DOI: 10.1002/cmdc.200900273

Aryldiketo Acids Have Antibacterial Activity Against MDR *Staphylococcus aureus* Strains: Structural Insights Based on Similarity and Molecular Interaction Fields

Branko J. Drakulić,*[a] Michael Stavri,[b] Simon Gibbons,[b] Željko S. Žižak,[c] Tatjana Ž. Verbić,[d] Ivan O. Juranić,[d] and Mire Zloh*[b]

Staphylococcus aureus is a major community- and hospital-acquired pathogen.[1] Reports of resistance to antibiotics, including the fluoroquinolone class,[2] have placed a greater emphasis on the development of new drugs for the treatment of both methicillin- (MRSA) and multidrug-resistant (MDR) *S. aureus* strains. Recently, mixed quinolonediketo acid derivatives, which are based on the scaffold of fluoroquinolone antibiotics, were shown to exert significant anti-HIV-1 potency.[3] The γ-diketo moiety is also found in tetracycline, and is therefore potentially important for antibacterial activity. GS-9137 (elvitegravir, CAS 697761-98-1), a 4-quinolone-3-carboxylic acid-based HIV-1 integrase inhibitor is presently in phase III clinical trials.[4] The similarity between these two scaffolds (Figure 1),

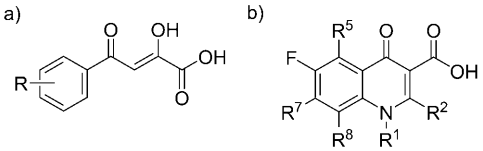

Figure 1. Scaffolds of a) prevailing keto-enol form of ADKs[6,7] and b) fluoroquinolone antibiotics, including substituent numeration.

both of which are integrase inhibitors, inspired our study of aryldiketo acids (ADK) as potential antibacterial agents. To the best of our knowledge, the antibacterial activity of ADK, the scaffold of an important class of HIV-1 integrase inhibitors,[5] has not been reported in the literature so far.

[a] *B. J. Drakulić*
Institute of Chemistry, Technology and Metallurgy, University of Belgrade
Njegoševa 12, 11000 Belgrade (Serbia)
Fax: (+381) 112636061
E-mail: bdrakuli@chem.bg.ac.yu

[b] *M. Stavri, S. Gibbons, M. Zloh*
The School of Pharmacy, University of London
29–39 Brunswick Square, London WC1N 1AX (UK)
Fax: (+44) 2077535964
E-mail: mire.zloh@pharmacy.ac.uk

[c] *Ž. S. Žižak*
Institute for Oncology and Radiology of Serbia
Pasterova 14, 11000 Belgrade (Serbia)

[d] *T. Ž. Verbić, I. O. Juranić*
Faculty of Chemistry, University of Belgrade
Studentski Trg 12-16, 11000 Belgrade (Serbia)

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.200900273.

4-Phenyl-2,4-dioxobutanoic acids (**1**–**19**) were tested for antibacterial activity against clinical isolates of MDR bacterial strains (Table 1). The synthesis and characterisation of **1**–**7**, **13**–**19** was previously reported.[6,7] Characterisation data for **8**–**12** and **20** are given in the Supporting Information. All compounds (**1**–**10, 12**–**20**) were initially tested for their antibacterial activity against SA-1199B, a strain of *S. aureus* possessing the NorA MDR efflux protein that confers resistance to hydrophilic fluoroquinolones, including norfloxacin.[8] Minimum inhibitory concentration (MIC) values were also compared with those of norfloxacin (**21**),[8] tetracycline, erythromycin and reference fluoroquinolone antibiotics, ciprofloxacin (**22**), levofloxacin (**23**) and moxifloxacin (**24**)[9] (scheme 1S in the Supporting Information).

Table 1. Structures and antibacterial activities of ADKs **1**–**20** against selected MDR *S. aureus* strains.

Compd	R	MIC [μM]						
		SA-1199B[a]	IS-58	XU-212	RN-4220	CD-1281	ATCC-25923	EMRSA-15
1	H	>2660	–	–	–	–	–	–
2	4-Me	>2480	–	–	–	–	–	–
3	4-Et	>2320	–	–	–	–	–	–
4	4-*i*Pr	>2320	–	–	–	–	–	–
5	4-*t*Bu	1030	–	–	–	–	–	–
6	2,5-di-Me	>2320	–	–	–	–	–	–
7	3,4-di-Me	>2320	–	–	–	–	–	–
8	2,4-di-*i*Pr	232	116	464	232	232	232	232
9	2,5-di-*i*Pr	232	232	464	464	464	464	464
10	2,4,6-tri-Et	116	232	232	232	232	232	232
11[b]	2,4,6-tri-*i*Pr	–	–	–	–	–	–	–
12	4-Ph	477	–	–	–	–	–	–
13	β-naphthyl	>2110	–	–	–	–	–	–
14	3-OH	>2460	–	–	–	–	–	–
15	4-OH	>2460	–	–	–	–	–	–
16	4-MeO	>2300	–	–	–	–	–	–
17	4-NO_2	>2160	–	–	–	–	–	–
18	4-F	>2440	–	–	–	–	–	–
19	4-Cl	>2260	–	–	–	–	–	–
20	4-(1*H*-indol-3-yl)	>2220	–	–	–	–	–	–
Norfloxacin[c]		106	–	–	–	–	6.6	3.3
Tetracycline[c]		–	72	288	–	72	–	–
Erythromycin[c]		–	–	–	175	–	–	–

[a] Compounds that exerted an MIC against SA-1199B in concentrations higher than 400 μM were not screened against other strains. [b] Compound was unstable. [c] The MIC determination for these compounds was measured only against relevant resistant strains. – Not measured.

The most promising potency against bacterial strains overexpressing efflux pumps was observed for compounds **8**–**10**, with MIC values comparable to clinically used antibiotics susceptible to MDR. Compounds **5** and **12** exhibited only moderate potency against SA-1199B and were not subjected to studies against other strains. The MIC value of the most potent aryldiketo acids (**8**–**10, 12**) did not change significantly against strains overexpressing efflux pumps or for the standard laboratory strain (ATCC 25923); this indicates that the compounds are possibly not substrates for these efflux pumps. Compounds **8** and **10** were twice as potent as erythromycin and/or tetracycline against bacteria resistant to those antibiotics.

The whole set (**1**–**20**) was also tested for the ability to modulate the activity of norfloxacin against the norfloxacin-resistant NorA-overexpressing strain SA-1199B. The results are given in Table 2; only compounds that had any potentiating effect are given. The presence of several compounds at 25% of their MIC (max 100 $\mu g\,mL^{-1}$) improved the potency of norfloxacin (**21**) by decreasing its MIC value to 53 μM. A further decrease in the concentrations of **15** and **17** did not affect their modulation ability, while compounds **7**, **12** and **20** lost their ability to modulate norfloxacin activity at a lower concentration.

Table 2. Antibacterial activity of norfloxacin against norfloxacin-resistant NorA-overexpressing *S. aureus* strain SA-1199B in the presence of ADKs at two different concentrations.

Compd[a]	R	ADK [μM]	MIC [μM]
Norfloxacin		–	106
7	3,4-di-Me	454	53
		50	106
12	4-Ph	119	53
		50	106
15	4-OH	240	53
		50	53
17	4-NO_2	211	53
		50	53
20	4-(1*H*-indol-3-yl)	430	53
		50	106

[a] No improvement in antibacterial activity of norfloxacin was observed for compounds **1**–**6**, **8**–**11**, **13**–**14**, **16**, **18**–**19**.

Six compounds were selected from for evaluation in a cytotoxicity assay: compounds **8, 10**, **12** with antibacterial activity (MIC) values lower than 400 μM; compounds **15**, **17** able to potentiate the antibacterial action of **21**; indolyl derivative **20**. All tested compounds inhibited 90% of healthy human cell growth at concentrations higher than their MIC value (Supporting Information). A moderate selectivity was observed that could potentially be improved by appropriate structural modifications on the aryl ring.

A common feature for the activity of the two classes of drug is the requirement for metal complexation. Inhibition of HIV-1 integrase by aryldiketo acids is mediated by Mg^{2+} ion complexation to the diketo moiety.[10] We reported the influence of the phenyl substitution pattern in aryldiketo acids on Mg^{2+} complexation.[7] In a similar manner, Mg^{2+} bound to the fluoroquinolone carboxylic acid group at position 3 is necessary for the interaction with prokaryotic gyrase A (GyrA).[11] Notably, ionisation constants for moieties responsible for Mg^{2+} binding in fluoroquinolone antibiotics and aryldiketo acids are similar. Ionisation constants of **5**, **8**–**10**, **12**, **15**, **17**, **19**, **21**–**24** are given in Table 3. pK_a values of **5**, **8**–**10**, **15**, **17**, **19**, were experimentally obtained, pK_a of **12** was calculated by MoKa, while values of **21**–**24** were taken from the literature.

Table 3. Ionisation constants of **5**, **8**–**10**, **12**, **15**, **17**, **19**, **21**–**24**.

Compd	pK_{a1}	pK_{a2}	pK_{a3}	HA^- (%)[c]	A^{2-} (%)[c]	Ref.
5	2.21 ± 0.03	7.77 ± 0.06	–	72.45	27.55	[6]
8	2.04 ± 0.04	7.29 ± 0.03	–	46.51	53.49	–
9	2.33 ± 0.03	7.13 ± 0.04	–	37.59	62.41	–
10	1.99 ± 0.03	6.72 ± 0.03	–	18.98	81.02	–
12	2.35 ± 0.33[a]	6.94 ± 0.59[a]	–	28.01	71.99	–
15	2.29 ± 0.05	7.73 ± 0.01	–	70.42	29.58	[6]
17	1.87 ± 0.06	6.63 ± 0.02	–	83.92	16.03	[6]
19	2.09 ± 0.04	7.30 ± 0.03	–	47.17	52.83	[6]
21	–	6.32 ± 0.005	8.56 ± 0.005	Zwitterion		[18]
22	–	6.23 ± 0.004	8.58 ± 0.004	Zwitterion		[18]
23	–	6.24 ± 0.02	8.26 ± 0.05	Zwitterion		[18]
24	–	6.25 ± 0.02	9.29 ± 0.04	Zwitterion		[19]

[a] Estimated by MoKa v1.0.9;[20] [b] For compounds **21**–**24** pK_{a2} and pK_{a3} refers to ionisations of carboxyl group at C3 and distal nitrogen of heteroalicyclic ring at C7, respectively;[21] [c] % of HA^- refers to the estimated percentage of monoanionic form, % A^{2-} refers to the estimated percentage of dianionic form at physiological pH (7.35).

Furthermore, structural similarity between **1**–**20** and norfloxacin **21** was observed by overlaying the representative conformations of the monoanions of **1**–**20** generated by OMEGA,[12] with the representative conformations of **21** in its zwitterionic form, using ROCS.[13] Visual analysis of the overlays was carried out,[14] and a qualitative correlation between the overlap of important pharmacophoric features of **21**[15,16] with **1**–**20**, and their antibacterial potency was observed (overlaid 3D structures in msv file format are available as Supporting Information). Substituents in positions 1, 3, 4 and 7 of the quinolone core (Figure 1) are a prerequisite for antibacterial activity. The $C(O)CH{=}C(OH)COO^-$ moiety of **1**–**20** overlaps well with the 3-carboxylate and 4-carbonyl positions of the norfloxacin core, which are responsible for binding to cleaved or perturbed DNA and are critical for antimicrobial activity.

Compounds only exhibited notable antibacterial activity if there was additional overlap with the other two pharmacophoric points of **21**, the R^1 ethyl moiety responsible for hydrophobic interaction with the major grove of DNA, and R^7 piperazinyl moiety that directly interacts with GyrA or topoisomerase IV. The *ortho*-phenyl substituents of compounds **8**–**10** overlap with the important R^1 of **21**; those compounds possess MIC values between 116 and 232 μM. As the overlap decreases, and the R^1 position is no longer mimicked, the potency decreases as can be seen for **12** and **5**, with MIC values of 477 and 1030 μM, respectively. Activity diminishes for the remaining compounds lacking phenyl substituents able to overlay the

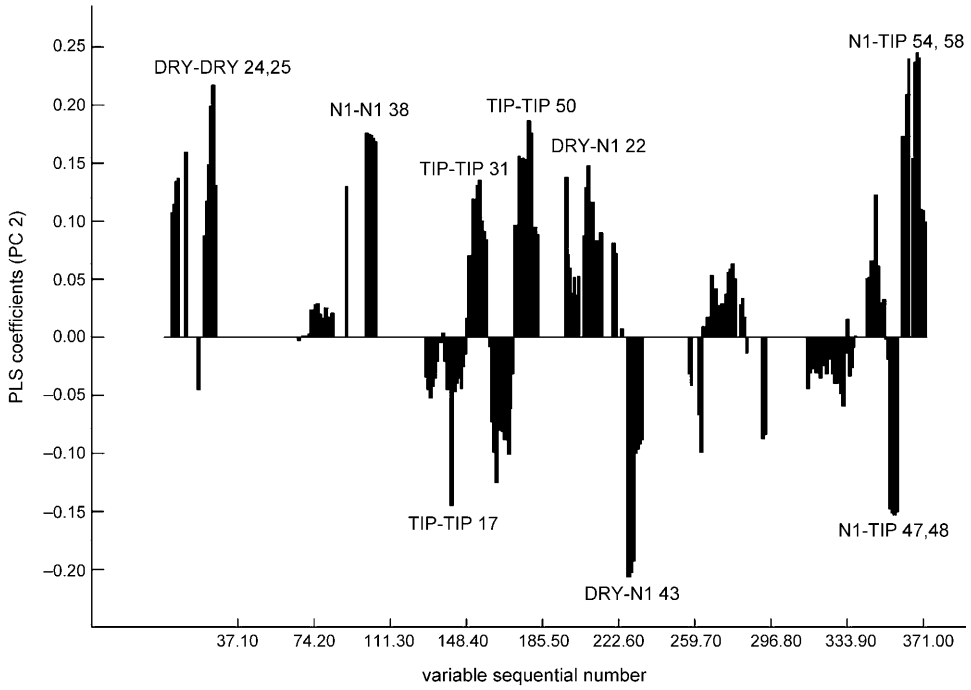

Figure 2. PLS coefficient plot (2 PC) of the ALMOND model show which GRIND variables are correlated to the potency of **5**, **8**–**10**, **12**, **15**, **17**, **19** and **21**–**24**, in their ionised forms against SA-1199B. MIF regions around molecules associated with variables are given in Figures 3 and 4, as well as in panels I and II in the Supporting Information.

R^1 and R^7 positions of **21** (msv files showing overlaid structures of **21**/**15** and **21**/**17** can be found in the Supporting Information).

Further SAR analysis was carried out by including factors other than shape similarity. The protonation states under physiological pH (Table 3) of **5**, **8**–**10**, **12**, **15**, **17**, **19, 21**–**24** were considered as more accurate representation of pH effects on conformation. The molecular interaction fields (MIF) of the active ADKs were compared to those of the fluoroquinolones. The alignment-free 3D QSAR models developed on the basis of GRid INdependent Descriptors (GRIND)[17] were built to provide quantitative representation of pharmacophoric points that are common for these two classes of compounds. The best model was obtained with active molecules (**5**, **8**–**10**, **12**, **15**, **17**, **19**) and reference commercial antibiotics (**21**–**24**) in ionised form at physiological pH (7.35). A necessary approximation was introduced during in silico structure preparation; if a particular molecule (**5**, **8**–**10**, **12**, **15**, **17**, **19**) under physiological pH existed in more than 50% in the A^{2-} form, this molecule was considered to be a dianion. The remaining molecules in the set were treated as monoanions (HA^-). The most favourable interaction regions extracted from molecular interaction fields obtained with N1, DRY and TIP probes (hydrogen bond donor (HBD), hydrophobic and shape, respectively) were correlated with potency of compounds by partial least square (PLS) analysis, and a corresponding PLS coefficient plot is shown in Figure 2. Information about statistics of the model, observed versus calculated log (1/MIC), and detailed descriptions of moieties associated with variables with a high impact on the model and association of variables to particular compounds are given in the Supporting Information. For comparison, a similar model that included the same molecules in their neutral form is also discussed in the Supporting Information.

A structural similarity between moieties of the studied aryldiketo acids (**5**, **8**–**10**, **12**, **15**, **17**, **19**) and fluoroquinolone antibiotics (**21**–**24**) was observed and all of those similar regions were positively correlated with potency. Similarity between the 4-phenyl-4-oxo-2-butenoic moiety of the aryldiketo acids and the quinolone core of compounds **21**–**24** is shown by variables DRY–DRY 24 and 25 (Figure 3a and b). One node of the variable is positioned in proximity to the phenyl rings of both the ADK and quinolone core (interaction energy (IE) $= -1.76$ kcal mol^{-1}), while the other node is positioned in proximity to the 4-oxo-2-butenoic moiety or pyridone C=C double bonds (IE $= -2.08$ kcal mol^{-1}).

The spatial arrangement between the carboxyl group and the *ortho*-alkyl substituent of aryldiketo acids **8**–**10** is similar to the arrangement of the quinolone core 3-carboxyl group and the alkyl substituent in its position 1 (Figure 3c and d), as depicted by the variable TIP–TIP 31.

The similarity between the aroyl moiety (Ar–C(O)–) of the ADKs and the quinolone moiety of compounds **21**–**24** is described by the variable DRY–N1 22 (Figure 4). For aryldiketo acids, the N1 node is associated with the aroylketo group, while the DRY node (IE $= -3.03$ kcal mol^{-1}) is associated with the phenyl ring of ADKs. For the fluoroquinolone molecules, the N1 node (IE $= -6.60$ kcal mol^{-1}) is associated with the keto group in position 3, while the DRY node is situated in close proximity to both the aromatic quinolone core and the alkyl substituent in position 1.

The distinction between fluoroquinolones **21**–**24** and the aryldiketo acids is given by the variable N1–TIP 58. The non-zero value of this variable is found only for the fluoroquinolones (table 7S in the Supporting Information). ADKs, in both neutral and ionised forms, with longer or branched *ortho*-alkyl substituents on the phenyl ring (**8**–**10**), adopted conformations that matched well with the quinolone core and the 1-, 3-, and 4-substituents of compounds **21**–**24**. Along with the quinolone 7-substituent, these are the most important moieties of of the fluoroquinolones, responsible for antibacterial activity.

Although the inclusion of the MG+2 probe (Mg^{2+} ion) did not improve the ALMOND models, it was found that the most favourable interaction regions of this probe with both aryldiketo acids and fluoroquinolones are located in proximity to the

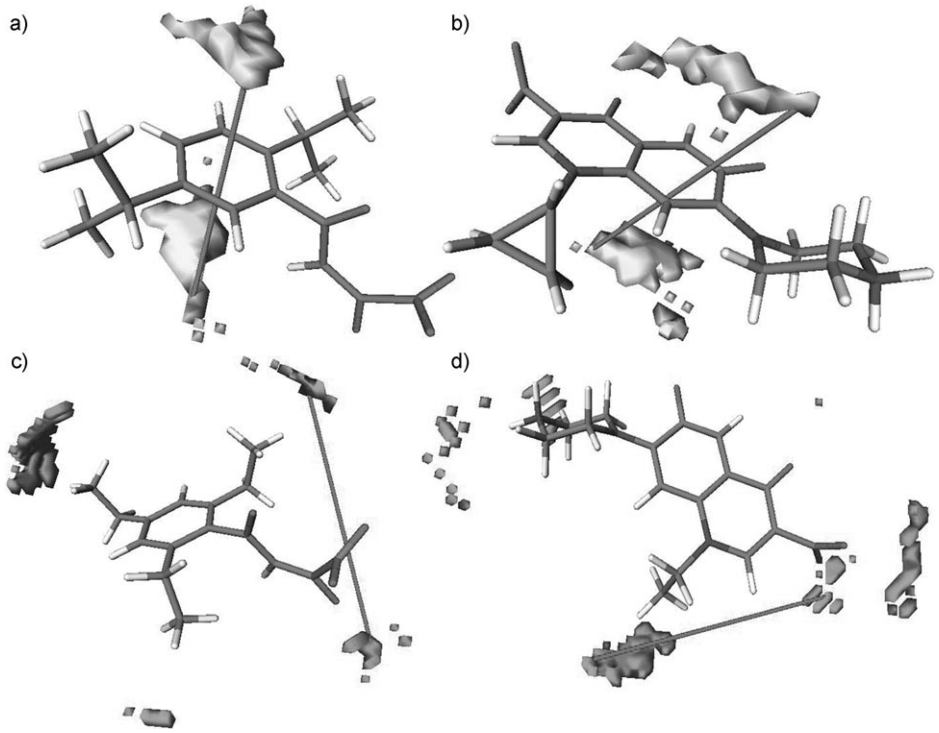

Figure 3. Favourable interaction fields with hydrophobic (up) and shape probes (down). Variable DRY–DRY 25 for a) **9** and b) **22** in their ionised forms at physiological pH. Variable TIP–TIP 31 for c) **10** and d) **21** in their ionised forms at physiological pH.

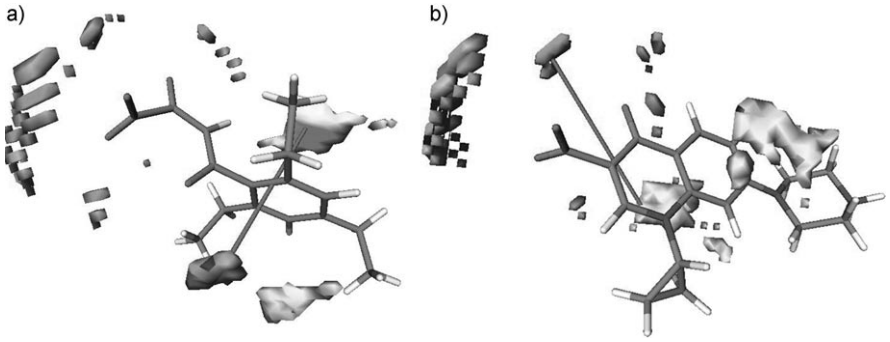

Figure 4. Variable DRY-N1 22 for a) **10** and b) **22** in their ionised forms at physiological pH.

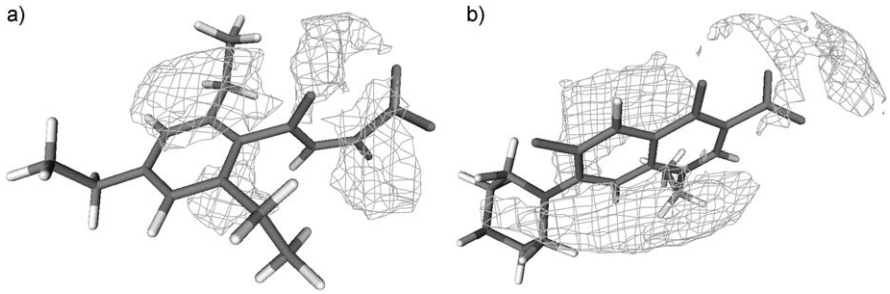

Figure 5. MG+2 GRID probe isocontour levels on −22.2, −4.2 kcal mol^{-1} for **10** and **21**, respectively, in their ionisation states at physiological pH (7.35).

aromatic rings of **1**–**24**. The carboxylate moieties of both aryldiketo acids and fluoroquinolones, which bind the Mg^{2+} ion in the active sites of their respective target enzymes, have to some extent less favourable interaction energies. Similarities of the GRID MG+2 probe isocontour levels on slightly less favourable (less negative) interaction energies are given in Figure 5. So far, only one complex of an aryldiketo acid with the Mg^{2+} ion has been reported.[22] In this complex, the spatial arrangement of the diketo acid C(O)CH=CH(OH)COOH moiety and the Mg^{2+} ion is almost identical to the region around the aryldiketo acids reported here, as predicted by the GRID Mg+2 probe (see 3D msv and mol2 files in the Supporting Information for examples). The observed regions of MG+2 probe favourable interactions will be the subject of a further study.

The potentiation ability of compounds **7**, **12**, **15**, **17** and **20**, can not be explained by the possible complex formation between **21** and ADKs (Supporting Information).[23] The loss of potentiation with decreased compound concentrations indicates that competitive binding of aryldiketo acids might be responsible for overcoming the efflux pumps. The examined aryldiketo acids probably bind to the efflux pumps in a similar position to norfloxacin (**21**) prior to removal from the cell, emphasising the importance of similarities between norfloxacin (**21**) and compounds **15** and **17**.

The antibiotic activity of aryldiketo acids has been reported here for the first time. Some compounds have antibiotic activity against MDR *S. aureus* strains comparable to norfloxacin; furthermore, other compounds have the ability, albeit weak, to potentiate the activity of norfloxacin against effluxing strains. Similarity studies have revealed the importance of the nature of substituents and their position on the aryldiketo acid phenyl moiety on the type of activity (antibacterial/MDR modulation) and their potency. This class of compounds could be a good platform for designing

novel therapeutics that are able to act alone or in synergy with other antibiotics, namely fluoroquinolones. The design, synthesis and evaluation of novel congeners is currently underway.

Acknowledgements

BJD gratefully acknowledge Dr. Francesca Milletti and Prof. Gabriele Cruciani for pK_a estimations. This work was partially supported by the Ministry of Science of Serbia, grant 142010 (for BJD, TŽV and IOJ).

Keywords: antibiotics · aryldiketo acids · biological activity · molecular interaction fields · molecular similarity · MRSA

[1] T. M. Perl, *Am. J. Med.* **1999**, *106*, 26S – 37S.
[2] H. M. Blumberg, D. Rimland, D. J. Carroll, P. Terry, I. K. Wachsmuth, *J. Infect. Dis.* **1991**, *163*, 1279 – 1285.
[3] R. Di Santo, R. Costi, A. Roux, M. Gaetano, G. C. Crucitti, A. Iacovo, F. Rosi, A. Lavecchia, L. Marinelli, C. Di Giovanni, E. Novellino, L. Palmisano, M. Andreotti, R. Amici, C. M. Galluzzo, L. Nencioni, A. T. Palamara, Y. Pommier, C. Marchand, *J. Med. Chem.* **2008**, *51*, 4744 – 4750.
[4] L. A. Sorbera, N. Serradell, *Drugs Future* **2006**, *31*, 310 – 313.
[5] J. Deng, R. Dayam, L. Q. Al-Mawsawi, N. Neamati, *Curr. Pharm. Des.* **2007**, *13*, 129 – 141.
[6] T. Ž. Verbic, B. J. Drakulić, M. F. Zloh, J. R. Pecelj, G. V. Popović, I. O. Juranić, *J. Serb. Chem. Soc.* **2007**, *72*, 1201 – 1216.
[7] T. Ž. Verbic, B. J. Drakulić, M. Zloh, I. O. Juranić, *Lett. Org. Chem.* **2008**, *5*, 692 – 699.
[8] G. W. Kaatz, S. M. Seo, C. A. Ruble, *Antimicrob. Agents Chemother.* **1993**, *37*, 1086 – 1094.
[9] S. Gibbons, M. Oluwatuyi, G. W. Kaatz, *Aureus. J. Antimicrob. Chemoth.* **2003**, *51*, 13 – 17.
[10] C. Marchand, A. A. Johnson, R. G. Karki, G. C. Pais, X. Zhang, K. Cowansage, T. A. Patel, M. C. Nicklaus, T. R. Burke Jr. , Y. Pommier, *Mol. Pharmacol.* **2003**, *64*, 600 – 609.
[11] C. Sissi, E. Perdona, E. Domenici, A. Feriani, A. J. Howells, Maxwell, A. M. Palumbo, *J. Mol. Biol.* **2001**, *311*, 195 – 203.
[12] J. Boström, J. R. Greenwood, J. Gottfries, *J. Mol. Graph. Mod.* **2003**, *21*, 449 – 462; www.eyesopen.com/products/applications/omega.html (Last accessed: October 14, 2009).
[13] J. E. J. Mills, P. M. Dean, *J. Cold Reg. Eng. J. Comput-Aided Mol. Des.* **1996**, *10*, 607 – 622; www.eyesopen.com/products/applications/rocs.html (Last accessed: October 14, 2009).
[14] M. Zloh, S. Gibbons, *Int. J. Mol. Sci.* **2004**, *5*, 37 – 47.
[15] L. R. Peterson, *Clin. Infect. Dis.* **2001**, *33*, S180 – S186.
[16] S. Emami, A. Shafiee, A. Foroumadi, *Mini-Rev. Med. Chem.* **2006**, *6*, 375 – 386.
[17] a) M. Pastor, G. Cruciani, I. McLay, S. Pickett, S. Clementi, *J. Med. Chem.* **2000**, *43*, 3233 – 3243; b) F. Fontaine, M. Pastor, F. Sanz, *J. Med. Chem.* **2004**, *47*, 2805 – 2815; c) ALMOND version 3.3.0, Molecular Discovery Ltd., Perugia (Italy); www.moldiscovery.com (Last accessed: October 14, 2009).
[18] A. Llinàs, R. C. Glen, J. M. Goodman, *J. Chem. Inf. Model.* **2008**, *48*, 1289 – 1303.
[19] M-H. Langloisa, M. Montaguta, J-P. Dubosta, J. Grellet, M-C. Saux, *J. Pharm. Biomed. Anal.* **2005**, *37*, 389 – 393.
[20] F. Milletti, L. Storchi, G. Sforna, G. Cruciani, *J. Chem. Inf. Mod.* **2007**, *47*, 2172 – 2181; MoKa version 1.0.9, Molecular Discovery Ltd., Perugia (Italy); www.moldiscovery.com (Last accessed: October 14, 2009).
[21] M. S. Burkhead, H. Wang, M. Fallet, E. M. Gross, *Anal. Chim. Acta* **2008**, *613*, 152 – 162.
[22] A. Bacchi, M. Biemmi, M. Carcelli, F. Carta, C. Compari, E. Fisicaro, D. Rogolino, M. Sechi, M. Sippel, C. A. Sotriffer, T. W. Sanchez, N. Neamati, *J. Med. Chem.* **2008**, *51*, 7253 – 7264.
[23] M. Zloh, G. W. Kaatz, S. Gibbons, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 881 – 885.

Received: July 6, 2009
Revised: October 10, 2009
Published online on October 23, 2009

