Aryldiketoacids. Synthesis, high resolution mass spectra, and pharmacophoric similarity with floxacins

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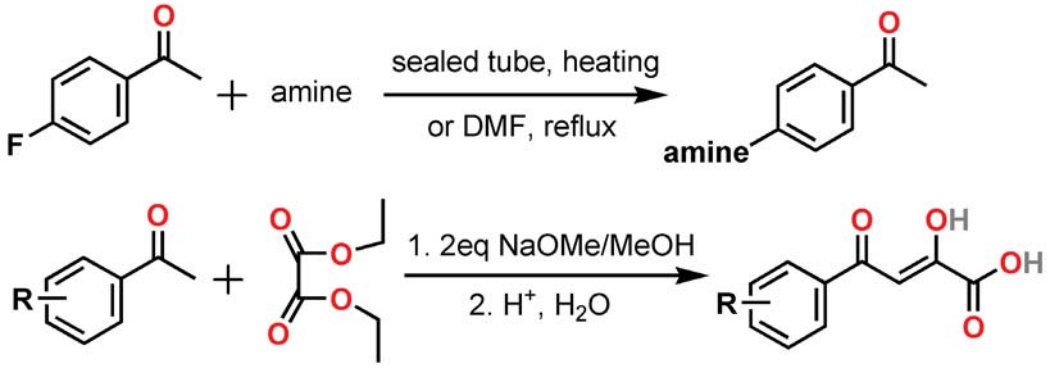
Introduction

Aryldiketoacids (ADKs) are proved as highly biologically active molecules, targeting several important enzymes associated with the life-threatening pathogens. One of the most important is Human imunodeficiency virus (HIV-1) intergase, the enzyme responsible for the integration of viral DNA in host genome.[1] Diketoacids bind to the active site Mg^{2+}/Asp domain in HIV-1 integrase, sequestrate Mg^{2+} ion, and inhibit the enzyme.

There is an urgent need for the new antibacterial drugs, because of the rapid development of bacterial resistant toward wide spectrum of commercially available antibacterial drugs. Methycilin-drug resistant *Staphylococcus aureus* (MDRSA) are one of the most spread bacteria, especially in hospital conditions. Discovery of the new drug targets in bacteria is crucial for overcoming resistance. Inhibition of isoprenoid biosynthesis, involved in lipid biosynthesis, could be accomplished by targeting farnesyl diphosphate synthase (FPPS), or undecaprenyl diphosphate synthase (UPPS). Dehydrosqualene synthase (CrtM) is another prenyl transferase involved in cell wall biosynthesis. UPPS and CrtM posses similar active site Mg^{2+}/Asp domain as HIV-1 intergase. Several well-known HIV-1 intergase inhibitors are proved as very potent inhibitors of prenyl transferases.[2]

Results and discussion

Ciprofloxacin and other floxacins are antibacterial drugs, highly potent against gram-positive and gram-negative strains, and inhibit DNA replication by targeting DNA-topoisomerase complex. We observed structural similarity between floxacins and aryldiketoacids. Biological test showed that several ADK, synthesized by us, act against MDRSA in micromolar range of concentrations.[3] In order to design more potent inhibitors of MDRSA, new series of ADK have been synthesized. Along with this, we showed that *meta*-alkyl substituted ADK have higher affinity toward Mg^{2+} ion, compared to *ortho*- and *para*-substituted congeners, and form complexes with ML_2 stoichiometry ($\log\beta_2$ ~ 10).[4] The indication on different complexation ability within congeneric series of ADK, was initially obtained by analyzing high resolution mass spectra. *Meta*-alkyl substituted ADK show peak corresponding to 2(M-1)+Na of high intensity, where M is a mass of molecular ion.



R = 3-Me-, 2,4-di-Me-, 2,4,5-tri-Me-, 2,3,5,6-tetra-Me-, 2,4,6-tri-*i*-Pr-, 2,5-di-cyclohexyl-, 3-fluorenyl-, 2-tetralinyl-, 3-Br-, 4-Br-2,5-di-Me-, 4-MeO-2,5-di-Me-, 4-OH-3,5-di-Me-, 2-MeO-, 3-MeO-, 3-CF_3-, 4-piperidinyl-, 4-pyrolidinyl-, 4-morpholinyl-, 4-(*N*,*N*-di-Me)-, 4-*N*-Me-piperazinyl-, 4-imidazolyl-, 4-*N*-cyclohexyl-

Figure 1. *Synthesis of compounds reported.*

ADKs were synthesized by Claisen condensation of substituted acetophenones with diethyl-oxalate, followed by *in-situ* base hydrolysis of ethyl ester formed, to give aryldiketoacids (Figure 1). Substituted

acetophenones used were commercially available (3-CH_3, 2-OCH_3, 3-OCH_3, 3-CF_3), or synthesized using different procedures. Alkyl- and halogen- substituted acetophenones were obtained by Friedel-Crafts acylation of corresponding substituted benzenes, using $AlCl_3$ as a catalyst in CH_2Cl_2 as a solvent. The 4-amino-substituted acetophenones were obtained starting from commercially available 4-F acetophenone. Condensation with cyclic secondary amines (piperidine, *N*-methylpiperazine, morpholine, pyrolidine, imidazole) proceeded easily, with yields over 90%, mixing the excess of amine with 4-F acetophenone and heating for 2-3 hours on 130-140 °C in the pressure-resistant steel tube, without any solvent. Similarly, refluxing the mixture of 4-F acetophenone, amine, and K_2CO_3 in DMF on 110-120 °C for 24^h, we obtained the same products in high yields. Synthesized compounds were purified by crystallization or by dry-flash chromatography, and characterized by ^1H and ^{13}C NMR, ESI-MS, IR spectra and melting points.

The difference of complexation ability of ADK was confirmed in the newly prepared set by ESI-MS spectra. Selected examples are shown on Figure 2 and in Table 1. Obviously, compounds bearing *meta*-alkyl substituents show much more intensive [2(M-1)+Na] ions that the rest in the set. Highest ratio is observed for the 3-Me- derivative. Presence of the both *meta*-alkyl and *ortho*-alkyl substituents, as in 2,4,5-tri-CH_3-, and 2,3,5,6-tetra-CH_3- derivatives, attenuate such effect in some extent, probably because aryl to Ar-C(O)- torsion. *Ortho*- substituents increase this torsion angle, and probably decrease the resonance between diketo moiety and aryl ring. Alkoxy and halogens, as substituents in *meta*-position, did not influence in the same manner as alkyl substituents.

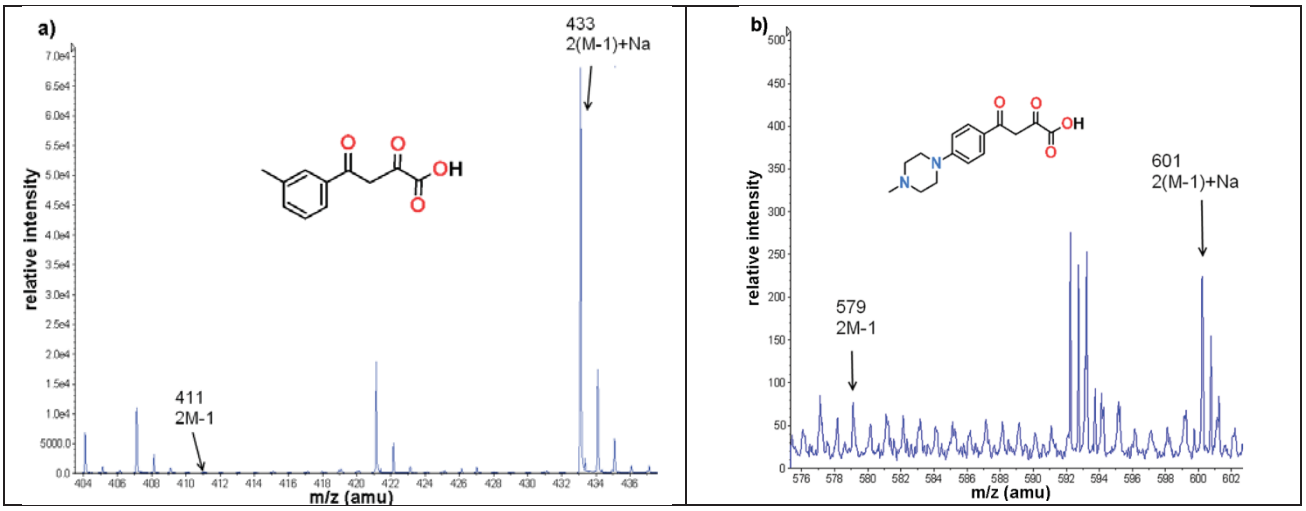


Figure 2. Selected regions of ESI-MS spectra. (a) 3-CH_3ADK, and (b) 4-N-Me-piperazinyl ADK.

Table 1. Intensities of observed peaks in ESI-MS spectra and their relative ratios, displayed for selected compounds.

Compound	Ion intensity		$\frac{2(M-1)+Na}{2M-1}$
	2M-1	2(M-1)+Na	
3-CH_3-	211	57222	271
2,4,5-tri-CH_3-	766	85509	112
3-OCH_3-	227	14011	62
4-OCH_3-2,5-di-CH_3-	1226	102381	84
2,3,5,6-tetra-CH_3-	847	151487	179
3-Br-	707	33065	47
4-(*N*-CH_3-pyperazinyl)-	35	69	2

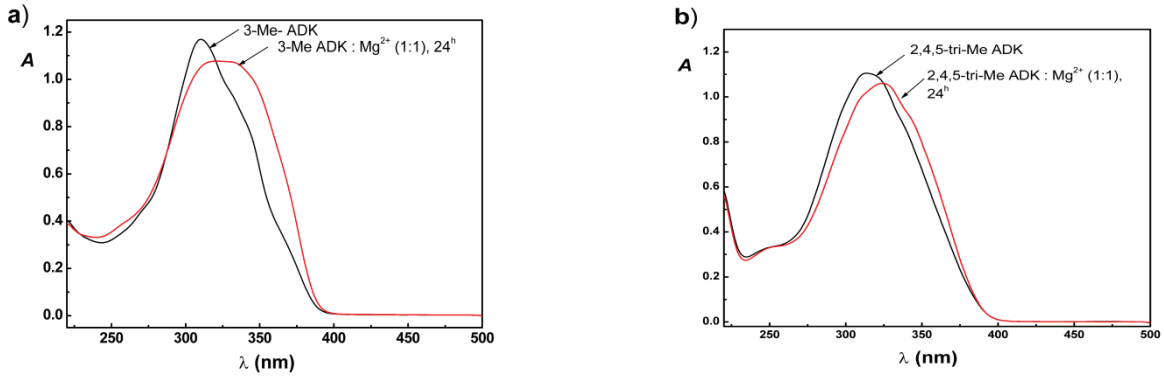


Figure 3. UV/Vis absorption spectra of a) 3-CH_3-, and b) 2,4,5-tri-Me- derivatives; before (black) and after the addition of Mg^{2+} (red).

Complexation ability of *meta*-substituted ADK with Mg^{2+} was determined by UV/Vis spectroscopy (Figure 3); batochromic shift of absorption maximum was recorded upon complexation. Those results are in agreement with our previous findings.[4]

Pharmacophoric similarity of floxacins and reported compounds:

Reported set is prepared as continuation of our work on ADK derivatives that can overcome multidrug resistance in MDRSA.[3] Pharmacophoric similarity with known fluoroquinoline antibiotics (Figure 4) was examined by superimposition in the ROCS program.[5] The crystal structure of the norfloxacin (Figure 4a)[6] is used as a template. Ten conformations of the each compound studied were obtained by OMEGA,[7] from the SMILES notation, and 'rms' keyword was set to 0.15. 100 random starts per molecule were used. Both template and queries are treated in their neutral forms. Tanimoto and Tanimoto combo (include shape and OpenEye 'color' force field (ff)) similarity scores were used for the quantification of results.

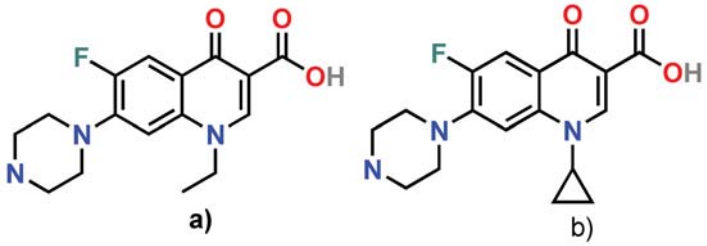


Figure 4. Structures of norfloxacin (a) and ciprofloxacin (b)

The highest similarity between norfloxacin, by both shape and pharmacophore (color ff) similarity, not surprisingly, were observed for derivatives having 4-hetero(ali)cyclic substituents. Best overlap was found for 4-*N*-Me-piperazinyl derivative (Tanimoto Combo 1.074, Shape Tanimoto 0.696, Color Tanimoto 0.378) Figure 5a, followed by 4-pyrolidinyl- derivative (Tanimoto Combo 1.037, Shape Tanimoto 0.733, Color Tanimoto 0.304).

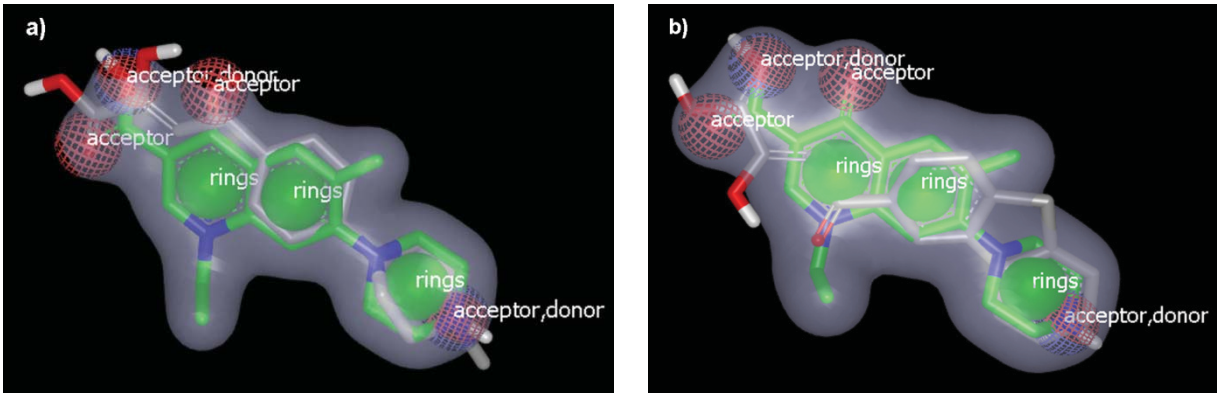


Figure 5. a) 4-N-Me-piperazinyl- and b) 3-fluorenyl derivatives superimposed on norfloxacin.

It should be noted that among highly ranked derivatives we found 3-fluorenyl- derivative (Tanimoto Combo 1.030, Shape Tanimoto 0.742, Color Tanimoto 0.287), that show better shape similarity than previous two, but is positioned with their diketo moiety opposite to the -C(O)-C-COOH moiety of the norfloxacin, Figure 5b. Favored overlap of norfloxacin Ph-piperazinyl moiety with fluorenyl moiety of ADK contributes to relatively high shape score.

Conclusion

We reported preparation and characterization of 22 aryldiketo acids, designed to confirm significant observations obtained by our group on congeners in the same series. High-resolution ESI-MS spectra showed better complexation of monovalent metal ions for *meta*-alkyl substituted derivatives. Introduction of *ortho*-alkyl substituents attenuate such effect in some extent. The same derivatives exert good complexation of Mg^{2+} ion, and this is proved by UV/Vis spectroscopy. This observation has pharmacological relevance, due to significance of Mg^{2+} ion in HIV-1 integrase active site. 4-Hetero(ali)cyclic substituted derivatives show significant pharmacophoric similarity with floxacin antibiotics. Biological tests of prepared compounds against multidrug resistant *Staphylococcus aureus* are in preparation.

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Арилдикетокиселине. Синтеза, масени спектри високе резолуције и фармакофорна сличност са антибиотицима из класе флоксацина

Арилдикетокиселине (АДК) су биолошки активни молекули који делују на ензим интегразу HIV-1 вируса. Наша претходна истраживања су показала већи афинитет мета-супституисаних АДК ка комплексирању Mg^{2+} јона у односу на остале супституционе обрасце. Још важније, АДК показују антибактеријску активност према сојевима бактерија Staphylococcus aureus *резистентним према више антибиотика (MDRSA). Као наставак ових истраживања, синтетисали смо серију од 22 нова конгенера, као потенцијално боље антибактеријске агенсе (MDRSA). У овом саопштењу је укратко описана њихова синтеза, афинитет ка комплексирању једно- и двовалентних металних јона, као и фармакофорна сличност са антибиотицима из класе флоксацина.*

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