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The Relationship between Physicochemical Properties, In Vitro Activity and Pharmacokinetic Profiles of Analogues of Diamine-Containing Efflux Pump Inhibitors

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Abstract—Following the optimization of diamine-containing efflux pump inhibitors with respect to in vitro potentiation activity, in vivo stability and acute toxicity, we addressed the question of how to control the pharmacokinetic properties of the series. Upon intravenous administration in the rat, tissue levels of MC-04,124 (the lead compound) were high and prolonged compared to those in the serum. The lipophilicity and basicity of analogues of this compound were systematically varied, and effects on potency and pharmacokinetics explored. The ratio of drug levels in tissue versus serum was not significantly reduced in any of the active analogues examined.

A number of recent publications have described the discovery and characterization of the biological, microbiological and pharmacological properties of a novel class of broad-spectrum bacterial RND efflux pump inhibitors (EPIs).[1–4] Their optimization with respect to potentiation of the activity of levofloxacin verses *Pseudomonas aeruginosa* (both in vitro and in vivo) and stability have been described, and analogues have been reported with significantly reduced acute toxicity. This effort culminated in MC-04,124 (**1**, Fig. 1) as the optimal agent.[3]

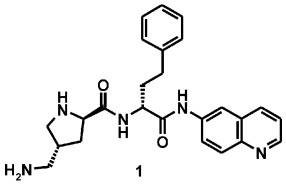

Figure 1. MC-04,124 (**1**).

The pharmacokinetic profiles of **1** in the rat following single and multiple doses are summarized in Figures 2 and 3. In contrast to serum, tissue levels following a single dose remained high for prolonged periods, and this led to significant accumulation of the drug in a variety of organs upon repeated dosing.

Accumulation of polycationic compounds within acidic organelles such as lysosomes can lead to toxicity.[5–7] The observation of high levels of **1** in a variety of tissues, particularly the kidney, therefore, was a concern.

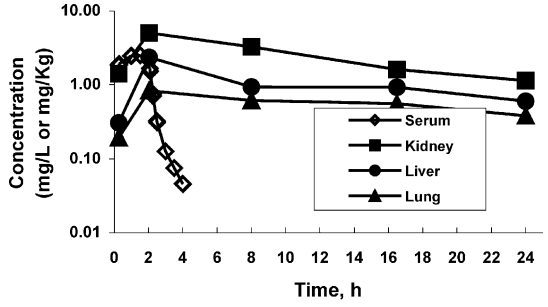

Figure 2. Serum and tissue concentrations of **1** in rats. Two-hour iv infusion of 8.4 mg/kg in three rats; serum data are shown for each rat, and tissue concentrations are the mean. CL = 1.6 L/h/kg, V_{ss} = 0.5 L/kg, $T_{1/2}$ = 0.73 h, protein binding = 63%.

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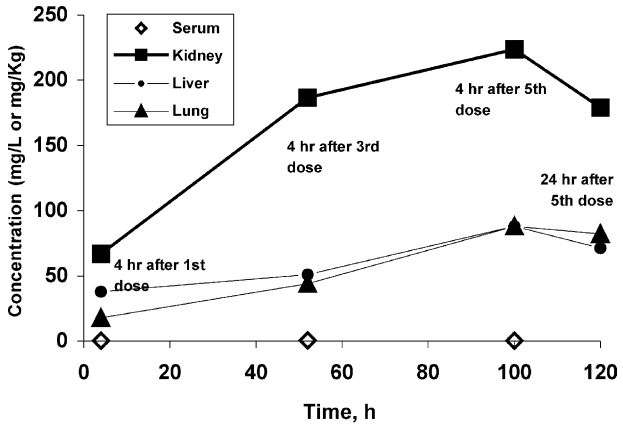
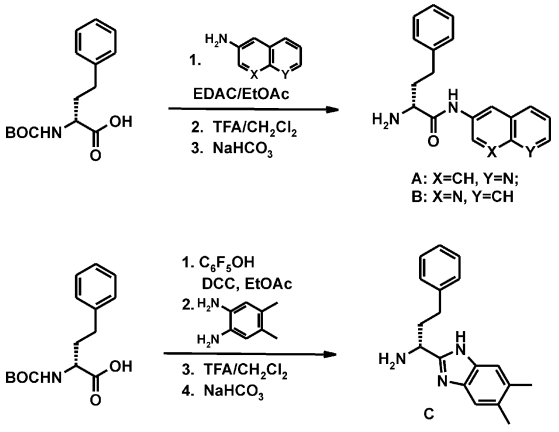


Figure 3. Serum and tissue concentrations of **1** following multiple doses in rats. Daily 10-min iv infusion of 25 mg/kg at 0, 24, 48, 72, and 96 h. Each point represents one rat.

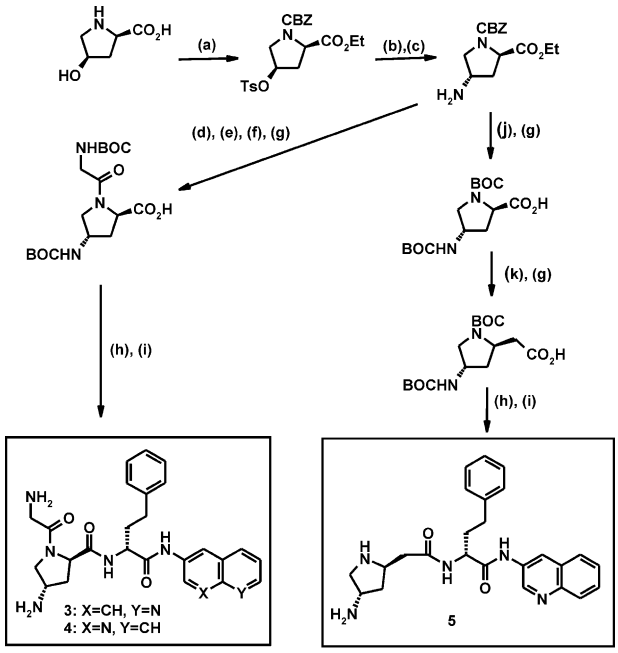


Scheme 1. Synthetic routes to amine intermediates.

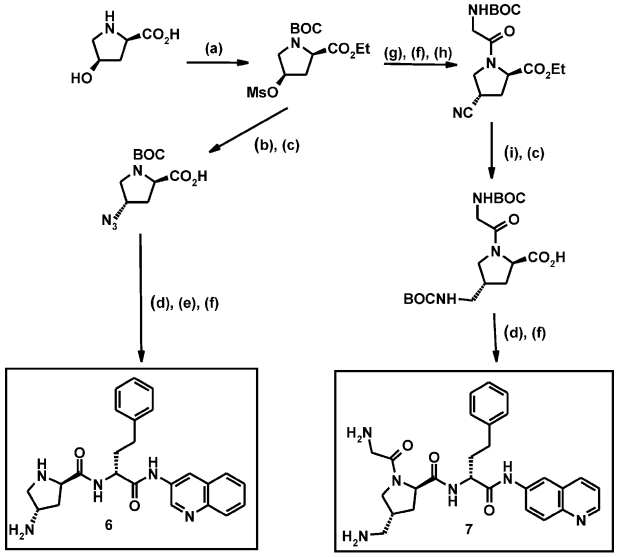
Mindful also of the need to control the pharmacokinetic profile in order to achieve maximum synergy with an antibacterial agent in vivo, a study of the physicochemical characteristics of the series with respect to both levofloxacin potentiation activity and pharmacokinetic properties was instigated. The results are reported here.

The partition coefficient at a given pH (logD) of a dibasic compound is a function of the partition coefficient (logP) and the ionization constants (pK_a) of both basic groups,[8] and may therefore be varied by changes in all three parameters. Initially, we were interested to explore compounds in which the substituted proline moiety was varied. Through incorporation of electron-withdrawing groups adjacent to the basic entities, and through juxtaposition of the basic groups themselves, we were able to modulate the ionization constants quite widely within the range of interest. The synthesis of **1** and **2** has been described previously.[3] The synthetic routes by which the remaining analogues in this study were generated are shown in Schemes 1–4.

Table 1 displays analogues in which the ionization constants are systematically varied, together with their potentiation activity. Analogues containing a 3-quinolyl substituent were marginally more potent than their 6-quinolyl comparators (cf. **2** vs **1** and **4** vs **3**)—perhaps

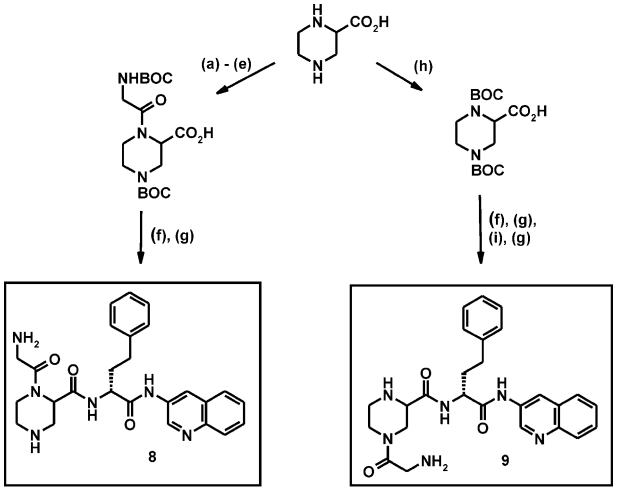


Scheme 2. Synthesis of **3**, **4**, and **5**. Reagents and conditions: (a) see ref 9; (b) NaN_3, $Bu_4N^+Cl^-$, DMF; (c) $SnCl_2$, PhSH, Et_3N, CH_3CN; (d) BOC_2O, aq NaOH, 1,4-dioxane; (e) H_2, Pd/C, MeOH; (f) BOC-Gly-OH, $^iPr_2NEt$, PyBrop, THF; (g) LiOH, THF, MeOH, H_2O; (h) C_6F_5OH, DCC, EtOAc, then amine A or B; (i) TFA; (j) H_2, Pd/C, BOC_2O, EtOH; (k) Et_3N, EtOCOCl, THF; CH_2N_2; $PhCO_2^-Ag^+$.



Scheme 3. Synthesis of **6** and **7**. Reagents and conditions: (a) see ref 10; (b) NaN_3, $Bu_4N^+Cl^-$, DMF; (c) LiOH, THF, MeOH, H_2O; (d) amine A or B, EDAC, HOBT, CH_2Cl_2; (e) H_2, Pd/C, EtOH; (f) TFA; (g) NaCN, DMSO; (h) BOC-Gly-OH, Et_3N, EtOCOCl, CH_2Cl_2; (i) PtO_2, BOC_2O, aq NaOH.

because of their greater lipophilicity (logP increased by 0.3–0.4). The activity is tolerant of considerable structural diversity in the diamine moiety, both with regard to the incorporation of extra amide bonds or hydroxyl groups, as well as the position of the two amines relative to each other and to the rest of the molecule: compare, for example, the similar potency of **1** and **7**, or **2** and **5**. This allows deliberate modulation of the ionization constants, for which comparison of the amine-contain-



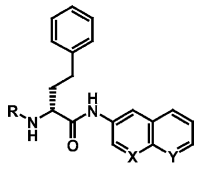
Scheme 4. Synthesis of **8** and **9**. Reagents and conditions: (a) BOC_2O (1 equiv), aq NaOH, 1,4-dioxane, then BnOCOCl; (b) CH_2N_2, THF; (c) H_2, Pd/C, MeOH; (d) BOC-Gly-OH, $^iPr_2NEt$, PyBrop, THF; (e) KOH, THF, MeOH, H_2O; (f) amine A, EDAC, CH_2Cl_2; (g) TFA; (h) BOC_2O (2.2 equiv), aq NaOH, 1,4-dioxane; (i) BOC-Gly-OH, EDAC, CH_2Cl_2.

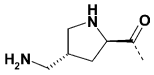
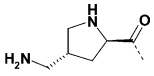
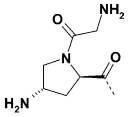
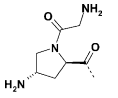
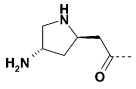
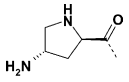
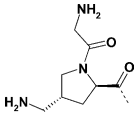
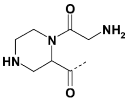
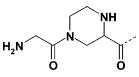
ing substituent at the pyrrolidine 4-position in **2**, **4**, and **6** is illustrative. In **2**, this primary amine is the most basic. In **4**, the methylene unit connecting this amine to the pyrrolidine ring is deleted. Consequently, the amine is closer to electron-withdrawing amide bonds, rendering it less basic; the observed pK_{a_1} is typical of an N-terminal glycine and is probably due to protonation of the other amine in the molecule. In **6**, however, the primary amine at the 4-position becomes the most basic again. Once ionized, its proximity to the ring has a profound effect on the ionization constant of the secondary amine. We attribute the lack of activity of **6** to the inadequate basicity of this secondary amine; incorporation of a methylene linker between it and the adjacent amide, as in **5**, raises pK_{a_2} and restores potency. Similarly, the differing activity of isomers **8** and **9** is attributable to the lower pK_{a_2} in **9**.

Compounds **1**–**9** encompass examples in which the logD is altered primarily by variation in the ionization constant. The tissue pharmacokinetics of a representative subset of these analogues, together with two additional examples (**10** and **11**), are summarized in Table 2. In **10**, the amido-quinolyl substituent is replaced by a much more lipophilic benzimidazole,[4] resulting in good activity (MPC_8 5 μg/mL). In **11**, one amine is removed altogether, resulting in an inactive analogue that is nonetheless an important comparator for establishing the structural features of the series that result in high drug levels in tissues.

Despite the variation of logD from −0.5 to 1.9, no diamine analogues with markedly different tissue distribution profiles from that seen with **1** were found. For **3**, **4**, and **7** lung levels were reduced, perhaps reflecting a somewhat lower logP, but the propensity for distribution to the liver and kidney remained. The profile of **10** appears even more extreme than other diamine analogues—perhaps because of the additional weakly basic

Table 1. Physicochemical parameters and potentiation activity of analogues of **1**



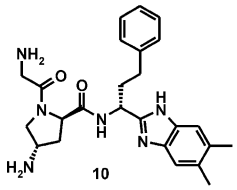
No.	R	X	Y	pK_{a_1}	pK_{a_2}	MPC_8 (μg/mL)
1		CH	N	9.6	6.9	10
2		N	CH	9.6	6.8	5
3		CH	N	8.2	7.2	20
4		N	CH	8.1	7.1	10
5		N	CH	9.3	6.3	5
6		N	CH	8.8	5.2	>40
7		CH	N	9.0	7.7	10
8		N	CH	8.2	6.3	20
9		N	CH	8.5	5.4	>40

benzimidazole moiety. For the (inactive) analogue **6**, the relative lack of basicity is such that at physiological pH it is calculated to exist largely (ca. 95%) as the monocation, although at pH 5 the dicationic form would predominate (75%).[8] In this case the kidney level is somewhat reduced compared to other diamines. The trend is emphasized by the monoamine **11**, which displays tissue levels that are greatly reduced relative to all the other compounds. Taken together, the data strongly implicates the diamine moiety, which is essential for EPI activity, in giving rise to the pharmacokinetic profile seen in **1**.

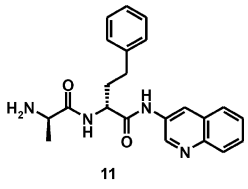
While it is unclear from these studies whether the distribution to tissues is attributable to pH differences within organelles or to some more specific binding phenomenon, none of the active analogues investigated in this study displayed the pharmacokinetic profile we desired. Furthermore, based on the results herein show-

Table 2. Comparative tissue levels of analogues of **1**

No.	logP	logD	$AUC_{0-24\ h}$ (mg h/kg)[a]		
			Kidney	Liver	Lung
1	2.3	0.5	56	22	15
3	1.4	0.5	96	12	4
4	1.8	0.9	79	20	3
6	2.3	1.1	25	13	14
7	1.2	−0.5	100	26	4
10[b]	2.8	1.9	133	176	5
11[c]	ND	ND	8.5	5.5	ND



10



11

[a]Normalized to 1 mg/kg dose; serum $AUC_{0-24\ h}$ for all compounds was ca. 1 mg h/L.
[b]Synthesized as for **3**, but coupling with amine C.
[c]Synthesized by coupling of BOC-D-Ala-OH with amine B ($^{i}Pr_2NEt$, PyBrop, CH_2Cl_2), followed by treatment with TFA.

ing that all dications accumulate regardless of structure type, it is highly probable that no such EPIs fulfilling this particular pharmacophore can be found.

All analogues were purified by reverse-phase MPLC and tested as their bis-TFA salts. The structural identity of each compound was confirmed by ^1H NMR and MS. Levofloxacin was provided by Daiichi Pharmaceutical Co., Ltd (Tokyo, Japan).

The ionization constants, partition coefficients and lipophilicity profiles of the compounds were determined in triplicate at pIon, Inc. (Cambridge, MA, USA), using the GLpKa™ Potentiometric System. LogP measurements are accurate to ±0.3, and pK_a measurements to ±0.15. LogD values are reported at physiological pH (7.4).

Levofloxacin MICs for *P. aeruginosa* strain PAM 1723 (MexAB-OprM overexpressed; *ΔmexCD-oprJ*, *ΔmexEF-oprN*)[11] were measured in the presence or absence of varying concentrations of efflux pump inhibitor (checkerboard format). Drugs were tested in Mueller–Hinton broth according to NCCLS reference methods. The in vitro potency of the EPI is expressed as the minimum potentiation concentration (MPC_8), which is the lowest concentration required for an 8-fold reduction in levofloxacin MIC. Assay controls established the variability of the MPC_8 as ±2-fold. All the compounds reported in this work had no intrinsic antibacterial activity (MIC >40 μg/mL).

Serum and tissue pharmacokinetics were measured in 5 male Sprague–Dawley rats with femoral and jugular venous catheters. EPIs were administered alone or in a cassette of up to five compounds as a 2 h constant rate infusion into the femoral vein at doses varying from 1 to 10 mg/kg. Blood samples were collected for up to 8 h from the jugular catheter. At 0.25, 2, 8, 16, and 24 h after the start of the infusion, a single rat was sacrificed and liver, kidney, and lung tissue were harvested. Serum and tissue homogenates were assayed using HPLC/MS/MS. Pharmacokinetic data were analyzed using compartmental and non-compartmental methods. Tissue $AUC_{0-24\ h}$ were normalized to a dose of 1 mg/kg for comparison.

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