CHEMMEDCHEM

CHEMISTRY ENABLING DRUG DISCOVERY

Accepted Article

Title: Synthesis and Microbiological Evaluation of Novel Tetracyclic Fluoroquinolones

Authors: Allan S Wagman, Ryan Cirz, Glenn McEnroe, James Aggen, Martin S Linsell, Adam A Goldblum, Sara Lopez, Marcela Gomez, George Miller, Lloyd J Simons, Thomas R Belliotti, Christina R Harris, Toni-Jo Poel, Michael J Melnick, Ricky D Gaston, and Heinz E. Moser

To be cited as: *ChemMedChem* 10.1002/cmdc.201700426

Link to VoR: http://dx.doi.org/10.1002/cmdc.201700426

A Journal of

ChemPubSoc Europe

WILEY-VCH

www.chemmedchem.org

Synthesis and Microbiological Evaluation of Novel Tetracyclic Fluoroquinolones

Dr. Allan S. Wagman[a][e], Dr. Ryan Cirz[b], Glenn McEnroe[a][f], Dr. James Aggen[a][g], Martin S. Linsell[a][h], Adam A. Goldblum[a][h], Sara Lopez[a][f], Marcela Gomez[a][i], Dr. George Miller[a], Lloyd J. Simons[d], Thomas R. Belliotti[d], Dr. Christina R. Harris[c], Toni-Jo Poel[d], Dr. Michael J. Melnick[d], Dr. Ricky D. Gaston[d], Dr. Heinz E. Moser*[a][f]

[a] Former or [b]current employees of Achaogen, Inc., 1 Tower Place, South San Francisco, CA 94080

[c] Former or [d]current employees of Kalexsyn, 4502 Campus Drive, Kalamazoo, MI 49008

[e] 3-V Biosciences, 3715 Haven Ave. Suite 220, Menlo Park, CA 94025

[f] Novartis Institute for Biomedical Research, 5300 Chiron Way, Emeryville, CA 94608

[g] Revolution Medicines, 700 Saginaw Drive, Redwood City, CA 94063

[h] Dice Molecules, 220 Penobscot Drive, Redwood City, CA 94063

[i] Emery Pharma, 1000 Atlantic Ave. Suite 110, Alameda, CA 94501

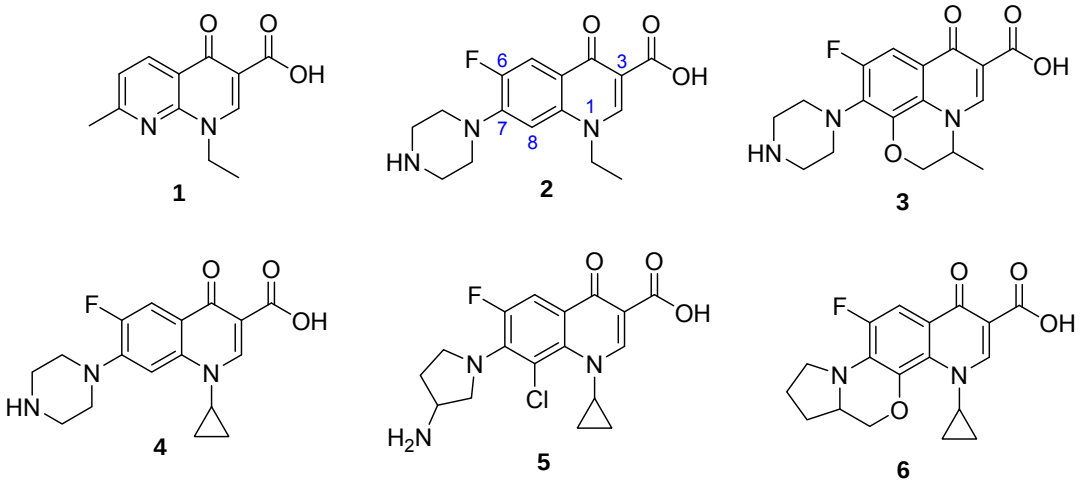
Abstract: Conformationally-constrained tetracyclic fluoroquinolones (FQs) were synthesized and profiled for their microbiological spectrum. The installation of a 7-membered ring between the pyrrolidine substituents and the C_8-position on the FQ core scaffold resulted in a remarkable enhancement of microbiological potency on both Gram-positive and Gram-negative bacteria. Focused optimization of 7-membered ring composition, stereochemistry, and amine placement led to the discovery of the two lead compounds that were selected for further progression.

The need for novel antibiotics, especially against multi-drug resistant (MDR) Gram-negative pathogens, is becoming increasingly dire.[1,2] After a golden era of antibacterial drug discovery roughly half a century ago, the field has been plagued over the past several decades with lack of success [3] due to commercial challenges, regulatory difficulties with the evaluation and approval process, and technical challenges identifying novel compounds that are active against Gram-negative pathogens.[4] In contrast to Gram-positive bacteria, Gram-negative bacteria have a second, outer membrane that lies beyond the cell wall and acts as an additional barrier, preventing highly hydrophobic molecules from crossing. In addition, Gram-negative bacteria generally have a larger array of multi-drug efflux pumps that expel many small molecules from the cell. Therefore target access is significantly more challenging in Gram-negative bacteria.[5]

To this end, we were seeking to make improvements to the existing FQs by exploring the installation of structural rigidity and how it might impact cell permeation and efflux-pump avoidance in Gram-negative pathogens. This class of therapeutics had a long history with the initial member – **1** (nalidixic acid) – introduced to clinical use in 1964 by Sterling.[6-9] The discovery and market introduction of **2** (norfloxacin, 1983), **3** (ofloxacin, 1985; the racemate of levofloxacin), and **4** (ciprofloxacin, 1986),

marked a new milestone for the quinolone class of antibiotics. The introduction of a fluorine atom in position 6 combined with the addition of a positive charge in the R_7 substituent resulted in excellent Gram-negative and good Gram-positive activity. At this time the emerging clinical need for agents active against *S. aureus* and methicillin-resistant *S. aureus* (MRSA) had many research groups focus exclusively on the improvement of Gram-positive antibacterial activity which had an instrumental impact on the evolving structure activity relationship (SAR) understanding of FQs (for reviews see[6,10,11]). Since ciprofloxacin/levofloxacin, a focused effort on optimizing Gram-negative activity of the FQs has not occurred to the best of our knowledge.

Scheme 1. Reference quinolone antibiotics and first tetracyclic analog **6**



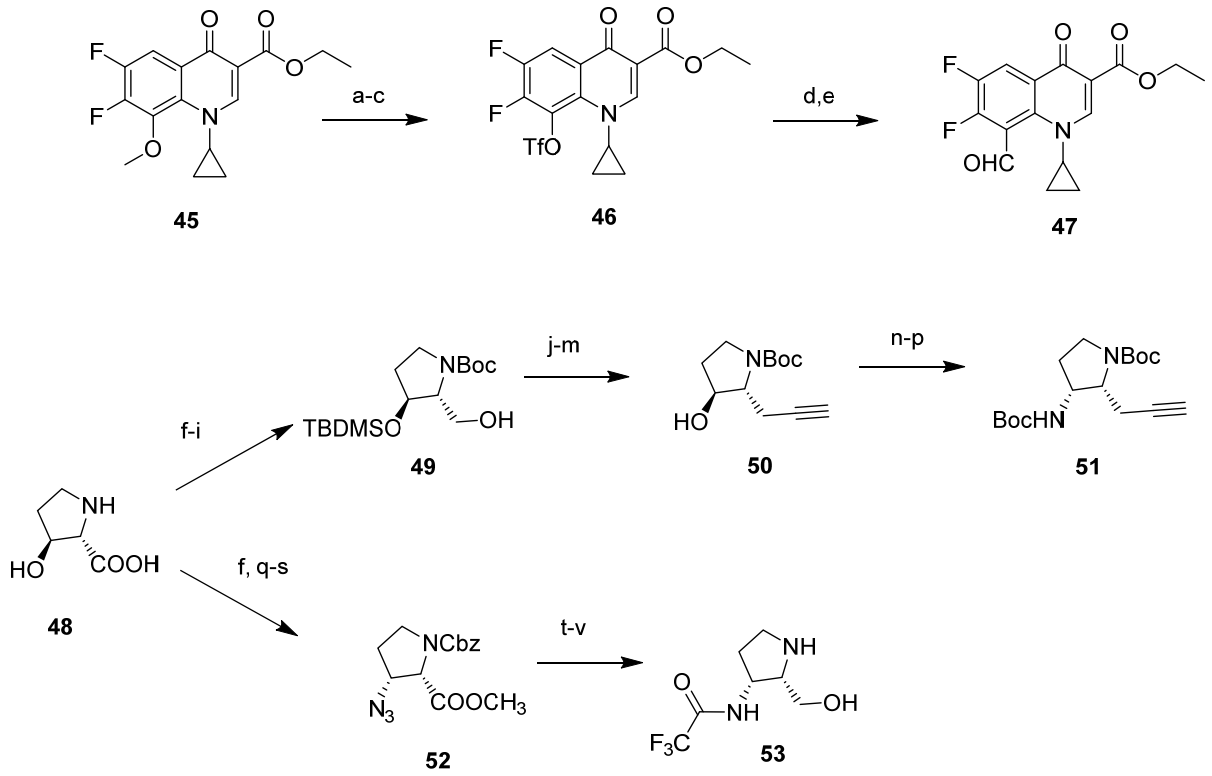
We hypothesized that the conversion of a standard FQ scaffold such as in **4** (ciprofloxacin) or **5** (clinafloxacin) to the corresponding tetracyclic analogs by connecting the R_7 substituent with either the neighboring R_8 or R_6 position on the bicyclic scaffold should result in a preorganization of the saturated R_7 ring. By optimizing the R_7 substituent and its conformation an improvement in activity of the resulting FQ analogs may be seen.

To test this hypothesis, we synthesized a few simple, exemplary derivatives that indicated more promise for the R_7-R_8 than the R_6-R_7 linked derivatives (data not shown) and our attention consequently focused on these scaffolds. Based on the synthesis and examination of a few tetracyclic examples using either piperazine or pyrrolidine as the R_7 group, we found the pyrrolidine derivatives generally to be more potent (data not shown) and we therefore decided to exclusively focus on the pyrrolidine family of compounds to better understand the corresponding SAR. An extensive literature research for 7-8 linked tetracyclic FQs yielded only one literature hit, a patent published in 1987 describing a tetracyclic scaffold linking the R_7 piperazine substituent to the 8-position via a 6-membered morpholine ring.[12] We consequently decided to synthesize first key reference scaffolds by omitting basic substituents on the pyrrolidine ring and preparing racemic compounds (Table 1) to gain better understanding on the SAR of the 7-membered ring composition.

The detailed synthesis of these tetracyclic FQ derivatives has been published previously[13] and we chose to outline only the synthesis of the two identified lead compounds **36** and **40**. The preparation of the key pyrrolidine and quinolone building blocks are shown in Scheme 2. The commercially available quinolone **45** was de-methylated by treatment with hot aqueous HBr, esterified in ethanol by the addition of thionyl chloride, and converted to the triflate **46** by treatment with *N*,*N*-

bis(trifluoromethylsulfonyl)aniline. The triflate was converted to the aldehyde **47** by vinyl addition followed by Lemieux–Johnson oxidative cleavage of the double bond. Both pyrrolidine coupling partners were synthesized starting from *trans*-3-hydroxy-L-proline. Esterification with methanol was followed by Boc-protection of the ring nitrogen, silylation of the 3-hydroxy group and reduction of the methyl ester to yield alcohol **49**. The required two carbon atom extension was achieved by Swern oxidation to the aldehyde, Wittig one-carbon addition to the corresponding enol ether, hydrolysis to the aldehyde followed by Seyferth–Gilbert homologation afforded the terminal alkyne employing the *in-situ* generated Bestmann-Ohira reagent. The alkyne **50** was further transformed to the bis-Boc protected aminopyrollidine **51** by replacement of the alcohol by the azido group with inversion of configuration (Taber and Deker modification of the Mitsunobu reaction),[14] reduction to the corresponding amine and Boc-protection. Preparation of the second pyrrolidine building block **53** started from the *trans*-3-hydroxy-L-proline methyl ester. Cbz-protection was followed by hydroxyl group activation (*m*-nosylate), azide introduction with inversion of stereochemistry, reduction to the amine with *in-situ* Cbz-capture to prevent cyclic urea formation followed reduction of the methyl ester with $LiBH_4$ and global Cbz-deprotection by hydrogenation, and finally selective trifluoracetylation of the primary amine.

Scheme 2. Synthesis of key quinolone and pyrrolidine intermediates

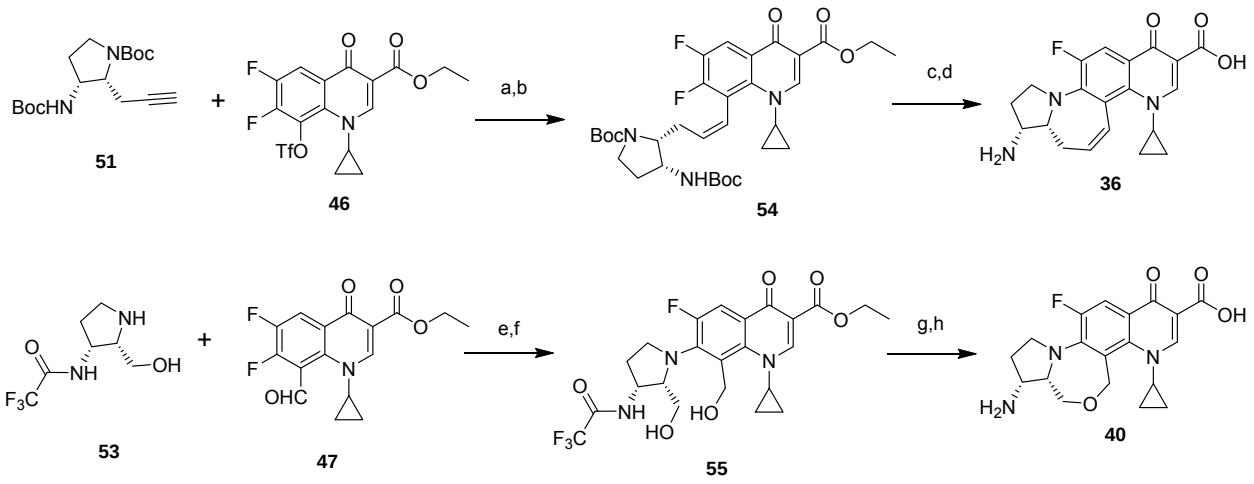


a) 48% aq. HBr, 100 °C, 18 h, 86% (plus roughly 10% decarboxylated by-product); b) Anh. EtOH, 20 eq. $SOCl_2$, 0 °C to RT to reflux (15 min), 75%; c) 2 eq. Hünig's base in THF, 1.05 eq. $C_6H_5N(SO_2CF_3)_2$, RT, 24 h, 66% (recrystallized); d) 1.7 eq. $Bu_3SnCHCH_2$, 2.5 eq. LiCl, 0.1 eq. $Pd(PPh_3)_2Cl_2$, dioxane, 80 °C, 15 h, 25%; e) 0.02 eq. OsO_4, 1 eq. NMO, dioxane/water 3:1, 40 °C, 20 h, then 1 eq. $NaIO_4$, RT, 6 h, 87%; f) 1.44 eq. AcCl in MeOH, 0 °C to 65 °C, 20 h, 95%; g) 2.5 eq. $NaHCO_3$, 1.5 eq. Boc_2O, THF, 0 °C to RT, 10 h, 97%; h) 1.8 eq. imidazole, 1.35 eq. TBDMSCl, DMF, RT, 4 h, 96%; i) 1.5 eq. $LiBH_4$, THF, 0 °C to RT, 18 h, 96%; j) 2 eq. $(COCl)_2$ in $MeCl_2$, -78 °C, then add 4 eq. DMSO followed by **49**, <-65 °C,

20 min, then 4 eq. TEA, 86%; k) 2.5 eq. (Methoxymethyl)triphenylphosphonium chloride, 2.3 eq. tBuOK, THF, 0 °C to RT, 14 h l) 5% TFA in MeCN, RT, 2 h, 58% (2 steps); m) 1.5 eq. Dimethyl-2-oxopropylphosphonate, 3 eq. K_2CO_3, 1.5 eq. 4-methylbenzenesulfonyl azide, MeCN, 0 °C to RT, then addition of SM in MeOH, RT, 10 h, 86%; n) 1.5 eq. DIAD, 1 eq. Ph_3P, $MeCl_2$, 0 °C, then 1.65 eq. DPPA, RT, 12 h; o) 3 eq. Ph_3P in $MeCl_2$, RT, conc. in vacuo, THF/water 20:3, 55 °C, 6 h; p) 1.2 eq. Boc_2O, 0.1 eq. DMAP, RT, 16h, 29% (3 steps); q) 2.4 eq. $NaHCO_3$, THF/water 10:1, 1.27 eq. CbzCl, 0 °C to RT, 23 h, 96%; r) 1.5 eq. *m*-Nitrobenzenesulfonyl chloride, pyridine, 0 °C to RT, 64 h, 91%; s) 4 eq. NaN_3, DMF, 50°C, 16 h, 75%; t) 1.5 eq. Ph_3P, THF, 0 °C to RT, then 56 eq. water, 55°C, 18 h, 3 eq. $NaHCO_3$, 1.5 eq. CbzCl, RT, 3 h, 91%; u) 2 eq. $LiBH_4$, THF, 0 °C to RT, 42 h, 85%; v) 10% Pd/C, MeOH, 1 atm H_2, RT, 2 h, 1 eq. CF_3COOEt, THF/MeOH 4:3, 0 °C to RT, then 16 h, quant.

The preparation of the two final tetracyclic lead compounds is illustrated in Scheme 3. Palladium/copper catalyzed Sonogashira coupling of triflate **46** with alkyne **51** yielded the linked intermediate that was hydrogenated to give the cis-alkene **54**. Deprotection of the pyrrolidine nitrogens with TFA and deprotonation of the resulting ammonium ions led to cyclization under mild conditions. Saponification of the ethylester provided **36**. The other lead compound **40** was assembled by S_NAr reaction to link building blocks **47** and **53**, reduction of the aldehyde, acid-catalyzed cyclization of the diol with concomitant TFA deprotection and finally basic hydrolysis of the ethyl ester.

Scheme 3. Synthesis of lead compounds **36** and **40**

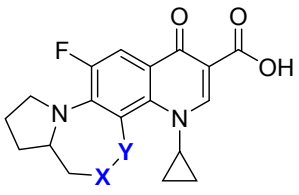


a) 0.25 eq Ph_3P, 0.1 eq. $Pd(PPh_3)_4$, 2 eq. Hünig's base, 0.35 eq. CuI, THF, 55 °C, 12 h, 60%; b) 0.36 eq. Et_3N, 0.44 eq. quinoline, Pd/C, EtOH, 1 atm. H_2, 5 h, 47%; c) 7% v/v TFA in $MeCl_2$, RT, 12 h, 5 eq. Hünig's base, MeCN, RT, 16 h, 89%; d) 1.3 eq. NaOH in MeCN/water 12:1, 60 °C, 3 h, 62%; e) 1.25 eq. **53**, NMP, 1.39 eq. Hünig's base, 50 °C, 3 h, 57%; f) 2 eq. $Na(MeCO_2)_3BH$, $MeCl_2$, RT, 22 h, 79%; g) 4 % TFA in MeCN, RT, 70 h, 42%; h) 4 eq. K_2CO_3, MeOH/water 5:2, 132 h, 77%.

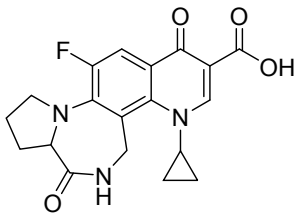
Based on known SAR and understanding the importance of basic amino groups appended to the pyrrolidine substituent especially for Gram-negative potency, the significant loss of activity by the morpholine derivative **6** was not surprising (see Table 1). However, ring expansion by the addition of a methylene group to a saturated 7-membered ring dramatically increased the activity (**7**,**8**), indicating the benefit such an expansion likely has on the relative positioning of the pyrrolidine ring in relation to the bicyclic quinolone core. Replacement of the oxygen by a methylene (**9**) seemed to slightly

negatively affect the activity while the corresponding unsaturated ring system was comparable (**10**). Replacing the middle position of the 3-atom linker by nitrogen was not only tolerated (**11**-**14**) but also resulted in excellent wild-type activities in *E. coli*, *P. aeruginosa* and *A. baumannii* especially for the sp^3-hybridized and charged amines (**12**,**13**). Compound **14**, the acetylated derivative of **12**, did lose a substantial amount of activity, indicating the importance of the charged species for cellular activity. Sulfur was tolerated at the same position (thioether **15**) with insignificant changes on potency with the exception of *P. aeruginosa*. Oxidation to the sulfoxide **16** or sulfone **17** resulted in a decrease of cellular activity with the latter displaying a larger difference. Ketone **18** is comparable to sulfoxide **16** and the activity was improved 4-16 fold by conversion to oxime **20**. Addition of a methyl group to the oxime (**21**) slightly reduced the potency. The related amide **19**, replacing a methylene in **18** by an amine, almost completely abolished the activity, likely due to conjugation with the aromatic core and hence different geometry. Avoiding this conjugation as in the isomeric amide **23** fully restored the activity and resulted in one of the more active compounds. Diol **22** was prepared from the unsaturated derivative with the notion to increase polarity; however, this modification clearly resulted in a reduction of activity.

Table 1. MIC values in µg/mL of tetracyclic FQs, cipro- and clinafloxacin



6 - 22



23

Entry	X-Y	SA001	SP001	EC001	EC003	PA002	PA006	AB1065
4	-	0.52[a]	0.81[b]	0.027[a]	0.018[a]	0.27[a]	0.046[a]	0.13[c]
5	-	0.021[a]	0.051[c]	0.009[a]	0.006[a]	0.12[a]	0.009[a]	0.045[a]
6	O	11[b]	-	32[b]	1.3[b]	>64[b]	64[b]	>64[d]
7	CH_2O	0.06[d]	2[d]	0.63[d]	0.25[d]	40[d]	0.75[d]	2.5[d]
8	OCH_2	0.21[b]	0.67[b]	0.42[b]	0.008[b]	21[b]	0.67[b]	0.38[d]
9	CH_2CH_2	0.5	1	1	1	>64	1	32
10	CHCH	0.10[b]	0.5[b]	0.83[b]	0.18[b]	17[b]	0.42[b]	2[d]
11	NCH	0.5	2	0.03	0.03	1	0.06	>16
12	$NHCH_2$	0.25	1	0.25	0.25	2	0.125	0.25
13	$N(CH_3)CH_2$	0.25	4	0.125	0.125	16	0.25	0.25
14	$N(COCH_3)CH_2$	32	>64	8	2	>64	8	64
15	SCH_2	0.082[b]	6.3[b]	1[b]	0.20[b]	>16[b]	1.67[b]	2[d]
16	$SOCH_2$[e]	1[d]	>16[d]	4[d]	0.16[d]	>16[d]	3[d]	-
17	SO_2CH_2	4[d]	>16[d]	16[d]	1[d]	>16[d]	12[d]	>16[d]

18	$COCH_2$	1	32	4	0.25	>64	2	8
19	CONH	>64	-	>64	4	>64	>64	>64
20	$C(NOH)CH_2$	0.125	2	1	0.03	>16	0.5	2
21	$C(NOCH_3)CH_2$	0.25	4	4	0.06	>32	2	4
22	CH(OH)CH(OH)	1	32	4	0.25	64	2	8
23	-	<0.06	0.5	0.5	<0.06	8	<0.06	2

a) Average from ≥10 measurements with outlier values removed b) average of ≥3 measurements c) average of ≥6 measurements d) average of two measurements e) mixture of stereoisomers. Organisms used in this study: SA001 (*S. aureus* ATCC 29213), SP001 (*S. pneumoniae* ATCC 49619), EC001 (*E. coli* ATCC 25922), EC003 (strain KD65 *ΔtolC*), PA002 (*P. aeruginosa* PAO1), PA006 (*ΔmexAB-oprM, ΔmexCD-oprJ, ΔmexEF-oprN*),[15] AB1065 (*A. baumannii* clinical isolate from 2006, USA, VA; aminoglycoside susceptible). Antibacterial activities were determined according to the broth microdilution method recommended by the Clinical and Laboratory Standards Institute.[16]

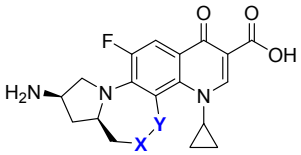
Next we focused on amino-substituted pyrrolidine derivatives to address the question of position and stereochemistry. Aminomethyl-substitued pyrrolidines are known to be highly potent substituents as well, but the increase in pK_a of the amine often results in an increased hERG activity that has to be addressed by additional modifications.[17] Based on the potency, broad spectrum activity, acceptable safety profiles and abundant literature knowledge, we decided to focus our exploration on the aminopyrrolidines. From the previous data set we decided to include only a subset of promising 3-atom linkers with the major focus of carbon, oxygen, and nitrogen analogs in the middle position. Table 2 summarizes the data of the 4-aminopyrrolidines (the nitrogen-containing linker analogs were not synthesized for this series) which provided the following conclusions:

i) The stereochemistry in position 2 of the pyrrolidine is essential and preferred in the *R*-configuration (as determined in compounds **28**-**31**), irrespective of the linker composition.

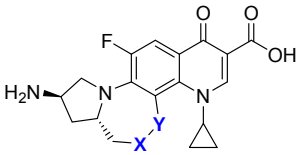
ii) The stereochemistry in position 4 of the pyrrolidine has a less dramatic impact on potency but is consistently preferred in the *S*-configuration, averaging a 4-8 fold improvement.

iii) From the compounds with optimal stereochemistry (**27**,**31**,**33**,**34**), the saturated rings with carbon and oxygen in the middle position (**31**,**33**) and the unsaturated carbon linker (**27**) looked most promising.

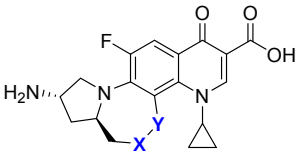
Table 2. MIC values in μg/mL of tetracyclic FQs: Evaluation of stereochemistry for clinafloxacin analogs (first series)



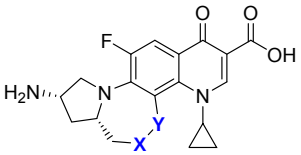
24 (XY = CHCH)
28 (XY = CH_2CH_2)



25 (XY = CHCH)
29 (XY = CH_2CH_2)
32 (XY = OCH_2)



26 (XY = CHCH)
30 (XY = CH_2CH_2)



27 (XY = CHCH)
31 (XY = CH_2CH_2)
33 (XY = OCH_2)
34 (XY = CH_2O)

Entry	SA001	SP001	EC001	EC003	PA002	PA006	AB1065
4	0.52[a]	0.81[b]	0.027[a]	0.018[a]	0.27[a]	0.046[a]	0.13[c]
24	16	8	8	8	>64	4	16
25	0.25	1	0.25	0.125	1	0.06	0.5
26	2	4	2	2	32	1	4
27	≤0.06[b]	≤0.06[b]	0.013[b]	0.008[b]	0.22[b]	0.09[d]	≤0.06[b]
28	8	16	16	16	64	4	16
29	0.25	1	0.25	0.25	1	0.125	0.5
30	8	16	8	8	32	2	8
31	0.038[b]	0.12[b]	0.02[b]	0.006[b]	0.45[b]	0.021[b]	0.067[b]
32	0.5	2	0.004	0.015	0.5	<0.06	0.25
33	0.015	<0.06	0.0005	0.008	0.25	<0.06	<0.06
34	0.125[d]	0.5	0.023[d]	0.034[d]	0.38	0.015	0.093[d]

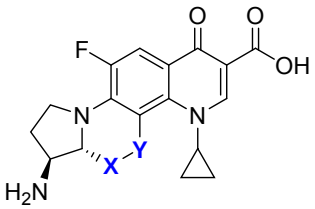
See footnote of Table 1 for specifics

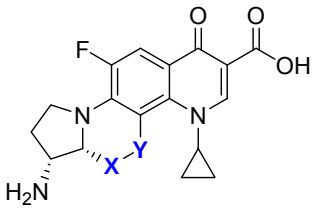
With this SAR understanding in mind, we also evaluated the 3-amino-pyrrolidines **35**-**44** with only compounds shown with the preferred stereochemistry in the 2-position of the pyrrolidine ring (the other diastereoisomers in this series confirmed the same trend as observed for the 4-amino-pyrrolidines, data not shown). While the S stereochemistry is generally preferred at the 3-position of the pyrrolidine ring for the derivatives examined, the difference is relatively small and certainly less pronounced than that demonstrated for the 4-amino pyrrolidines. From the linker composition in the 7-membered ring, the unsaturated carbon analog **36** and the saturated oxygen containing derivative **40** were the most promising compounds identified in this series with excellent activity against Gram-positive and Gram-negative bacteria. Comparison of the matched pairs of 4- and 3-aminopyrrolidines with optimized stereochemistry (Table 2 and 3; **27** vs. **36** and **33** vs. **40**) revealed no significant differences as judged by MIC values of this small bacterial panel.

Finally, we wanted to compare the optimized compounds **36** and **38** containing the 7-membered rings with their 6- and 8-membered ring analogs **45** - **49**. Removal or one methylene group in the bridging ring (**36** vs. **45** and **38** vs. **46**) resulted in a greater than hundred-fold loss of antibacterial potency against most strains examined, presumably due to a more coplanar preorganization of the pyrrolidine

ring in relation to the quinolone core and as permeability (data not shown) and efflux properties are comparable to the FQ comparators. Interestingly, replacement of one methylene by sulfur (**46** vs. **47**) could restore most of the potency observed for the 7-membered analog **38**. This experimental observation can be rationalized by the longer carbon-sulfur bonds (1.83 vs. 1.54 Å) and the preference of substituted thiophenols to position the substituent perpendicular to the aromatic plane. Expansion of the ring size to the 8-membered analogs **48** and **49** reveals a negative impact on antibacterial potency that is more pronounced for the saturated derivative **49** (roughly 100-fold decrease of activity for most strains compared to **38**) than for the unsaturated analog **48** (roughly 10-fold decrease of activity for most strains compared to **38**).

Table 3. MIC values in µg/mL of tetracyclic FQs: Evaluation of stereochemistry for bridged clinafloxacin analogs





35 (XY = CH_2CHCH)
37 (XY = $CH_2CH_2CH_2$)
39 (XY = CH_2OCH_2)
41 (XY = CH_2NCH)
43 (XY = CH_2NHCH_2)

36 (XY = CH_2CHCH)
38 (XY = $CH_2CH_2CH_2$)
40 (XY = CH_2OCH_2)
42 (XY = CH_2NCH)
44 (XY = CH_2NHCH_2)
45 (XY = $CHCH$)
46 (XY = CH_2CH_2)
47 (XY = CH_2S)
48 (XY = CH_2CH_2CHCH)
49 (XY = $CH_2CH_2CH_2CH_2$)

Entry	SA001	SP001	EC001	EC003	PA002	PA006	AB1065
4	0.52[a]	0.81[b]	0.027[a]	0.018[a]	0.27[a]	0.046[a]	0.13[c]
35	0.015	0.03	0.015	0.004	0.093[d]	0.002	0.06
36	0.007[b]	≤0.015[b]	0.0054[b]	0.0022[b]	0.16[c]	0.0083	≤0.015[b]
37	0.045[d]	0.125	0.045	0.06[d]	0.38[d]	0.023[d]	0.38[d]
38	0.015[d]	≤0.06[b]	0.012[d]	0.004[d]	0.29[b]	0.019[d]	0.045[d]
39	0.03	0.125	0.004	0.004	0.125	≤0.06	≤0.06
40	0.0082[b]	≤0.06[c]	0.0057[b]	0.002[b]	0.25[c]	0.010[b]	≤0.03[b]
41	2	2	0.004	0.004	0.5	≤0.06	1
42	2	2	≤0.06	≤0.06	0.5	≤0.06	2
43	4	8	0.03	0.125	1	0.125	4
44	1	1	≤0.06	≤0.06	0.5	≤0.06	2
45	1	8	1	0.25	16	2	4

46	8	64	4	1	>64	4	64
47	0.03	0.25	0.015	0.004	0.5	0.03	<0.03
48	0.125	0.5	0.06	0.03	4	0.25	0.06
49	4	16	1	0.25	32	2	2

See footnote of Table 1 for specifics

In conclusion we have identified multiple novel lead compounds with exquisite potency by bridging the 8-position on the quinolone core with the 2-position of amino-substituted pyrrolidines to form a 7-membered ring and optimizing the corresponding stereochemistry. Tetracyclic FQs **36** and **40** were chosen as lead compounds; their structure bound to *S. pneumoniae* topoisomerase IV has recently be published[18] and further in-depth microbial and preclinical profiling will be published elsewhere.

Acknowledgements: This work was partially funded by contract number HDTRA-07-C-0005. The authors would like to thank John Griffin, Phil Patten and Kevin Judice for support and valuable discussions.

Keywords: Antibiotics, gyrase and topoisomerase, fluoroquinolones, structure-activity relationships, antimicrobial spectrum

Bibliography:

[1] B. Spellberg, J. Bartlett, R. Wunderink, D. N. Gilbert, *Amer. J. Respir. Crit. Care Med.* **2015**, *191*, 135-140.

[2] Antimicrobial resistance interactive database (EARS-net), European Centre for Disease Prevention and Control: http://ecdc.europa.eu/en/healthtopics/antimicrobial-resistance-and-consumption/antimicrobial_resistance/database/Pages/database.aspx.

[3] D. J. Payne, M. N. Gwynn, D. J. Holmes, D. L. Pompliano, *Nat. Rev. Drug Discovery* **2007**, *6*, 29-40.

[4] H. W. Boucher, G. H. Talbot, J. S. Bradley, J. E. Edwards Jr., D. Gilbert, L. B. Rice, M. Scheld, B. Spellberg, J. Bartlett, *Clin. Infect. Dis.* **2009**, *48*, 1-12.

[5] L. L. Silver, *Bioorg. Med. Chem.* **2016**, *24*, 6379-6389.

[6] G. Bisacchi, *J. Med. Chem.* **2015**, *58*, 4874-4882.

[7] D. C. Hooper, *Emerging Infect. Dis.* **2001**, *7*, 337-341.

[8] G. A. Jacoby, *Clin. Infect. Dis.* **2005**, *41* (Suppl. 2), S120-S126.

[9] P. C. Appelbaum, P. A. Hunter, International Journal of Antimicrobial Agents 2000, 16, 5-15.

[10] J. M. Domagala, S. E. Hagen in *Quinolone Antimicrobial Agents* 3rd Edition (Eds.: D. C. Hooper, E. Rubenstein), ASM Press, Washington DC, **2003**, pp. 3-18.

[11] L. A. Mitscher, *Chem. Rev.* **2005**, *105*, 559-592.

[12] S. Jolidon, I. Kompis, R. Locher, E. Weiss, P.-C. Wyss, (F. Hoffmann-La Roche & Co., Basel, Switzerland), European Patent No. EP0216345A2, **1986**.

[13] A. Wagman, H. E. Moser, G. A. McEnroe, J. Aggen, M. S. Linsell, A. A. Goldblum, J. H. Griffin, L. J. Simons, T. R. Bellotti, C. R. Harris, T.-J. Poel, M. J. Melnick, R. D. Gaston, (Achaogen, Inc., South San Francisco, California USA), Int. PCT Pub. No. WO2009/137130 A2, **2009**.

[14] D. F. Taber, P. B. Deker, H. M. Fales, T. H. Jones, H. A. Lloyd, *J. Org. Chem*. **1988**, *53*, 2968-2971.

[15] O. Lomovskaya, A. Lee, K. Hoshino, H. Ishida, A. Mistry, M. S. Warren, E. Boyer, S. Chamberland, V. J. Lee, *Antimicrob. Agents Chemother.* **1999**, *43*, 1340-1346.

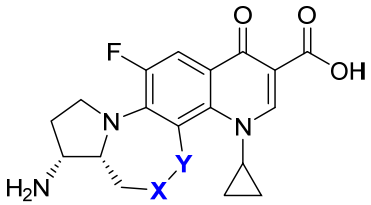
[16] Performance standards for antimicrobial susceptibility testing; 19th informational supplement. Clinical and Laboratory Standards Institute, Wayne, PA **2009**. pp. M100-S19.

[17] C. Jamieson, E. M. Moir, Z. Rankovic, G. Wishart, *J. Med. Chem.* **2006**, *49*, 5029-5046.

[18] I. Laponogov, X.-S. Pan, L. M. Fisher, D. A. Veselkov, M. R. Sanderson, R. T. Cirz, A. Wagman, H. E. Moser, Open Biol. 2016, 6, DOI: 10.1098/rsob.160157.

10.1002/cmdc.201700426

Graphical abstract:



36 (XY = CHCH) MIC (*S. aureus*) = 7 ng/mL
MIC (*E. coli*) = 5.4 ng/mL
MIC (*P. aeruginosa*) = 160 ng/mL

40 (XY = OCH_2) MIC (*S. aureus*) = 8.2 ng/mL
MIC (*E. coli*) = 5.7 ng/mL
MIC (*P. aeruginosa*) = 250 ng/mL

Conformationally constrained tetracyclic fluoroquinolones (FQs) were synthesized and profiled for their microbiological spectrum. The installation of a 7-membered ring between the pyrrolidine substituents and the C_8-position on the FQ core scaffold resulted in a remarkable enhancement of microbiological potency on both Gram-positive as well as Gram-negative bacteria. Focused optimization of 7-membered ring composition, stereochemistry and amine placement yielded the two lead compounds **36** and **40**.