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Substituted 2,5-diazabicyclo[4.1.0]heptanes and their application as general piperazine surrogates: synthesis and biological activity of a Ciprofloxacin analogue

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ABSTRACT

Piperazines and modified piperazines, such as homopiperazines and 2-methylpiperazines, are found in a wide range of pharmaceutical substances and biologically active molecules. In this study 2,5-diazabicyclo[4.1.0]heptanes, in which a cyclopropane ring is fused onto a piperazine ring, are described as modified piperazine analogues. Differentially *N*,*N*′-disubstituted and *N*-monosubstituted compounds can be readily prepared from 2-ketopiperazine in a few steps, using a Simmons–Smith reaction of 1,2,3,4-tetrahydropyrazines with diethylzinc and diiodomethane for the key cyclopropane ring formation. An analogue of the fluoroquinolone antibacterial Ciprofloxacin was synthesized using a palladium-catalyzed Buchwald–Hartwig cross-coupling to attach the diazabicyclo[4.1.0]heptane core to the 7-position of the fluoroquinolone core. The resultant analogue was demonstrated to have similar antibacterial activity to the parent drug Ciprofloxacin. X-ray crystallographic analysis of this analogue reveals a distorted piperazine ring in the diazabicyclo[4.1.0]heptane core. The pK_a of the conjugate acid of *N*-Cbz-monoprotected 2,5-diazabicyclo[4.1.0]heptane was determined to be 6.74±0.05, which is 1.3 pK_a units lower than the corresponding *N*-Cbz-monoprotected piperazine compound. The lower basicity of diazabicyclo[4.1.0]heptanes is due to the electron-withdrawing character of the adjacent cyclopropane rings. The modified physicochemical and structural properties of diazabicyclo[4.1.0]heptanes relative to piperazines are expected to lead to interesting changes in the pharmacokinetic and biological activity profile of these molecules.

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1. Introduction

Nitrogen heterocycles are found in the majority of clinically approved small molecule pharmaceuticals. A few privileged heterocyclic scaffolds, such as pyridines, piperidines, piperazines, pyrrolidines, imidazoles, pyrazoles, indoles, β-lactams, etc., dominate in most cases. There is general recognition of the need for the development of both new heterocyclic scaffolds,[1] as well as approaches to modify existing scaffolds in novel ways to confer desirable biological and pharmacological properties. The goal of modifying existing heterocyclic scaffolds is particularly challenging, however, since most modifications add molecular weight (MW), which often results in concomitant undesirable changes in physicochemical and ADMET behaviour.[2] Therefore for a novel structurally modified heterocyclic scaffold to have utility in a medicinal chemistry program, it must not add significantly to the MW of the system or introduce other structural features that might lead to physicochemical or ADMET problems. This notion is reinforced by well-known practical medicinal chemistry guidelines such as Lipinski's rules.[3]

One important pharmacologically relevant heterocycle is the piperazine ring system **1**, which is found in roughly 7% of all small molecule therapeutics approved by the FDA between the years 1998 and 2009 (Fig. 1).[4] Of these piperazine-containing therapeutics, roughly 25% contained modified piperazine cores. The piperazine ring is used as both a rigid three-dimensional diamine core structure, as well as a modifying group to introduce an amino residue in

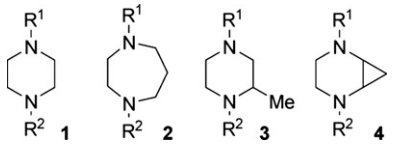

Figure 1. Piperazine and piperazine analogue core structures.

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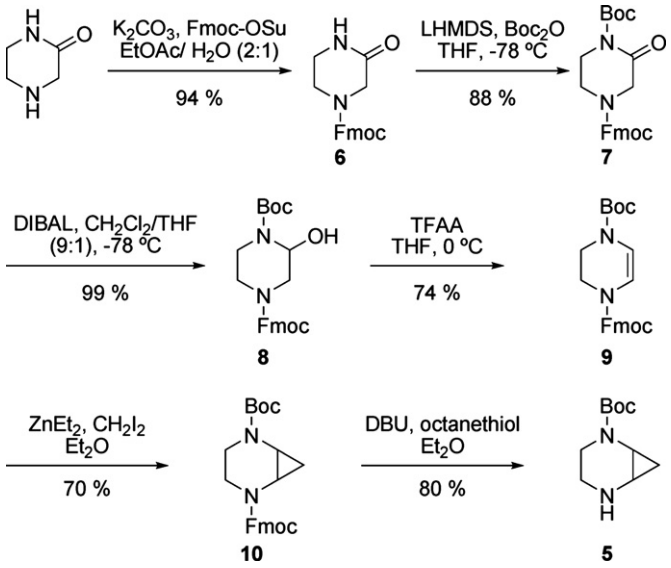
molecules. The latter approach is often taken in order to improve solubility or achieve changes in the ionization state of a potential drug. Structurally modified versions of the piperazine core structure found in pharmaceuticals are mainly limited to the ring-expanded homopiperazine ring system **2** and the 2-methylpiperazine ring system **3**. We now report the synthesis and use of 2,5-diazabicyclo[4.1.0]heptanes **4**, which are minimally modified analogues of the piperazine ring system possessing an embedded fused cyclopropane ring. In addition, we demonstrate the utility of this piperazine surrogate in an analogue of the antibiotic Ciprofloxacin.

Introduction of a fused cyclopropane ring onto the piperazine ring, as for 2,5-diazabicyclo[4.1.0]heptanes **4**, provides a novel piperazine core with only a modest increase in molecular weight. The presence of the cyclopropane ring in **4** would be expected to impose additional conformational constraints, relative to **1**, albeit with the introduction of two new chiral centres (for $R^1 \neq R^2$). Aminocyclopropanes are known to possess rigid structures, and have found extensive application in the synthesis of conformationally restricted polyamines and peptidomimetics.[5] More specifically **4** incorporates a *cis*-substituted 1,2-diaminocyclopropane functionality, a structural motif that is found in a number of biologically active molecules.[6] Thus we envisaged that the 2,5-diazabicyclo-[4.1.0]heptane ring system could be an attractive medicinal chemistry target, combining several biologically active elements within a highly compact, low molecular weight core.

Beyond this intriguing combination of structural features, the presence of a fused cyclopropane ring in **4** would be further expected to alter physicochemical and pharmacokinetic behaviour relative to the corresponding piperazines. In particular, the enhanced s-character of the peripheral cyclopropane bonds should reduce electron density at the ring-nitrogen atoms in **4** through inductive effects, leading to decreased basicity or lower pK_a's of the conjugate acids (ammonium salts). The additional aliphatic substitution imparted by the cyclopropane ring would be expected to modestly increase lipophilicity. Since the acid dissociation constant and lipophilicity figure strongly in determining a drug's pharmacokinetic properties, analogues based on **4** could have altered membrane permeability, distribution behaviour, etc.

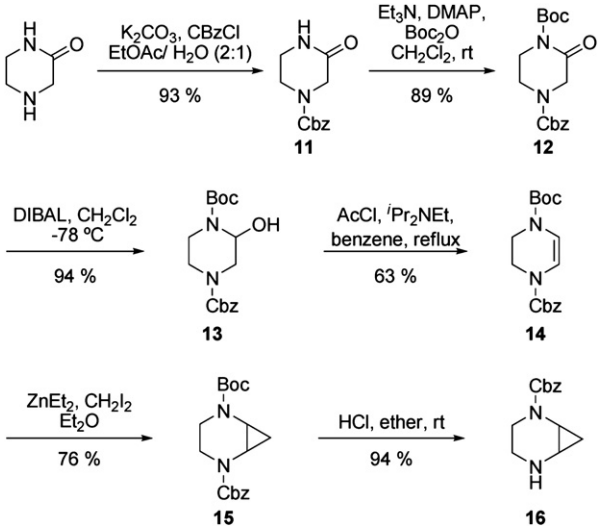
2. Results and discussion

The approach chosen for the synthesis of the 2,5-diazabicyclo[4.1.0]heptane core[7,8] was based upon a cyclopropanation of a 1,2,3,4-tetrahydropyrazine, as exemplified in the preparation of the mono-protected compound **5** (Scheme 1). Reaction of 2-ketopiperazine[9] with Fmoc-*o*-succinamide and potassium carbonate under biphasic conditions furnished Fmoc-2-ketopiperazine **6** in 94% yield. Subsequent treatment with Boc anhydride and LHMDS at −78 °C furnished the diprotected compound **7** in 88% yield. Reaction at low temperature using a strong sterically hindered base was required to prevent premature Fmoc cleavage. Due to the presence of the Fmoc protecting group, the remainder of the synthesis was achieved without the use of bases. Reduction of **7** with DIBAL at −78 °C proceeded smoothly to give the protected hemiaminal **8** in near quantitative yield. At low temperatures **7** had diminished solubility in dichloromethane, and the best yields were attained when a 9:1 dichloromethane/THF solvent system was used. Standard workup conditions for DIBAL reductions, which involve extended stirring with Rochelle's salt, were unsatisfactory for the isolation of **8**, and an expedient quench and workup were required to prevent decomposition of the product. Treatment of **8** with trifluoroacetic anhydride in THF at 0 °C for 20 min furnished the desired protected enamine **9** in 74% yield, via in situ formation of the activated trifluoroacetate ester of **8**, and subsequent trifluoroacetate induced elimination. Finally, cyclopropanation of **9** using a Simmons–Smith procedure with diethylzinc and diiodomethane,[10] afforded the differentially protected 2,5-diazabicyclo[4.1.0]heptane **10** in 70% yield. Typical procedures for Fmoc deprotection of **10**, involving treatment of the protected compound with an excess of piperidine in a polar solvent such as DMF, complicated the isolation of the desired free base **5** from the reaction mixture. Instead, deprotection of **10** using catalytic DBU with octanethiol as a scavenging agent,[11] followed by rapid column chromatography afforded **5** in 80% isolated yield. Generally this compound was freshly prepared from the stable, fully protected precursor **10** immediately prior to its use, as it degraded slowly under ambient conditions.



Scheme 1. Synthesis of Boc-protected 2,5-diazabicyclo[4.1.0]heptane **5**.

A similar protocol was used for the synthesis of the Cbz-protected compound. Thus, reaction of 2-ketopiperazine with benzyl chloroformate and potassium carbonate using biphasic conditions afforded **11** in 93% yield (Scheme 2). Installation of a Boc group using triethylamine and DMAP in the presence of Boc anhydride gave **12** in 89% yield. DIBAL reduction proceeded smoothly to give lactamol **13**, which was used in the subsequent reaction without further purification. As for the reduction of **7** to **8** above, an expedient quench and workup was necessary to minimize decomposition of lactamol **13**.



Scheme 2. Synthesis of Cbz-protected 2,5-diazabicyclo[4.1.0]heptane **16**.

The elimination step proved to be more challenging for this substrate. The use of the previous conditions, treatment of **13** with TFAA in THF, gave the desired enamine **14** in only 50% yield, and attempts to improve the yields were unsuccessful (e.g., by altering reaction temperature or the addition of bases such as triethylamine, DIPEA, 2,6-lutidine and 2,5-lutidine). The best conditions that were identified involved treatment of **13** with 4 equiv each of acetyl chloride and Hünig's base in refluxing benzene for 1.5 h, to give an improved 63% yield of **14**. Cyclopropanation of **14** proceeded smoothly to give **15** in 76% yield. The improved yield for this transformation compared to the analogous one for **10** is perhaps as a result of the improved base stability of the Cbz compared to the Fmoc protecting groups. Finally the Boc group was removed by treatment of **15** with 4 equiv of anhydrous HCl to afford **16** as the hydrochloride salt in 94% yield. One limitation with the current route is that the Simmons–Smith reaction is not enantioselective, leading to racemic products **10** and **15**. Although there is considerable interest in the development of chiral drugs as single enantiomers,[12] in the current study a racemic synthesis was sufficient in order to demonstrate the utility of 2,5-diazabicyclo[4.1.0]heptanes as piperazine surrogates.

With an approach to 2,5-diazabicyclo[4.1.0]heptanes established, we next wished to show their application in an analogue of a known drug. The second-generation fluoroquinolone[13] antibiotic Ciprofloxacin[14] was chosen as an appropriate target since it bears the piperazine motif, and modifications of this residue are known to be tolerated, as for example, in the analogues Trovafloxacin and Enoxacin (Fig. 2). Ciprofloxacin shows activity against a variety of Gram-positive and Gram-negative strains,[15] and through time, has maintained excellent activity against Gram-negative bacteria,[16] although resistance has developed in a number of Gram-positive strains.[17] Ciprofloxacin is commonly prescribed for the treatment of urinary tract infections, prostatitis and enteric typhoid fever,[18] and it is currently the most effective treatment for *anthrax* infections.[19] It has also been used to treat difficult cases of neonatal meningitis.[20] Like other fluoroquinolones, the accepted mechanism by which Ciprofloxacin exhibits antibacterial activity is by interaction with DNA gyrase and topoisomerase.[21] Structure–activity studies have demonstrated that variation at the 7-position of fluoroquinolones influences potency and spectrum,[22] and many analogues have been prepared with different substituents at this site. SAR studies have also demonstrated that the 6-fluoro substituent greatly enhances antibacterial activity,[23] while 8-aza analogues display enhanced pharmacokinetics, although their QSAR is substantially different from that of true quinolones.[24] The continued appearance of antibiotic resistant bacteria, as well as the importance of modulating pK_a and lipophilicity on pharmacological properties, suggested that the Ciprofloxacin analogue **17** would be a clinically relevant target. For example, like the other quinolone antibiotics, Ciprofloxacin enters the cerebrospinal fluid (CSF) via a passive transport mechanism,[25] and the distribution of such drugs into the CSF is mediated largely by lipophilicity and pK_a. Therefore modifications to Ciprofloxacin that would increase its lipophilicity and lower its pK_a, could lead to an increase in its CSF concentration, and perhaps improve its clinical efficacy in the treatment of cerebrospinal infections such as meningitis.

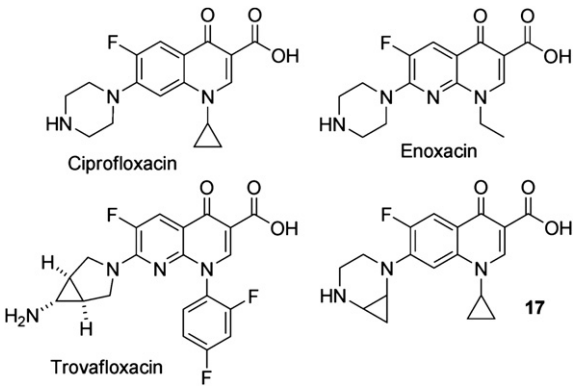
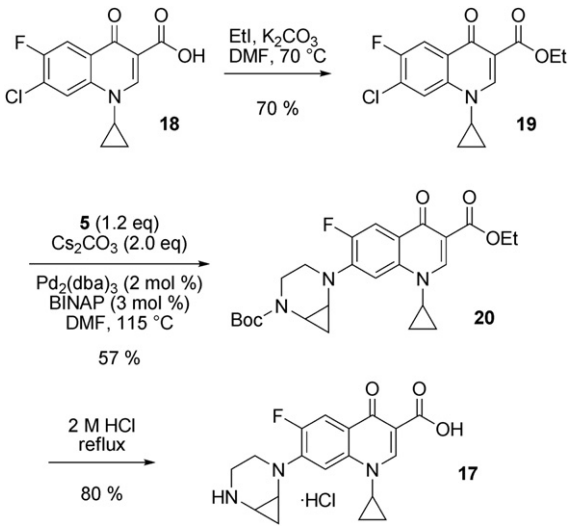


Figure 2. Representative fluoroquinolone antibiotics and 2,5-diazabicyclo[4.1.0]-heptane analogue **17**.

The synthesis of **17** was achieved from commercially available 7-chloro-1-cyclopropyl-6-fluoro-4-oxo-1,4,-dihydroquinoline-3-carboxylic acid **18** using an amination protocol as the key step (Scheme 3). Previous methods employed in the synthesis of Ciprofloxacin from **18** involve S_NAr conditions, and afford approximately 10% of the undesired 6-substituted side product.[26] The use of a boron chelate of **18** has been shown to afford the correct 7-isomer in higher yields and regioselectivity.[27] However, in our experience, this procedure afforded trace amounts of the incorrect 6-substituted product as an inseparable impurity. This impurity was unacceptable given the necessity of the 7-fluoro substituent for good antibacterial activity.



Scheme 3. Synthesis of Ciprofloxacin analogue **17**.

A further undesirable aspect of standard S_NAr conditions is that a large excess of the amine component is invariably required. In contrast, metal catalyzed Buchwald–Hartwig cross-coupling reactions[28] are selective for chlorinated sites, and 1 equiv of amine is often sufficient. A palladium-catalyzed route was therefore investigated for the key amine–quinolone coupling step. Interestingly, there is only one literature example of a Buchwald–Hartwig coupling using **18** as a substrate,[29] with a $Pd_2(dba)_3$/BINAP catalyst system. For our studies the ethyl ester **19** was employed due to its increased solubility. It was prepared in 70% yield by treatment of **18** with iodoethane and potassium carbonate (Scheme 3).

In an effort to improve the efficiency of the palladium-catalyzed coupling step, optimization studies were performed between **19** and a slight excess of *N*-Boc piperazine (1.2 equiv). A number of ligands including QUINAP, Dppf, PCy_3, DavePhos, dpePhos and Xphos were examined. Although dpePhos showed similar activity to BINAP, no ligand assayed offered any significant improvement over the literature procedure. Thus, reaction of *N*-Boc piperazine (1.2 equiv) with **19** using Cs_2CO_3 (2 equiv), $Pd_2(dba)_3$ (2 mol %) and BINAP (3 mol %) in DMF for 3 h gave the *N*-Boc protected ethyl ester of Ciprofloxacin, in 73% yield. The reaction conditions were such that there was no difference in yield using 1.2 and 3.0 equiv of the piperazine nucleophile, and none of the undesired 6-substituted side product was observed by ^1H NMR spectra analysis of the crude reaction mixtures, indicating excellent regioselectivity for

substitution at the 7-position. Application of these conditions to the reaction of **19** with **5** at 140 °C led to the desired product **20** in only 20% yield. ^{1}H NMR analysis of the crude reaction mixture revealed complete consumption of amine **5**. Suspecting decomposition to be the culprit for the diminished yield, the reaction was repeated at a lower temperature. At 115 °C the reaction took considerably longer but after 16 h, **20** was obtained in 57% yield. Further lowering of the temperature did not improve the yield of **20**, but increased the reaction time substantially. Having accomplished the key amination step, all that remained was to deprotect the ester and Boc groups. Refluxing **20** in 2 M HCl for 2 h, achieved global deprotection to give **17** as its hydrochloride salt in 80% yield.

The antibacterial activity of analogue **17** and Ciprofloxacin were compared as minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC),[30] against a number of clinical isolates, representing both Gram-negative and Gram-positive strains (Fig. 3).[31] All of the strains tested were sensitive to **17** at an MIC equal to or slightly higher than Ciprofloxacin. *Streptococcus pneumoniae* and *Escherichia coli* were the only species that required up to four times the concentration of **17** in order to inhibit growth, otherwise MICs were the same such as for *Staphylococcus aureus*, or only two-times higher as for *Neisseria gonorrhoeae*, *Neisseria meningitidis* and *Haemophilus influenzae*. For bactericidal effects, higher concentrations of both Ciprofloxacin and **17** were required for *S. pneumoniae*, *N. meningitidis* and *H. influenzae*. The MBC's of **17** and Ciprofloxacin were very similar for *S. aureus*, while the remainder of the species tested exhibited MBC's for **17** that were two to four times those of Ciprofloxacin. Even though the MIC and MBC values for **17** were somewhat higher than for Ciprofloxacin, they are within the reported range of MIC values of Ciprofloxacin for the various species.[32,33] These results indicate that **17** holds potential as an antimicrobial agent. Since a racemic cyclopropanation strategy was used in the synthesis of the 2,5-diazabicyclo[4.1.0]heptane core, the product **17** is racemic. It has been demonstrated previously that enantiomeric quinolones possessing a chiral heterocycle at position-7 show differences in activity, particularly when the chiral centre occurs alpha to a heteroatom.[34] It is therefore possible that the reduced antibacterial activity of racemic **17** compared to the parent Ciprofloxacin may be the result of differences in activity between the enantiomeric forms of **17**.

A more thorough knowledge of the structural and physicochemical parameters of the diazabicyclo[4.1.0]heptane system would be useful for it to have general medicinal chemistry utility. Representative structural data for this system were obtained from an X-ray crystallographic analysis of **17** (Fig. 4). The 2,5-diazabicyclo[4.1.0]heptane ring of **17** adopts a twist-like conformation, where the cyclopropane ring is somewhat distorted, with the exocyclic C–C bond proximal to the quaternary nitrogen being shortened (1.46 Å) relative to the other exocyclic and endocyclic bonds (1.50 Å). The presence of the cyclopropane forces the two piperazine nitrogens to within a distance of 2.77 Å, which is shorter than that for Ciprofloxacin monohydrate[35] for which the piperazine ring adopts a chair conformation with a distance of 2.86 Å between the nitrogen atoms.

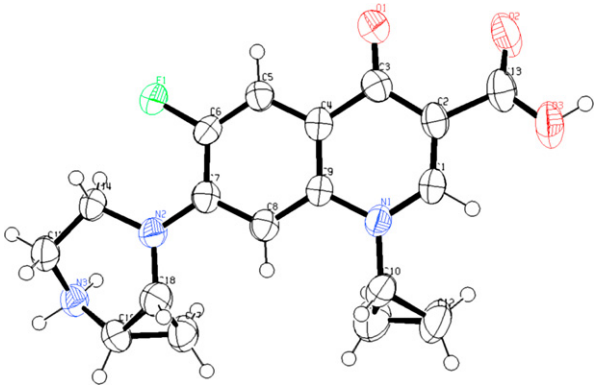


Figure 4. X-ray crystallographic structural analysis of **17** with 50% thermal ellipsoids.

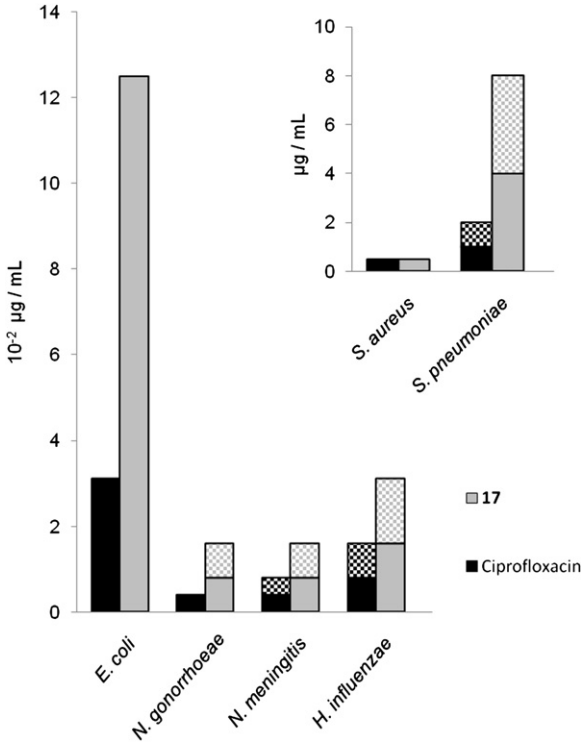


Figure 3. Minimum inhibitory concentrations (solid blocks), and minimum bactericidal concentrations (checkered box) for **17** and Ciprofloxacin against a number of bacterial strains. Results are the consensus of three trials.

As expected, the hydrochloride salts of 2,5-diazabicyclo[4.1.0]heptanes were more stable and easily stored than the corresponding free bases. The pK_a of **16** was determined to be 6.74±0.05 and that of *N*-Cbz piperazine·HCl to be 8.09±0.02,[36] using a multicomponent NMR titration method described by Perrin and Fabian.[37,38] A pK_a of 8.09±0.02 obtained for *N*-Cbz piperazine is in accordance with the reported pK_a of *N*-carbethoxypiperazine (8.28) and that of *N*-benzoylpiperazine (7.78).[39] Our results demonstrate that **16** is less basic than *N*-Cbz piperazine by approximately 1.3 pK_a units. This reduction in pK_a can be attributed both to the closer proximity of the ring-nitrogen atoms in **16**, which would lead to a greater electron-withdrawing through-space interaction, and the electron-withdrawing effects of the cyclopropane ring, mediated by the greater p-character of the cyclopropane C–C bonds. The latter effect has been noted previously by Roberts and Chambers,[40] who observed a 1.15 pK_a unit difference between cyclopropylamine (pK_a=8.66) and cyclohexylamine (pK_a=9.81). A similar reduction in pK_a was noted for a cyclopropylamine, when compared to an alkylamine situated within the same molecule.[41] Another relevant comparison can be made with the pK_a's of Trovafloxacin (pK_a=8.09),[42] Enoxacin (pK_a=8.50) and Ciprofloxacin (pK_a=8.62)[43] where the proximal cyclopropane results in a reduced pK_a for Trovafloxacin.[44] At physiological pH=7.4,[45] compound **16** would be expected to be approximately 83% in the neutral form, whereas only 17% of *N*-Cbz piperazine would be in the neutral form under these conditions. Given that passive transport across membranes relies heavily on the charge state of a given substance, with neutral species passing most easily, such alterations to the piperazine ring could be expected to display altered pharmacokinetic behaviour.[46]

3. Conclusions

This study has demonstrated the utility of the 2,5-diazabicyclo[4.1.0]heptane core as a piperazine surrogate, through an examination of a Ciprofloxacin analogue **17**, which shows comparable antibacterial activity to Ciprofloxacin. The formation of **17** required the use of differentially protected 2,5-diazabicyclo [4.1.0]heptanes, which could be synthesized using a Simmons–Smith cyclopropanation of a 1,2,3,4-tetrahydropyrazine as the key step. A palladium-catalyzed Buchwald–Hartwig cross-coupling was demonstrated to achieve regioselective attachment of the 2,5-diazabicyclo[4.1.0]heptane ring to the fluoroquinolone core.

Further studies will be necessary to validate the generality of the 2,5-diazabicyclo[4.1.0]heptane core as a piperazine surrogate in medicinal chemistry. It can be envisioned that this approach could be employed in order to modulate physicochemical or pharmacokinetic properties of piperazine-based systems. Notably the pK_a of the conjugate acid of 2,5-diazabicyclo[4.1.0]heptanes is lower than comparable piperazines by about 1 pK_a unit, an alteration achieved with only a modest increase in MW. Further studies will be required to develop an asymmetric route to the 2,5-diazabicyclo[4.1.0]heptane core, such as through an enantioselective cylopropanation strategy. Moreover the possibility of incorporating additional substitution on the 2,5-diazabicyclo[4.1.0]heptane scaffold, particularly at the exocyclic cyclopropane methylene position, may be attractive given the subtly different ring conformation expected relative to standard piperazines.

4. Experimental section

4.1. General

THF, ether and benzene were distilled over sodium under nitrogen. Dichloromethane and ethylenediamine were distilled over calcium hydride under nitrogen. All other solvents were obtained from Sigma–Aldrich and were ACS grade or better. All other reagents were obtained from Sigma–Aldrich and were used as received. Melting points were determined on a Fisher-Johns melting point apparatus and were uncorrected. Analytical TLC was performed on silica coated aluminium plates (Alugram SIL, G/UV_{254}), Silicycle Inc. Spots were visualized with UV_{254} or ninhydrin. Flash chromatography was performed using SiO_2 60 Å, 230–400 mesh low acidity silica gel and was obtained from Silicycle Inc. Eluting solvents were reagent grade and obtained from Fischer or Caledon. IR spectra were determined on a Perkin Elmer Spectrum 1000 FT-IR spectrometer, and samples were prepared as either a thin film on NaCl plates or as a KBr dispersion disc. NMR spectra were determined on a Varian Mercury 400 MHz spectrometer. Peaks were referenced to solvent residual peaks: $CDCl_3$ 7.27 ppm proton, 77.23 ppm carbon; D_2O 4.80 ppm proton, DMSO 2.50 ppm proton, 39.51 ppm carbon, dioxane 66.5 ppm carbon, TMS 0 ppm carbon, proton or TSP 0 ppm carbon. High-resolution mass spectra were obtained with an AB/Sciex QStar mass spectrometer. X-ray crystal structures were obtained using a Nonius KAPPA-CCD system. Crystallographic data (excluding structure factors) for the structures in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC 749764. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK (fax: +44 (0)1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

4.2. (9*H*-Fluoren-9-yl)methyl 3-oxopiperazine-1-carboxylate 6

Fmoc-OSu (11.3 g, 33.4 mmol) was added to a vigorously stirred biphasic mixture of 2-ketopiperazine (2.78 g, 27.8 mmol) and K_2CO_3 (31.0 g, 239 mmol) in 420 mL of ethyl acetate/water (2:1). The reaction mixture was stirred at room temperature for 4 h, until complete consumption of 2-ketopiperazine. The organic phase was collected and the aqueous portion extracted with ethyl acetate. After washing with brine, the combined organic extracts were dried over $MgSO_4$ and concentrated at reduced pressure. Purification by silica gel column chromatography eluting with ethyl acetate afforded 8.38 g (26.0 mmol, 94%) of **6** as a white solid: mp=150–151 °C from ethyl acetate. IR (chloroform): 3222, 2549, 2889, 1700, 1680, 1476, 1447, 1362, 1333, 1281, 1232, 1128, 980, 761, 739, 621 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, J=7.5 Hz), 7.56 (2H, d, J=7.5 Hz), 7.41 (2H, t, J=7.5 Hz), 7.41 (2H, dt, J=7.5 Hz, J=1.0 Hz), 6.77 (1H, br s), 4.49 (2H, br s), 4.25 (1H, t, J=6.5 Hz), 4.10 (2H, s), 3.68–3.54 (2H, br m), 3.38–3.27 (2H, br m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.4, 168.0, 154.7, 143.8, 141.4, 127.9, 127.2, 125.0, 120.1, 67.9, 67.6, 47.3, 40.9, 40.3. MS (EI) m/z 322 (1, M^+), 178 (100), 176 (14), 152 (8), 99 (3); HRMS (EI) calculated for $[M]^+$ $C_{19}H_{18}N_2O_3$ 322.1317; observed 322.1314.

4.3. 4-(9*H*-Fluoren-9-yl)methyl 1-*tert*-butyl 2-oxopiperazine-1,4-dicarboxylate 7

LHMDS (7.0 mL of 1.0 M solution in THF, 7.0 mmol) was added to a solution of **6** (2.27 g, 7.02 mmol) in 100 mL of THF cooled to −78 °C. The reaction mixture was allowed to stir at −78 °C for 20 min and Boc anhydride (2.30 g, 10.5 mmol) was added. After 1 h, TLC analysis indicated consumption of **6** and the reaction was quenched with distilled water and warmed to room temperature. The reaction mixture was extracted with dichloromethane, washed with brine and dried over $MgSO_4$. Evaporation of the solvent and purification by silica gel column chromatography eluting with 25% ethyl acetate in hexanes afforded 2.59 g (6.13 mmol, 88%) of **7** as a white solid: mp=109–110 °C from hexanes/ethyl acetate. IR (chloroform): 2987, 2891, 1778, 1712, 1472, 1446, 1424, 1365, 1297, 1237, 1153, 1118, 1049, 1029, 1004, 966, 943, 854, 761, 738 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, J=7.5 Hz), 7.56 (2H, d, J=7.0 Hz), 7.41 (2H, t, J=7.5 Hz), 7.34 (2H, dt, J=7.5, 1.0 Hz), 4.50 (2H, d, J=5.5 Hz), 4.23 (1H, t, J=7.0 Hz), 4.18–1.09 (2H, br m), 3.75–3.53 (4H, m), 1.55 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.6, 154.4, 151.4, 143.7, 141.4, 127.9, 127.2, 124.9, 120.2, 84.2, 67.9, 67.7, 49.0, 47.3, 44.2, 43.8, 41.8, 41.3, 28.2. MS (EI) m/z 423 (4), 422 (35, M^+), 322 (4), 321 (3), 178 (100), 152 (3); HRMS (EI) calculated for $[M]^+$ $C_{24}H_{26}N_2O_5$ 422.1842; observed 422.1842.

4.4. 4-(9*H*-Fluoren-9-yl)methyl 1-*tert*-butyl 2-hydroxypiperazine-1,4-dicarboxylate 8

DIBAL (10.0 mL of 1.0 M in hexanes, 10.0 mmol) was added slowly to a solution of **7** (3.62 g, 8.57 mmol) in 90 mL CH_2Cl_2 and 10 mL of THF at −78 °C. The reaction mixture was stirred at −78 °C for 2 h when an additional portion of DIBAL (2.6 mL of 1.0 M in hexanes, 2.6 mmol) was added. After an additional 2 h, the reaction was quenched with saturated aqueous Rochelle's salt (100 mL) and warmed slowly to room temperature. The resulting gel was filtered over a pad of Celite and washed with diethyl ether. The organic phase was collected, and the aqueous phase extracted with ether. The combined organic extracts were washed with water and brine and then dried over $MgSO_4$. After concentration to a volume of 10 mL, the crude extracts were passed through silica on Celite, eluting with ether. Evaporation of the solvent afforded 3.59 g (8.47 mmol, 99%) of **8** as a white solid. The product was used in the next step without further purification.

4.5. 1-(9*H*-Fluoren-9-yl)methyl 4-*tert*-butyl 2,3-dihydropyrazine-1,4-dicarboxylate 9

TFAA (1.2 mL, 8.8 mmol) was added to a solution of **8** (3.39 g, 8.00 mmol) in 90 mL THF all ready at 0 °C. After stirring at 0 °C for 20 min, the reaction was quenched with saturated aqueous $NaHCO_3$ and warmed to room temperature. The resulting mixture was diluted with distilled water and dichloromethane, and extracted with dichloromethane. The combined organic extracts were washed with brine and then dried over $MgSO_4$. Evaporation of the solvent and purification by column chromatography eluting with 10% ethyl acetate in hexanes afforded 2.40 g (5.93 mmol, 74%) of **9** as a white solid: mp=105–106 °C from hexanes. IR (chloroform): 3063, 2977, 2932, 2891, 1702, 1674, 1449, 1419, 1381, 1365, 1348, 1277, 1226, 1171, 1115, 994, 867, 761, 738 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, J=7.5 Hz), 7.60–7.55 (2H, m), 7.41 (2H, t, J=7.0 Hz), 7.32 (2H, dt, J=7.5, 1.0 Hz), 6.38–6.10 (2H, m), 4.51–4.46 (2H, m), 4.28 (1H, t, J=7.0 Hz), 3.74–3.65 (4H, m), 1.51 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.3, 151.4, 143.7, 141.3, 127.8, 127.1, 125.0, 124.8, 120.1, 109.7, 109.2, 107.6, 106.6, 81.3, 68.0, 67.8, 47.2, 41.8, 41.6, 41.1, 40.8, 40.4, 40.1, 28.3. MS (ESI) m/z 407 ($[M+H]^+$, 7%), 373 (5), 351 (11), 179 (100), 173 (9), 29(4); HRMS (ESI) calculated for $[M+H]^+$ $C_{24}H_{27}N_2O_4$ 407.1965; observed 407.1967.

4.6. (±)-2-(9*H*-Fluoren-9-yl)methyl-5-*tert*-butyl-2,5-diazabicyclo[4.1.0]heptane-2,5-dicarboxylate 10

To a solution of $ZnEt_2$ (13.7 mL of 1.0 M in hexanes, 13.7 mmol) in 25 mL ether was added a solution of **9** (1.32 g, 3.25 mmol) in 40 mL ether via cannula. Diiodomethane (3.16 g, 11.79 mmol) was added, and the reaction mixture was stirred for 18 h at room temperature. After quenching with saturated aqueous NH_4Cl, the reaction mixture was filtered over a pad of Celite and washed with ether. The aqueous portion was extracted with ether. The combined organic extracts were washed with water and brine, dried over $MgSO_4$ and concentrated at reduced pressure to afford the crude product. Purification by silica gel column chromatography eluting with 20% ethyl acetate in hexanes afforded 0.953 g (2.27 mmol, 70%) of **10** as a white solid: mp=52–56 °C from hexanes. IR (chloroform): 3063, 2977, 2876, 1699, 1449, 1406, 1358, 1255, 1229, 1171, 1118, 1057, 1006, 986, 961, 860, 759, 741 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (2H, d, J=7.5 Hz), 7.65–7.55 (2H, m), 7.39 (2H, t, J=7.5 Hz), 7.30 (2H, t, J=7.0 Hz), 4.51–4.42 (2H, m), 4.26 (1H, t, J=6.5 Hz), 3.54–2.94 (6H, m), 1.49 (9H, s), 1.18–0.88 (1H, br s), 0.47 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.8, 156.0, 144.0, 141.4, 127.7, 127.0, 125.0, 124.9, 120.0, 80.4, 80.2, 67.4, 47.3, 42.2, 41.7, 40.8, 40.5, 28.4, 13.8. MS (EI) m/z 420 (1, M^+), 363 (27), 320 (5), 185 (17), 178 (100), 165 (30), 152 (25), 141 (77), 126 (7), 97 (20), 89(1), 68 (14), 57 (39); HRMS (EI) calculated for $[M]^+$ $C_{25}H_{28}N_2O_4$ 420.2049; observed 420.2051.

4.7. (±)-*tert*-Butyl 2,5-diazabicyclo[4.1.0]heptane-2-carboxylate 5

To a solution of **10** (0.533 g, 1.27 mmol) in 15 mL of diethyl ether was added octanethiol (1.85 g, 12.7 mmol). The reaction mixture was allowed to stir for 10 min at room temperature, and then DBU (0.077 g, 0.507 mmol) was added. After 4 h the starting material was consumed and diethyl ether and excess octanethiol were removed at reduced pressure. Purification by column chromatography eluting with 5% methanol in ethyl acetate afforded 0.201 g (1.02 mmol, 80%) of **5** as a colourless oil. IR (thin film): 3349, 3086, 2993, 2932, 2878, 1688, 1535, 1404, 1367, 1263, 1191, 1132, 1057, 1003, 864, 772 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 3.54–3.35 (1H, m), 3.02–2.61 (5H, m), 1.62 (1H, s), 1.49 (9H, s), 0.88–0.76 (1H, m), 0.53–0.48 (1H, m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.8, 156.3, 79.7, 79.4, 42.5, 41.6, 40.9, 30.9, 30.7, 28.5, 26.6, 26.3, 12.8. MS (EI) m/z 198 (2, M^+), 141 (100), 125 (11), 97 (36), 86 (9), 84 (15), 70 (8), 68 (14), 57 (20); HRMS (EI) calculated for $[M^+]$ $C_{10}H_{18}N_2O_2$ 198.1368; observed 198.1360.

4.8. Benzyl 3-oxopiperazine-1-carboxylate 11

Benzyl chloroformate (9.73 g, 57.1 mmol) was added to 2-ketopiperazine (4.80 g, 47.9 mmol) dissolved in 400 mL ethyl acetate and 200 mL water. The reaction mixture was stirred vigorously at room temperature for 16 h. The organic phase was separated and collected, and the aqueous phase extracted with ethyl acetate. The combined organic extracts were washed with water and brine, and dried over magnesium sulfate. Purification by column chromatography eluting with 5% methanol in ethyl acetate afforded 10.5 g (44.8 mmol, 93%) of **11** as a white solid: mp=119–120 °C from ethyl acetate. IR (chloroform): 3478, 3235, 3063, 2942, 2881, 1704, 1671, 1431, 1335, 1234, 1127, 977, 757, 696 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.65–7.40 (1H, br s), 7.38–7.30 (5H, m), 5.16 (2H, s), 4.14 (2H, s), 3.68 (2H, t, J=5.0 Hz), 3.37 (2H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 168.3, 154.8, 136.3, 128.8, 128.5, 128.3, 67.9, 47.5, 41.1, 40.4. MS (ESI) m/z 234 (16 $(M)^+$), 143 (3), 99 (10), 91 (100), 77 (2), 65 (7), 56 (2); HRMS (ESI) calculated for $[M]^+$ $C_{19}H_{18}N_2O_3$ 322.1317; observed 322.1314.

4.9. 4-Benzyl 1-*tert*-butyl 2-oxopiperazine-1,4-dicarboxylate 12

Boc anhydride (4.41 g, 20.2 mmol) was added to a solution of **11** (3.15 g, 13.5 mmol), triethylamine (1.36 g, 13.5 mmol) and DMAP (1.65 g, 13.5 mmol) in 135 mL dichloromethane. The reaction mixture was stirred at room temperature for 16 h, and then diluted with dichloromethane and washed with saturated sodium bicarbonate. The organic phase was washed with water and brine and dried over magnesium sulfate. Purification by column chromatography eluting with 40% ethyl acetate in hexanes afforded 4.0 g (12.0 mmol, 89%) of **12** as a colourless oil. IR (thin film): 2976, 2935, 2894, 1776, 1708, 1419, 1295, 1233, 1154, 9947, 849, 766, 698 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.35 (5H, m), 5.15 (2H, s), 4.24 (2H, s), 3.81 (2H, t, J=5.0 Hz), 3.69 (2H, t, J=4.9 Hz), 1.54 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 154.4, 151.3, 136.1, 128.7, 128.4, 128.2, 84.2, 67.8, 49.1, 44.1, 41.8, 41.4, 28.0. MS (ESI) m/z 357 (50 $(M+Na)^+$), 257 (36), 235 (19), 91 (100); HRMS (ESI) calculated for $[M+Na]^+$ $C_{17}H_{22}N_2O_5Na$ 357.1420; observed 357.1426.

4.10. 4-Benzyl 1-*tert*-butyl 2-hydroxypiperazine-1,4-dicarboxylate 13

A solution of **12** (10.7 g, 32.1 mmol) in 150 mL dichloromethane was cooled to −78 °C. DIBAL (48.0 mmol, 1.0 M in hexanes) was added slowly and the reaction mixture was stirred at −78 °C for 5 h, when all **12** had been consumed as judged by TLC (dichloromethane). The reaction was quenched with 150 mL of saturated aqueous Rochelle's salt and allowed to warm to room temperature. The resulting gel was filtered over Celite and rinsed with ether. The organic phase was collected and the aqueous phase extracted with ether. The combined organic extracts were washed with brine and dried over sodium sulfate. After concentration to a volume of 50 mL, the crude extracts were passed through silica on Celite, eluting with ether to afford 10.19 g of **13** (30.3 mmol, 94%) as a colourless oil, which was used in the next step without further purification.

4.11. 1-Benzyl 4-*tert*-butyl 2,3-dihydropyrazine-1,4-dicarboxylate 14

A solution of **13** (9.08 g, 27.0 mmol), acetyl chloride (10.6 g, 135 mmol) and Hünig's base (17.4 g, 135 mmol) in 150 mL benzene was heated to reflux for 1.5 h. The reaction mixture was cooled to

room temperature, diluted with dichloromethane and washed with saturated sodium bicarbonate, water and brine. Purification by column chromatography eluting with 10% ethyl acetate in hexanes afforded 5.41 g (17.0 mmol, 63%) of **14** as a colourless oil. IR (thin film): 3139, 3033, 2977, 2891, 1707, 1451, 1416, 1376, 1348, 1279, 1224, 1176, 1117, 996, 867, 759, 728, 696 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.38–7.31 (5H, m), 6.41–6.13 (2H, br m), 5.19 (2H, s), 3.72–3.68 (4H, br m), 1.49 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.5, 151.6, 136.2, 128.7, 128.4, 128.2, 109.8, 109.0, 107.8, 107.0, 81.4, 67.9, 41.8, 41.3, 41.0, 40.5, 40.3, 28.5. MS (ESI) *m/z* 341 (84 $(M+Na)^+$), 319 (55 $(M+H)^+$), 285 (16), 263 (81), 219 (56), 175 (23), 128 (5), 91 (100) HRMS (ESI) calculated for $[M]^+$ $C_{17}H_{23}N_2O_4$ 319.1652; observed 319.1650.

4.12. (±)-2-Benzyl 5-*tert*-butyl 2,5-diazabicyclo[4.1.0]heptane-2,5-dicarboxylate 15

A solution of **14** (2.52 g, 7.95 mmol) in 60 mL of dry ether was added slowly via cannula to a solution of diethylzinc (32.6 mmol, 1.0 M in hexanes) in 50 mL ether under nitrogen. The reaction mixture was stirred at room temperature for 10 min, and diiodomethane (7.65 g, 28.6 mmol) was added. The reaction mixture was stirred at room temperature for 18 h, and then cooled to 0 °C. Saturated ammonium chloride was added, and the resulting mixture was filtered over Celite. The Celite pad was washed with water and ether. After extraction with ether, the combined organic extracts were washed with water and brine, and then dried over magnesium sulfate. Evaporation of the solvent and purification by column chromatography eluting with 20% ethyl acetate in hexanes afforded 2.02 g (6.07 mmol, 76%) of **15** as a colourless oil. IR (thin film): 3588, 3518, 3387, 2978, 2932, 2878, 1702, 1454, 1399, 1354, 1295, 1237, 1178, 1119, 1057, 1003, 964, 866, 772, 702 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.37–7.31 (5H, m), 5.20–5.12 (2H, br m), 3.53–2.99 (6H, br m), 1.48 (9H, s), 1.18–0.96 (1H, br m), 0.54 (1H, br s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.9, 156.2, 136.9, 136.6, 128.7, 128.2, 127.8, 80.6, 80.3, 67.4, 42.3, 41.9, 40.9, 40.7, 28.9, 28.6, 28.3, 14.0. MS (ESI) *m/z* 355 (100 $(M+Na)^+$), 333 (5), 315 (4), 299 (22), 277 (12), 255 (10), 233 (15); HRMS (ESI) calculated for $[M]^+$ $C_{18}H_{24}N_2O_4Na$ 355.1628; observed 355.1629.

4.13. (±)-5-(Benzyloxycarbonyl)-2,5-diazabicyclo[4.1.0]heptane hydrochloride 16

Anhydrous hydrochloric acid (29.1 mmol, 4.0 M in dioxane) was added to a solution of **15** (1.61 g, 4.86 mmol) in 20 mL of ether. The reaction mixture was stirred at room temperature for 18 h, and the resulting white precipitate was collected by filtration and rinsed with ether. The combined ether rinses were concentrated to dryness, and the residue triturated with ethyl acetate. The combined solids were recrystallized from ethyl acetate/methanol to afford 1.19 g (4.42 mmol, 94%) of **16** as a white solid: mp=165–167 °C (decomp.). IR (KBr disc): 3384, 2857, 2672, 2420, 2258, 2028, 1975, 1706, 1582, 1413, 1359, 1331, 1200, 1152, 1074, 1029, 951, 827, 766, 741, 697, 677, 607 cm^{-1}. ^1H NMR (400 MHz, DMSO-d_6): δ 10.24 (2H, br s), 7.36–7.29 (5H, m), 5.15–5.10 (2H, m), 3.73–3.55 (1H, m), 3.22–2.97 (5H, m), 1.21–1.07 (2H, m). ^{13}C NMR (100 MHz, DMSO-d_6): δ 155.6, 155.1, 136.7, 136.4, 128.4, 128.2, 127.8, 127.6, 127.3, 66.5, 37.6, 37.1, 27.0, 26.9, 26.1, 25.7, 9.0, 8.6. MS (ESI) *m/z* 233 (15 $(M)^+$), 189 (26), 142 (19), 91 (100); HRMS (ESI) calculated for $[M]^+$ $C_{13}H_{17}N_2O_2$ 233.1284; observed 233.1296.

4.14. (±)-Ethyl 7-(5-(*tert*-butoxycarbonyl)-2,5-diazabicyclo-[4.1.0]heptan-2-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylate 20

A mixture of **19** (0.040 g, 0.130 mmol), Cs_2CO_3 (0.084 g, 0.260 mmol), **5** (0.031 g, 0.160 mmol), $Pd_2(dba)_3$ (0.0020 g, 0.002 mmol), BINAP (0.0020 g, 0.004 mmol) and 1.0 mL DMF was placed in a recovery flask fitted with a reflux condenser and purged with argon. The reaction mixture was heated to 115 °C for 16 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature, and DMF removed at reduced pressure. The residue was taken up in dichloromethane, and filtered over Celite. Evaporation of the solvent and purification by column chromatography eluting with 20% ethyl acetate in hexanes afforded 0.035 g (0.073 mmol, 57%) of **20** as a white solid: mp=205–210 °C from ethyl acetate/methanol. IR (chloroform): 3084, 2978, 1721, 1619, 1508, 1366, 1238, 1163, 1062, 895, 827, 800, 775, 732 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.49 (1H, s), 7.99 (1H, d, *J*=14.5 Hz), 7.39 (1H, br m), 4.38 (2H, q, *J*=7.0 Hz), 3.77–3.05 (7H, br m), 1.50 (9H, s), 1.41 (3H, t, *J*=7.0 Hz), 1.29–1.28 (2H, m), 1.16–1.19 (3H, m), 0.66–0.54 (1H, br m). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.1, 166.0, 156.2, 152.7, 152.5, 150.3, 150.0, 148.1, 141.9, 141.6, 138.2, 121.2, 113.9, 113.6, 110.3, 102.4, 80.5, 80.2, 60.8, 45.9, 43.3, 41.6, 34.4, 33.4, 33.2, 29.5, 28.5, 14.5, 14.0, 8.2, 8.0. MS (EI) *m/z* 471 (3, M^+), 414 (100), 398 (3), 370 (9), 343 (24), 324 (12), 303 (8), 257 (6), 243 (2), 229 (2), 202 (2); HRMS (EI) calculated for $[M]^+$ $C_{25}H_{30}FN_3O_5$ 471.2169; observed 471.2168.

4.15. (±)-7-(2,5-Diazabicyclo[4.1.0]heptan-2-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid hydrochloride salt 17

A mixture of **20** (0.018 g, 0.038 mmol) in 1 mL of 2 M HCl was heated to reflux for 2 h under a nitrogen atmosphere. The reaction mixture was cooled to room temperature a small amount of ethanol was added. The mixture was allowed to crystallize at −12 °C. The solid was filtered and washed with dichloromethane and ice cold ethanol to afford 0.011 g (0.030 mmol, 80%) of **17** as a white solid: mp=241–242 °C (decomp.). IR (KBr disc): 3413, 3038, 2916, 2759, 2653, 1714, 1628, 1507, 1378, 1307, 1274, 1113, 1034, 958, 893, 827, 802, 766, 741 cm^{-1}. ^1H NMR (400 MHz, D_2O): δ 8.41 (1H, s), 7.41 (1H, br d, *J*=6.5 Hz), 7.26 (1H, d, *J*=14.5 Hz), 4.00 (1H, m), 3.98–3.14 (6H, br m), 1.59 (1H, m), 1.41–1.18 (5H, m). ^{13}C NMR (100 MHz, D_2O): δ 177.8, 171.8, 155.3, 152.8, 150.6, 145.7, 141.8, 118.4, 113.7, 113.5, 108.0, 105.9, 44.5, 43.3, 38.8, 33.1, 32.1, 12.3, 10.4, 10.2. MS (ESI) *m/z* 366 (5 $(M+Na)^+$), 344 (100 $(M-Cl^-)^+$), 326 (15), 306 (5), 285 (6), 215 (3), 197 (3), 179 (4), 158 (5), 149 (25), 141 (5), 113 (19), 105 (8), 95 (7); HRMS (ESI) calculated for $[M-Cl^-]^+$ $C_{18}H_{19}FN_3O_3$ 344.1404; observed 344.1417.

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Supplementary data

Additional experimental data, synthetic procedures, NMR titration procedures, copies of ^1H and ^{13}C NMR spectra for all new compounds, crystallographic information file (CIF) for compound **17**. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.tet.2010.02.046.

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