12}O_4$, M_w 220.22; M^+ 219 (100 %); 147 (70 %). IR ν (cm^{-1}): 1701.0; 1619.0; 1292.0; 1251.0. ^{1}H NMR (500 MHz, DMSO-d_6) δ 12.4 (*b*); 7.38 (*b*, *s*, 1H); 7.24 (*m*, 2H); 2.40 (*s*, 3H); 2.32 (*s*, 3H); 2.26 (*s*); 2.25 (*s*). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.26; 137.40; 135.36; 133.67; 131.69; 131.50; 129.08; 102.54; 20.60; 20.02. ESI-MS, m/z: 219 [M-1], 100 %.

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(**16**) 4-Naphthalen-2-yl-2,4-dioxobutanoic acid: M.p. 184-186 ºC, dec. yellow powder (8.73 g, 72 %, AcO-Et/$CHCl_3$), $C_{14}H_{10}O_4$, M_w 242.23; M^+ 241 (100 %); 169 (90 %). IR $\bar{\nu}$ (cm^{-1}): 3452.0; 1710.0; 1624.0; 1386.0; 1276.0; 1230.0. ^1H NMR (500 MHz, DMSO-d_6) δ 8.69 (*b*, *s*, 1H); 8.15 (*b*, *dd*, $J_{1,2}$ = 3.09, $J_{1,3}$ = 7.02, 1H); 8.03 (*b*, *s*, 2H); 7.99 (*b*, *d*, 1H); 7.72-7.56 (*m*, 2H, overlapped 8 signals: 7.72, 7.70, 7.67, 7.65, 7.64, 7.62, 7.61, 7.58, 7.56); 6.75 (*b*); 4.63 (*b*). ^{13}C NMR (125 MHz, DMSO-d_6) δ 164.39; 134.91; 133.62; 132.63; 129.65; 128.57; 127.84; 127.08; 123.69; 99.74. ESI-MS, m/z: 241 [M-1], 100 %.

FORMATION CONSTANTS DETERMINATION

Formation constants for Mg^{2+} complexes of **14**, **15** and **16** were spectrophotometrically determined at t = 25 ± 1 ºC, according to Job's method of continuous variation [21, 22]. Stock solutions of **14**, **15**, **16** and $MgCl_2$ were prepared in methanol (c = $1.0 \cdot 10^{-2}$ mol/L). Aliquots of stock solutions were mixed in different proportions to give four sets with total concentrations of Mg^{2+} and **14**, **15** or **16** as follows: c_1 = $1.8 \cdot 10^{-4}$ mol/L, c_2 = $1.5 \cdot 10^{-4}$ mol/L, c_3 = $1.2 \cdot 10^{-4}$ mol/L, and c_4 = $1.0 \cdot 10^{-4}$ mol/L. UV/VIS spectra were recorded over 220-500 nm range, with a scan speed of 500 nm/min. Wavelengths for determination ($\lambda_{comp\mathbf{14}}$ = 346.4 nm, $\lambda_{comp\mathbf{15}}$ = 329.3 nm and $\lambda_{comp\mathbf{16}}$ = 356.6 nm) were chosen as wavelengths of the absorption maxima in solutions with equimolar ratio of Mg^{2+} and **14**, **15**, **16**. Equation for ML_2 complex type was derived and results fitted according to Rose-Drago method [22, 23].

CALCULATIONS

Equations (1-4) were obtained by using BILIN program [24]. Values given after coefficients are twofold standard deviations. Statistical parameters are given as follows: n = number of observations, r = correlation coefficient, s = standard deviation (sd), F = Fisher test; Q^2 = Square of leave one-out correlation coefficient and s_{PRESS} = standard deviation of Q. All reported structures were optimized by semiempirical molecular orbital PM6 method [25] implemented in MOPAC 2007 [26], and implicit solvation model (COSMO). Keywords: EF, GNORM=0.01 (or 0.1 for **1**+DMSO couple), PRECISE, VECTORS, ESP=48.000, NSPA=92. Graphical presentation of molecular orbital was obtained by Jmol [27], from MOPAC output (mgf file). Other structures were visualized and H bond energies were calculated by VegaZZ 2.1.0 [28], using CHARMM force field.

RESULTS AND DISCUSSION

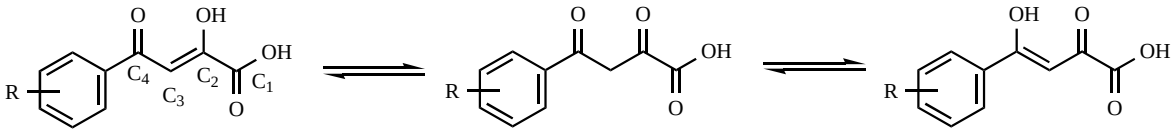
Sixteen 2-, 3- or 4-alkyl-, alkoxy-, hydroxy-, nitro-, or halo-phenyl substituted 4-phenyl-2,4-dioxobutanoic acid derivatives were used to examine phenyl substituent effects on butanoic carbons. Hammett type correlations of corresponding peaks in ^{13}C NMR spectra obtained in dimethyl sulfoxide (DMSO-d_6), were derived. In all so far reported pharmacophore models [8, 29], as well as in docking studies [8], it is proposed that enol or diketo moieties of 4-aryl-2,4-dioxobutanoic acids interact with metal ions or active site residues, loosing the intramolecular H-bonding ability. DMSO was chosen as hydrogen bond acceptor with high dielectric constant, supposing that it can interrupt intramolecular H-bonding in enolic forms, depicted as prevailing ones by NMR spectra of compounds **1**-**16**.

Recently, an exhaustive analysis (based on Cambridge Structural Database [30] bond lengths and bond angles) of β-diketone intra– and intermolecular H-bonding ability in solid state has been reported [31]. The analysis offers data related to subtle differences in recognition properties of β-diketones – pharmacophore of retroviral drugs or leads. Authors had shown that class of compounds corresponding to **1**-**16** exists mainly in enol forms, locked by strong intramolecular bonds of enolic moieties (β-ketoenol). 2070 β-Ketoenol fragments from 1524 structures, stabilized mainly by intramolecular H-bonding, are discussed. Approximately nine percent comprise of β-ketoenol moieties on "aliphatic" carbons and are classified as extremely inefficient with respect to intermolecular H-bonding, particularly toward hydrogen bond acceptors (O or N). Private communication with authors revealed additional data: only two structures [32, 33] within studied set can strictly be compared with the derivatives reported in this article (**1**-**16**), lacking >S=O as hydrogen bond donor. Furthermore, one structure [4–($MeSO_2$–Ph–NH–)C(O)–C(CN)=C(OH)–Me] [34] that can be related to our set, having $–SO_2Me$ fragment on phenyl ring, shows the ketoenol contacts with >S=O group, making strong H-bonding O–H···O=S. This supports our selection of DMSO as a solvent that can disrupt intramolecular H-bonding within keto-enol moiety of studied compounds. In this way the environment of studied compounds should be comparable with proposed pharmacophore models, as well as with docking studies (as described above).

4-Aryl-2,4-dioxobutanoic acids can exist in enol or keto form (Scheme **1**).

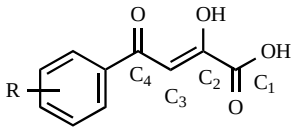
Enol form I (Scheme **1**) is the prevailing one in DMSO. Therefore, only the carbons of butanoic moiety of the enol I (Scheme **1**) are discussed. The enol/keto tautomers ratio is determined as the ratio of areas under ^1H NMR signals that correspond to one H atom at C_3 in enol and two H atoms at C_3 in keto form (sharp singlets for compounds **1**-**13**, Table **1**).

The peaks of butanoic moiety were assigned using DEPT, HMQC and HMBC spectral data.



Scheme 1. Enol (**I** and **III**) and diketo (**II**) tautomers of 4-phenyl-2,4-dioxobutanoic acids.

Table 1. Chemical Shifts of Carbons C_1 – C_4 Used in Correlations 1-4. The Enol/Keto Ratio Based on ^{1}H NMR Spectra Recorded at $t = 25 \pm 1$ °C in DMSO-d_6 is Shown in the Last Column



Com N^{o}	R-	δ (C_4)	δ (C_1)	δ (C_2)	δ (C_3)	Enol/Keto
1	H-	190.55	170.45	163.37	98.08	15.55
2	4-Me-	190.67	169.88	163.39	97.90	9.87
3	4-Et-	190.61	170.01	163.41	97.92	15.34
4	4-*i*-Pr-	190.44	170.20	163.43	97.93	16.40
5	4-*t*-Bu-	190.43	170.19	163.415	97.91	7.16
6	4-Ph-	189.91	170.57	163.42	98.11	15.74
7	4-NO_2-	186.65	172.28	163.21	98.88	16.77
8	4-OH-	190.64	168.30	163.51	97.665	16.44
9	3-OH-	190.31	170.10	163.37	98.12	14.29
10	4-MeO-	190.44	168.81	163.51	97.81	[a]
11	4-F-	189.74	169.65	163.34	98.17	11.12
12	4-Cl-	189.30	170.35	163.29	98.18	14.43
13	4-Br-	188.82	171.11	163.32	98.156	14.85
14	3,4-di-Me-	[b]	[b]	164.42	97.93	/
15	2,5-di-Me-	[b]	[b]	164.26	102.54[c]	/
16	β-naphtyl-	[b]	[b]	164.39	99.74[c]	/

[a]The enol signal fully overlaps with the aromatic ones; peak deconvolution was impossible. [b]Missing in spectrum. [c]Broad signals.

Table 2. The σ_p and σ_m Values Used for Derivation of Hammett Correlations 1-4

Com N^{o}	σ_p (C_4)	σ_p (C_1)	σ_p (C_2)	σ_p (C_3)
1	0[a]	0	0	0
2	–0.14	–0.14	–0.14	–0.14
3	–0.15	–0.15	–0.32[b]	–0.15
4	–0.15	–0.15	–0.28[b]	–0.15
5	–0.13[d]	–0.13	–0.26[b]	–0.13
6	–0.01	0.01	–0.34[b]	–0.01
7	0.78	0.78	0.78	0.78
8	–0.22	–0.92[b]	–0.92[b]	–0.37
9	–0.04[c]	–0.04[c]	–0.04[c]	0.01
10	–0.11[d]	–0.78[b]	–0.78[b]	–0.27
11	0.06	–0.09[b]	0.06	0.05
12	0.19[d]	0.11[b]	0.28	0.11[b]
13	0.25[d]	0.25[d]	0.25[d]	0.15[b]

[a]Omitted from equation derivation. [b]σ_p^+. [c]σ_m^+. [d]σ_p^-.

Correlations were built by using classical Hammett approach [35, 36]:

$$\delta = \rho\,\sigma + c$$

where, δ represents ^{13}C NMR chemical shifts of butanoic carbon atoms C_1 – C_4, ρ is "reaction constant" – measure of the efficiency of substituent effects transmission to particular carbon, and σ is substituent constant. Only sets of literature

Hammett sigmas (σ_p, σ_m) obtained for carboxylic acids dissociation, or derived either from NMR, or IR determinations, were used (all sigma values were taken from [37]).

The σ_p and σ_m values used for the derivation of Hammett correlations (1-4), are shown in Table **2**.

The following correlations were obtained by using Hammett approach:

(1) $\delta(C_4) = -4.169\ (\pm 0.510)\ \sigma_{p,m} + 190.000\ (\pm 0.130)$

(n = 13; r = 0.983; s = 0.215; F = 318.175; Q^2 = 0.951; s_{PRESS} = 0.259)

(2) $\delta(C_1) = 2.259\ (\pm 0.350)\ \sigma_{p,m} + 170.400\ (\pm 0.140)$

(n = 13; r = 0.974; s = 0.228; F = 206.093; Q^2 = 0.932; s_{PRESS} = 0.265)

(3) $\delta(C_2) = -0.185\ (\pm 0.017)\ \sigma_{p,m} + 163.400\ (\pm 0.008)$

(n = 13; r = 0.991; s = 0.012; F = 589.804; Q^2 = 0.991; s_{PRESS} = 0.012)

(4) $\delta(C_3) = 1.036\ (\pm 0.080)\ \sigma_{p,m} + 98.070\ (\pm 0.021)$

(n = 13; r = 0.993; s = 0.035; F = 812.151; Q^2 = 0.983; s_{PRESS} = 0.039)

^{13}C NMR shifts predicted by Equations 1-4 are given in Table **3**.

As expected, the highest ρ absolute value is obtained for C_4, an atom directly connected to the phenyl ring. Values of "reaction constant" ρ that quantifies the efficiency of substituent effects transmission have negative signs for aroyl keto carbon C_4 and for C_2 that is in β-position to C_4 and bears "enolic" hydroxyl group. Both carbons have direct conjugation with phenyl ring, but good correlation for C_2 was obtained for the whole set only when σ_{p+} values were introduced, as indicated in Table **2**. Otherwise good correlation was obtained only for compounds having substituents that bear lone pairs; alkyl substituted compounds were dispersed from such correlation. This could also indicate different average (or dominant, as perceived by NMR spectra) conformations of alkyl-substituted compounds with respect to other molecules within studied set. Hydroxyl group on C_2 theoretically could form H-bond with either aroyl keto group or carboxyl group. "Enolic" hydrogen on C_3 has sharp and strong signal in ^1H NMR spectra (compounds **1**-**13**), which implicitly indicate that there is no intramolecular H-bonding, furthermore, there is no exchange with aprotic solvent.

The next highest absolute value of ρ is obtained for carboxylic carbon that is the most distant one from aryl moiety. This behavior is not expected, since the effect of phenyl substituents through the bonds decreases with distance (even when conjugation is present). However, enolic forms of studied compounds optimized by PM6 semiempirical molecular orbital method, using implicit solvation model (conductor-like screening model - COSMO) in DMSO, showed that the most stable conformations, as well as local minima for almost all compounds, have carboxyl group significantly twisted with respect to the rest of the molecule probably due to repulsion with hydrogen atom on C_3. Non-planarity of molecules is causing high localization of intrinsic π-orbitals in certain parts of molecular structure. Consequently, influence on carboxylic carbon is perceived in a very comparable manner for all the compounds. As illustration, the HOMO (highest occupied molecular orbital), HOMO–1 and HOMO–2 of parent compound (**1**) obtained by energy minimization, as described above, are shown in Fig. (**1**).

It is obvious that these most polarizable orbitals are highly localized and are not suitable for efficient transfer of resonance effects to C_1. Butanoic moiety carbon atoms, C_1 and C_4, have lower electron densities than C_2 and C_3, and are relatively more affected by substituent electronic demand. Charges of carbons C_1 – C_4 on butanoic moiety of parent compound (**1**) are: 0.615, 0.344, –0.576, and 0.633, respectively.

This confirms that the effect which carboxylic carbon "experiences" through molecular skeleton, comes from far

Table 3. ^{13}C NMR Shifts Predicted by Equations 1-4

Com N^{o}	δ (C_4)	δ (C_1)	δ (C_2)	δ (C_3)
1	189.991	170.363	163.359	98.073
2	190.575	170.047	163.385	97.928
3	190.617	170.025	163.418	97.918
4	190.617	170.025	163.411	97.918
5	190.533	170.070	163.407	97.939
6	190.033	170.386	163.422	98.063
7	186.739	172.125	163.215	98.881
8	190.908	168.285	163.529	97.690
9	190.158	170.273	163.367	98.084
10	190.450	168.602	163.503	97.794
11	189.741	170.160	163.348	98.125
12	189.199	170.612	163.307	98.187
13	188.949	170.928	163.313	98.229

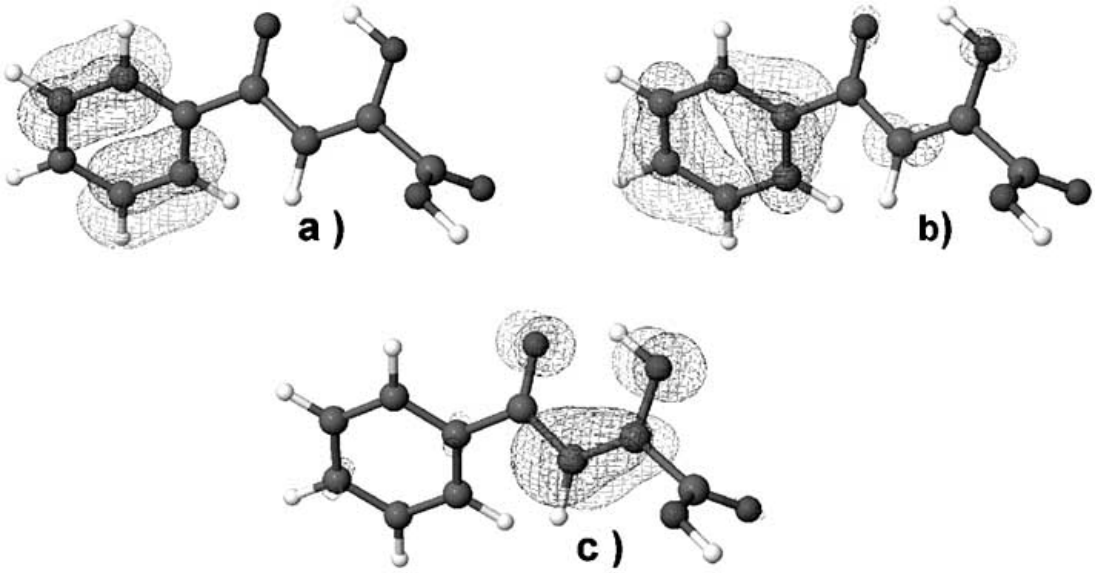


Fig. (1). a) The HOMO (–10.1125 eV), **b**) HOMO–1 (–10.1899 eV) and **c**) HOMO–2 (–10.7557 eV) of parent compound (**1**). Energy minimization of **1** was done by semiempirical MO PM6 method, with implicit solvation model (COSMO), using DMSO as a solvent. Rendering by Jmol.

phenyl substituents. Again, intramolecular H-bonding between phenyl substituents having lone pairs and carboxyl group could account for the presence of σ_p^+ for compounds **8**, **10**-**12** as well as σ_m^+ for **9**. Good correlation was also obtained for C_3 with ρ close to 1. The substituent influence on C_3, a carbon atom that follows aroyl keto carbon, is transmitted to it, regardless of variable electronic densities on C_4. Variation in C_4 is caused by small deviation from mutual coplanar arrangement of aroyl keto group and phenyl ring, with consequent minor changes of carbonyl oxygen electron densities.

Introduction of one explicit molecule of DMSO in model could be illustrative. The one DMSO molecule is optimized on semiempirical MO PM6 level and manually (and arbitrary) positioned by its oxygen above the >C=O···H-O- moiety of **1** (optimized as described above), on 25 % distance out of vdW radii of discussed atoms. The system was treated by implicit DMSO solvation (COSMO) and full optimization was carried out. After energy minimization of described couple to root mean square (RMS) gradient below 0.1, the enol H- of **1** is almost transferred to DMSO oxygen, as shown in Fig. (**2**). The H-bond distances and energies are significantly changed in comparison to **1**. Also, the torsion between phenyl carbon and aroyl keto group rises to 45°.

The =CH– group, depicted as C_3 atom, behaves like the anchor of whole butanoic moiety in studied compounds and

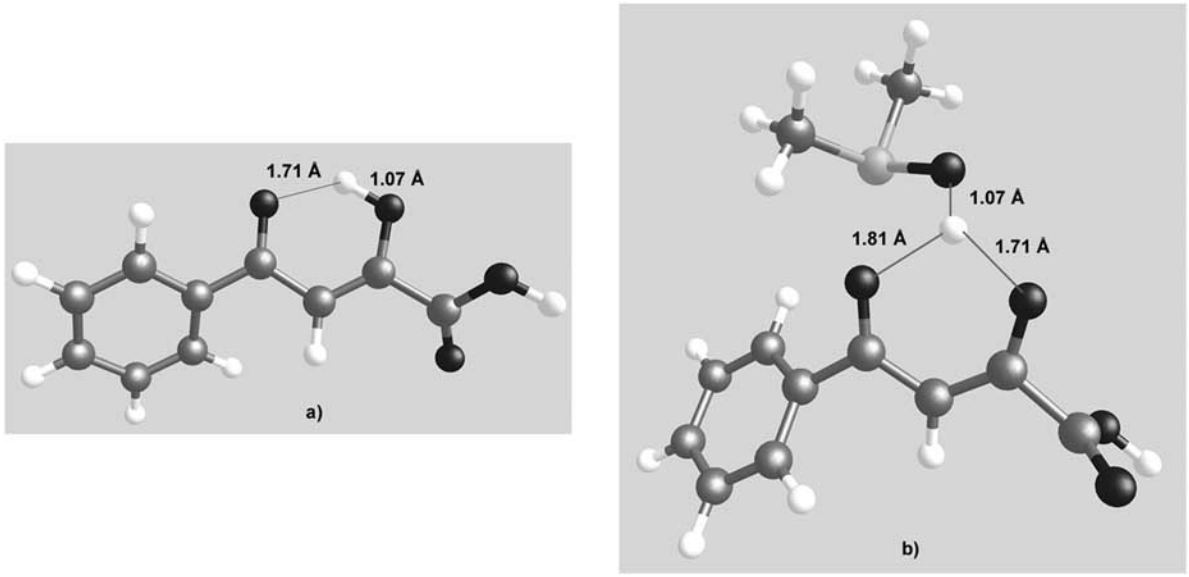


Fig. (2). a). The structure of **1** optimized by semiempirical MO PM6 method using implicit solvation in DMSO. The >C=O···H–O- distance is 1.71 Å, with ΔH_f = –0.3 kcal/mol. **b**) The structure of **1**+DMSO optimized by semiempirical MO PM6 method. The >C=O···H–O- distance is 1.81 Å, ΔH_f = –0.06 kcal/mol. The (DMSO) >S=O···H–O- (**1**) distance is 1.07 Å, ΔH_f = –0.48 kcal/mol. The enol –O-H distance of **1** is 1.71 Å (*vs.* 1.07 Å of **1** alone, as depicted in a)). The enol hydrogen repositioning is emphasized.

is most regularly influenced by changes in rest of the molecule. ^{13}C NMR chemical shifts for C_2 and C_3 carbon atoms are the only signals that are reported for compounds **14**-**16** (2,5- or 3,4-disubstituted) (Table **1**). Indeed, spectra of these compounds in DMSO-d_6 lack signals corresponding to C_1 and C_4 atoms. Accordingly, the ^1H NMR signals of H_3 in enolic form I or II and two H_3 in keto form (see Scheme **1**) give broad signals indicating fast exchange. ^{13}C NMR spectra acquired in solutions with higher concentration (0.1 mol/L), which allows shorter time for data acquisition (20-30 minute periods), were very similar to those recorded in solutions with lower concentration (0.04 mol/L) and were reproducible over several repeated measurements. This confirms that peak broadening in ^1H spectra and absence of peaks in ^{13}C spectra are not the consequence of aggregation. Compounds having 3-alkyl substituents (or a part of aryl ring as in **16**), despite the presence of 4-alkyl substituent (**15** *vs.* **14** and **16**), show fast exchange that cannot be quantified on NMR time scale in polar solvent like DMSO. ^{13}C NMR data of compounds similar to **1**-**16** in DMSO are rare in the literature [38].

For all studied compounds (**1**-**16**), mass spectra obtained by electrospray ionization, show presence of 2M–1 ions and 2(M–1)+Na. In spectra of compounds **14**-**16**, 2(M–1)+Na are more intensive than 2M–1 peaks. In turn, in MS spectra of all other studied compounds, 2M–1 peaks are more intensive. This could indicate significantly better complexation ability of 3-alkyl substituted compounds as compared to 4-substituted ones (Fig. **3a** and **b**).

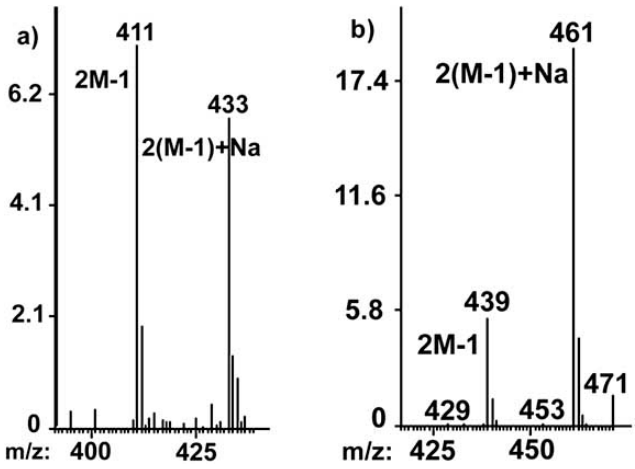


Fig. (3). Regions of ESI-MS spectra depicting 2M-1 and 2(M-1)+Na peaks of **2** (**a**), and **14** (**b**).

In order to further examine the ability of 3-alkyl substituted congeners for complex formation, we have studied compounds **2**, **14**, **15** and **16** mixed with $MgCl_2$ in methanol. As mentioned above, Mg^{2+} ion is a cofactor for HIV-1 integrase activity, and 4-aryl-2,4-dioxobutanoic acids inhibit integrase activity upon complexing with Mg^{2+} in enzyme active site [39, 40].

The Job's spectrophotometric method was used to confirm the complex stoichiometry (M:L=1:2 [40]) and to compare the abilities of **2**, **14**, **15** and **16** to complex with Mg^{2+}. Representative Job's plot for compound **14**, is shown in Fig. (**4**). Spectrum of pure compound **2** was only slightly changed, when mixed with Mg^{2+} in 2:1 molar ratio which indicates that there is no significant complex formation between **2** and Mg^{2+} ion (Fig. **5a**). Conversely, the batochromic shifts of 10 to 20 nm were observed in spectra of **14** (Fig. **5b**), **15** and **16**, after Mg^{2+} addition to get L:M=2:1 molar ratio. Formation constants for Mg^{2+} complexes with **14** $\left(\log\beta_2 = 9.20^{+0.28}_{-0.20}\right)$ **15** $\left(\log\beta_2 = 9.20^{+0.28}_{-0.20}\right)$, and **16** $\left(\log\beta_2 = 9.60^{+0.70}_{-0.30}\right)$ were determined by Job's method.

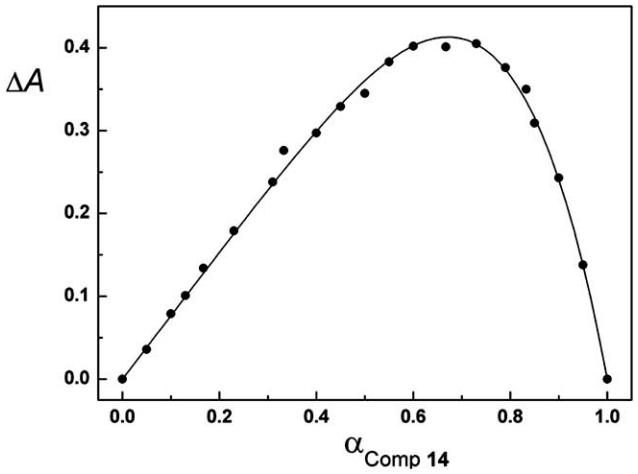


Fig. (4). Job's plot for Mg^{2+} complex of compound **14**.

CONCLUSIONS

The goal of this study was to give structural insight in the properties of each carbon, in butanoic moiety of 4-aryl-2,4-dioxobutanoic acids. These carbons are influenced by resonance and inductive effects of all other atoms within molecules as well as by "through the space" effects of proximal atoms. The ^{13}C NMR shifts should account for the summary of all those effects, and usage of Hammett sigmas derived from different sets of congeneric compounds (obtained from different spectral or kinetic measurements), offer very highly probable and discrete values that could be incorporated in correlations. Every carbon atom of butanoic moiety was considered in derivation of correlations, enabling us to separately examine the effect of substituents. This is comparable to commonly reported correlation of substituents effects on carboxylic moiety among several sets of phenyl-substituted phenylalkylcarboxylic acids with different alkyl chain length.

Linear free energy relationships of substituent effects on ^{13}C NMR chemical shifts of all butanoic carbons of sixteen 3- or 4-phenyl-substituted 4-phenyl-2,4-dioxobutanoic acids in their enolic forms, show that transmission of substituent effects could be rationalized in an acceptable extent using Hammett approach. It is proposed that compounds substituted with groups that have through-conjugation with the rest of molecule might have different conformations in DMSO, as compared to the corresponding alkyl substituted derivatives. Additionally, fast exchange between enol and keto forms is observed for 3,4- (**14**), 2,5-alkyl-disubstituted (**15**) and β-naphthyl (**16**) derivatives. This is ascribed to the pres-

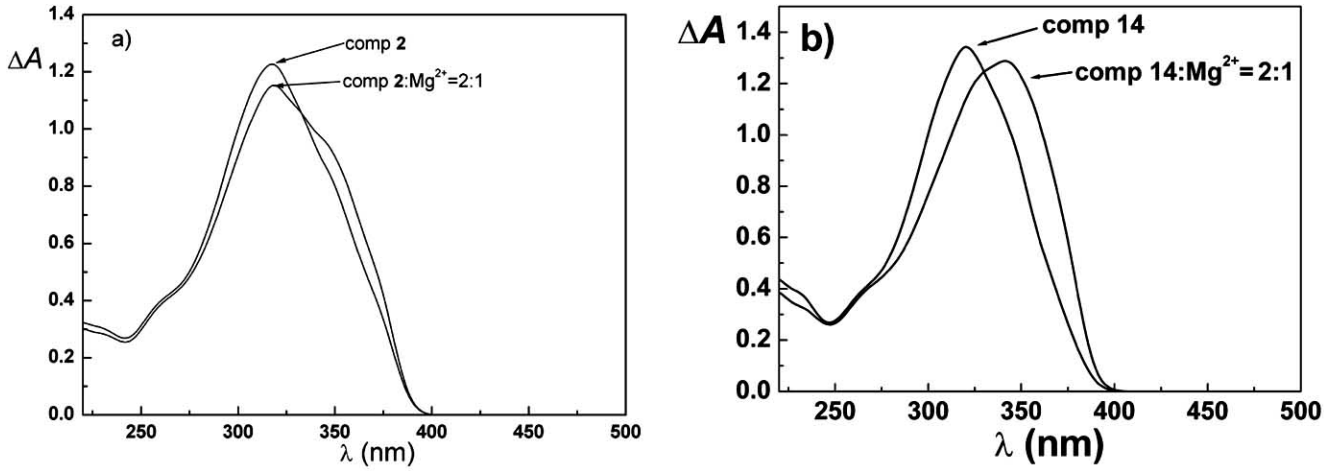


Fig. (5). Absorption spectra of compounds **a**) **2**, and **b**) **14** alone, and mixed with Mg^{2+} ($C_{Mg^{2+}} = 5.08 \cdot 10^{-5}$ mol/L; $C_2 = 1.01 \cdot 10^{-4}$ mol/L; $C_{\mathbf{14}} = 1.01 \cdot 10^{-4}$ mol/L).

ence of *meta*-alkyl substituents. ESI-MS spectra of compounds **14-16** *vs*. **1-13** could indicate significantly better complexation ability of 3-alkyl substituted compounds in comparison to 4-substituted ones. Formation constants for Mg^{2+} complexes with **14**, **15** and **16** were determined by using UV/VIS spectroscopy. The observed complexation ability will be subject of further studies due to potential pharmacological implications.

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ABBREVIATIONS

HIV = Human immunodeficiency virus

COSMO = Conductor-like Screening Model

DEPT = Distortionless Enhancement by Polarization Transfer

HMQC = Heteronuclear Multiple Quantum Coherence

HMBC = Heteronuclear Multiple Bond Correlation

HOMO = Highest occupied molecular orbital.

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