Synthesis and Evaluation of 3-Fluoro-2-piperazinyl-5,8,13-trihydro-5-oxoquino[1,2-*a*][3,1]benzoxazine-6-carboxylic Acids as Potential Antibacterial Agents

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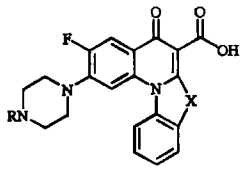
Key Words: *N_1-arylquinolones; antibacterial agents; DNA gyrase inhibitors; active conformation for N_1-arylquinolone antibacterials; 5,8,13-trihydro-5-oxoquino[1,2-a][3,1]benzoxazine-6-carboxylic acids*

Summary

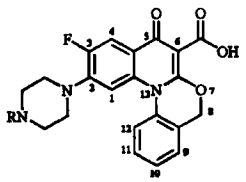
3-Fluoro-2-piperazinyl-5,8,13-trihydro-5-oxoquino[1,2-*a*][3,1]-benzoxazine-6-carboxylic acids were designed and synthesized as potential DNA gyrase inhibitors and antibacterial agents. The design rationale rests on the proposition made by Ohta and Koga that in order for N_1-aryl substituted quinolones to posses antibacterial activity the N_1-aryl ring should be oriented out of the plane of the quinolone ring. α-[Bis(methylthio)methylene]-2,4,5-trifluoro-β-oxobenzenepropanoic acid *tert*-butyl ester (**6**) obtained by the treatment of 2,4,5-trifluoro-β-oxobenzenepropanoic acid *tert*-butyl ester (**5**) with carbon disulfide and methyl iodide in the presence of cesium carbonate was used as a key intermediate, yielding *tert*-butyl 2,3-difluoro-5,8,13-trihydro-5-oxoquino[1,2-*a*][3,1]benzoxazine-6-carboxylate (**8**) upon treatment with 2-aminobenzyl alcohol. The coupling of **8** with piperazines followed by the hydrolysis of the ester under acidic conditions afforded the desired product (**3**). Contrary to expectation, both compounds (**3a,b**) were, however, devoid of antibacterial activity, suggesting that for N_1-aryl substituted quinolones to exhibit antimicrobial activity important structural feature(s) other than the conformational requirement of the N_1-aryl ring with respect to the quinolone nucleus should also be satisfied.

Introduction

Since the discovery of nalidixic acid [1], a prototypical quinolone antibacterial, massive research efforts aimed at improvement of the antibacterial property of the lead compound have resulted in syntheses of a vast number of quinolones having widely varying substituents. Numerous modifications of the ring skeleton have also been undertaken [2]. We have been interested in quinolones having an aromatic ring at the N_1-position. Encouraged by the discovery made by Chu *et al.* that the conformationally restricted analogs of the N_1-aryl quinolones, such as **1** (A-57,207) obtained by bridging the N_1-aryl ring to the 2-position of the quinolone nucleus by a sulfide bridge also possess potent antibacterial activities of a broad spectrum [3], we synthesized 2-(4-alkylpiperazin-1-yl)-3-fluoro-5,12-dihydro-5-oxobenzoxazolo[3,2-*a*]quinoline-6-carboxylic acids (**2**), oxygen isosteres of **1**[4,5]. However, these compounds failed to show any meaningful antibacterial activity in *in vitro* assay, but instead showed cytotoxic activity against human tumor cell lines [4]. The lack of antibacterial activity of **2** was unexpected but may be rationalized on the basis of structure-activity relationships reported by Ohta and Koga [6]. They suggested that the active conformers of N_1-phenylquinolones are those in which the phenyl ring is oriented roughly perpendicular to the quinolone ring [6]. A similar conclusion was also drawn by Domagala *et al.* [7]. The PM3 calculations showed that while the phenyl ring of **1** is oriented out of the plane of the quinolone nucleus with a dihedral angle of 23°, the two rings in **2** remain almost in the same plane. It was therefore thought that insertion of a methylene unit in the bridge linking the two aromatic nuclei in **2** would cause the phenyl ring to move out of the plane of the quinolone ring, satisfying the proposition of Ohta and Koga. They are then expected to display antibacterial activity. Indeed, the PM3 calculations indicated that the angle between the two aromatic rings in **3** is 43° which is substantially larger than 23° in the case of **1**. This communication describes the synthesis and results of antibacterial evaluation of 2-piperazinyl-3-fluoro-5,8,13-trihydro-5-oxoquino[1,2-*a*][3,1]benzoxazine-6-carboxylic acids (**3**).



1; X = S (A57207)
2; X = O



3

Chemistry

The synthesis of target compounds is illustrated in Scheme 1. Treatment of 2,4,5-trifluorobenzoyl chloride (**4**) with malonic acid mono *tert*-butyl ester [8] as described by Wierenga and Skulnick [9] afforded β-ketoester **5** in good yield. The conversion of **5** into **6** was carried out according to the procedure reported by Wentland *et al.* [10]. Thus, the successive addition of carbon disulfide and iodomethane to a cooled (–10 °C) solution of **5** and cesium carbonate in THF afforded **6** in 82% yield together with a by-product (**7**) in a very minute quantity. Higher reaction temperature or prolonged addition of methyl iodide increased the formation of **7** up to 40%. The key intermediate **8** was obtained in 28% yield by allowing **6** to react with 2-aminobenzyl alcohol in the presence of powdered potassium carbonate in refluxing dioxane. We originally synthesized **8** in the form of the ethyl ester, but since attempted hydrolysis of the ethyl ester moiety to the corresponding acid resulted in the cleavage of the oxazine ring as well, we changed our strategy to utilize **8**

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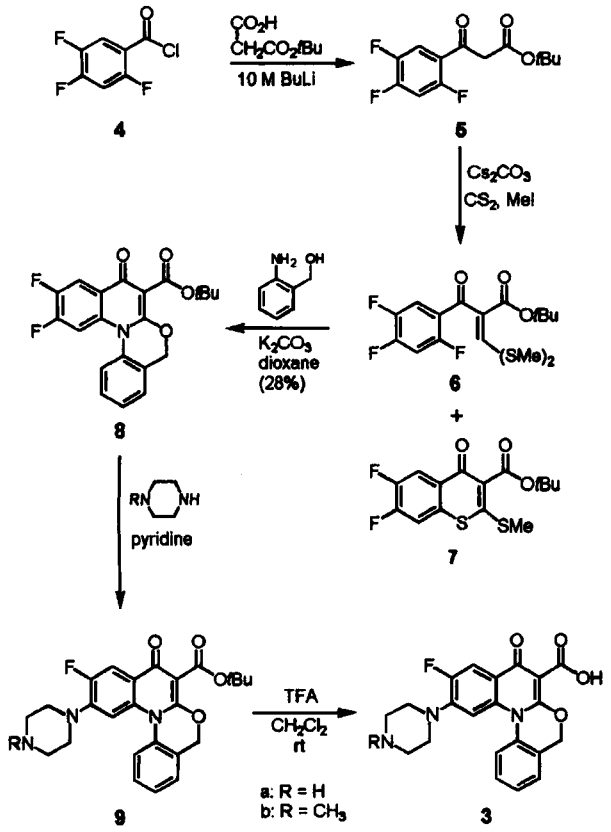
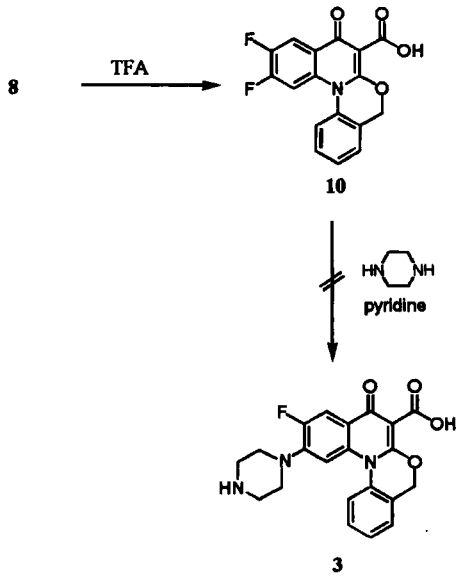


Scheme 1

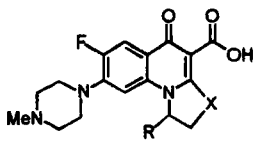
bearing the *tert*-butyl ester moiety which can be readily hydrolyzed under milder conditions. We treated **8** with piperazine under standard conditions to give **9a** which was subsequently treated with trifluoroacetic acid in methylene chloride at room temperature to afford **3a.** In a similar manner, **3b** was prepared. Attempts to introduce piperazine at the 2-position after hydrolysis of the ester moiety were unsuccessful (Scheme 2).



Scheme 2

Results and Discussion

Compounds **3a,b**, which are first examples of a new ring system, were synthesized and tested for *in vitro* antibacterial activity [11], but no meaningful activity was observed at concentrations of up to 200 μM. This was a surprise to us since these compounds were expected to exhibit antibacterial activity by satisfying the conformational requirement of Ohta and Koga [6]. The present observations suggest that in order for quinolone antibacterials having an aryl group at the N_1-position to exhibit the biological activity they must also satisfy other structural feature(s) in addition to the conformational requirement of the two aromatic nuclei. It has been widely known that in quinolone antibacterials the substitution at the 2-position would lead to a loss of antibacterial activity [12]. The lack of antibacterial activity observed with **2** and **3** conforms to the general trend. The potent antibacterial activity shown by **1** is an exception to the general pattern, suggesting that the sulfide linkage behaves differently from other substituents, causing **1** to exhibit antibacterial activity. A recent discovery by Kondo *et al.* that **11** possesses much greater DNA gyrase inhibitory activity than **12** and **13**[13] constitutes an additional example which demonstrates the uniqueness of the sulfide linkage of quinolones. The exact nature of the role played by the sulfide bridge is, however, not immediately apparent and remains to be elucidated.



11: R = H, X = S
12: R = CH_3, X = O
13: R = H, X = NMe

In conclusion, we have synthesized **3a** and **3b** which were expected to have antibacterial activity on the basis of the structure-activity relationships reported by Ohta and Koga [6] but disappointingly they were devoid of such activity when assayed. It appears therefore that there may be present an important factor(s) other than the steric one suggested by Ohta and Koga in exhibiting the biological activity of the N_1-aryl quinolones, and furthermore that the sulfide linkage present in **1** plays a unique role, surpassing the steric requirement in the gyrase inhibition.

Acknowledgment

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Experimental Part

Melting points were determined in a capillary tube with a Thomas Hoover melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded on a Bruker FT-NMR (300 MHz) in $CDCl_3$, TFA-*d* or DMSO-d_6 solution. Chemical shifts are reported in ppm (δ) values relative to TMS as internal reference. IR absorption spectra were obtained in a KBr pellet using a Perkin-Elmer Model 843 spectrometer. Mass spectra were obtained with

Kratos MS 25 RFA spectrometer. Elemental analyses were performed by the Korea Basic Science Center using a Carlo Erba elemental analyzer type CE 1108, and were within ± 0.4% of the theoretical values.

*2,4,5-Trifluoro-β-oxobenzenepropanoic Acid tert-Butyl Ester (**5**)*

A mixture of 2,4,5-trifluorobenzoic acid (17.6 g, 0.1 mole), thionyl chloride (11 ml, 17.8 g, 0.15 mole), and a catalytic amount of DMF was refluxed for 4 h. The excess thionyl chloride was removed *in vacuo* and the residue was distilled under reduced pressure to give **4** (bp 85 °C/17 Torr; 18.4 g, 94.6% yield).

To a solution of malonic acid mono *tert*-butyl ester (25.7 g, 0.1 mole) and 3 mg of 2,2'-bipyridyl (indicator) in THF (800 ml) was added slowly 10 M *n*-butyllithium in hexane, during which the temperature of the reaction mixture was allowed to rise to about –5 °C, and kept at that temperature for 5 min (the pink color of the reaction mixture persisted for 5 min). Thirty-three ml of 10 M *n*-butyllithium (0.33 mole) was consumed. The resulting mixture was chilled to –78 °C, and a solution of **4** (18.4 g) in THF (100 ml) was added dropwise over a period of 10 min. After stirring for 3 h, the reaction mixture was poured into a mixture of ether (800 ml) and 1N HCl (330 ml). The organic layer was separated and washed with a saturated aqueous solution of sodium bicarbonate (100 ml × 2), and with brine. The organic layer was dried over anhydrous $MgSO_4$ and the solvent was evaporated to give a yellowish solid which was recrystallized from hexane to afford 24 g of white crystalline **5**. (The product is a mixture of keto/enol form (in a ratio of 1/2.3) as shown by the ^1H NMR spectrum.): 88% yield; mp 73–75 °C.– IR (KBr): 1634 (C=O) cm^{-1}.– ^1H NMR ($CDCl_3$): δ = 1.45 (s, 2.7 H, C(CH_3)$_3$, keto form), 1.55 (s, 6.3 H, C(CH_3)$_3$, enol form), 3.88 (d, *J*= 3.9 Hz, 0.6 H, COCH_2CO), 5.75 (s, 0.7 H, C(OH)=C*H*CO), 7.0 (m, 1 H, aromatic H_3), 7.5 (m, 1 H, aromatic H_6), 12.9 (s, 0.7 H, enol O*H*).– MS; *m/z* = 274 (M^+), 256 (M^+ – H_2O). -Anal. ($C_{13}H_{13}F_3O_3$).

*α-[Bis(methylthio)methylene]-2,4,5-trifluoro-β-oxobenzenepropanoic Acid tert-Butyl Ester (**6**)*

To a mixture of 5 (11.5 g, 46.7 mmole) and Cs_2CO_3 (38.12 g, 117 mmole) in THF (600 ml) was added CS_2 (20 g, 15.7 ml, 261 mmole) with vigorous stirring at –10 °C. After 5 min methyl iodide (7.3 ml, 16.6 g, 117 mmole) was added in one portion and the reaction mixture was stirred for 12 h while allowing the reaction temperature to reach to room temp. The reaction mixture was diluted with ether (300 ml) and filtered. Crude product obtained by concentration of the filtrate was recrystallized from ethyl acetate-hexane to afford 13 g of **6**: 82% yield; mp 95–97 °C.– IR (KBr): 1691 (C=O), 1684 (C=O) cm^{-1}.– ^1H NMR ($CDCl_3$): δ 1.34 (s, 9 H, C(CH_3)$_3$), 2.28 (s, 3 H, SCH_3), 2.48 (s, 3 H, SCH_3), 6.99 (ddd, $J_{H\text{-}F(l)}$ = 15.6 Hz, $J_{H\text{-}F(2)}$ = 9.6 Hz, $J_{H\text{-}F(3)}$ = 6 Hz, 1 H, aromatic H_3), 7.77 (dd, $J_{H\text{-}F(1)}$ = 17.1 Hz, $J_{H\text{-}F(2)}$ = 9 Hz, 1 H, aromatic H_6).– MS; *m/z* = 378 (M^+), 322 (M^+ – C_4H_8 (isobutene)).– Anal. ($C_{16}H_{17}F_3O_3S_2$).

Concentration of the mother liquor *in vacuo* afforded an oily residue which was purified by column chromatography (silica gel) to give 0.5 g of *6,7-difluoro-2-methylthio-4-oxo-4H-1-benzothiopyrane-3-carboxylic acid tert-butyl ester (**7**)*: 3.1 % yield, mp 95–97 °C (ethyl acetate–hexane).– IR (KBr): 1727 (C=O), 1595 (C=O) cm^{-1}.– ^1H NMR ($CDCl_3$): δ = 1.62 (s, 9 H, OC(CH_3)$_3$), 2.65 (s, 3 H, SCH_3), 7.34 (dd, $J_{H\text{-}F(1)}$ = 9.36, $J_{H\text{-}F(2)}$ = 6.24, 1 H, aromatic H_8), 8.25 (dd, $J_{H\text{-}F(1)}$ = 10.59, $J_{H\text{-}F(2)}$ = 8.1, 1 H, aromatic H_5).– MS; *m/z* = 344 (M^+).

*2,3-Difluoro-5,8,13-trihydro-5-oxoquino[1,2-a][3,1]benzoxazine-6-carboxylic Acid tert-Butyl Ester (**8**)*

A mixture of **6** (3.5 g, 10 mmole), 2-aminobenzyl alcohol (1.58 g, 12 mmole), and K_2CO_3 (3.3 g, 24 mmole) in dioxane (150 ml) was stirred at room temperature for 1 h and refluxed for 18 h. The reaction mixture was concentrated to dryness under reduced pressure, and the resulting oily residue was dissolved in dichloromethane (200 ml). The insoluble materials were filtered off and an oily residue which was obtained by evaporation of the filtrate under reduced pressure was purified by column chromatography (silica gel), eluting with 50% ethyl acetate in hexane to afford pale yellow crystals. The crude product was recrystallized from ethanol to afford 1 g of white crystalline **8**: 28% yield; mp 209–209.5 °C.– IR (KBr): 1723 (C=O), 1612 (C=O) cm^{-1}.– ^1H NMR ($CDCl_3$): δ = 1.59 (s, 9 H, C(CH_3)$_3$), 5.22 (s, 2 H, OCH_2), 7.38 (m, 2 H, aromatic H), 7.48 (m, 2 H, aromatic H), 7.64 (dd, $J_{H\text{-}F(1)}$ = 10.2 Hz, $J_{H\text{-}F(2)}$ = 6.2 Hz, 1 H, aromatic H_1), 8.19 (dd, $J_{H\text{-}F(1)}$ = 10.0 Hz, $J_{H\text{-}F(2)}$ = 8.7, 1 H, aromatic H_4).– MS; *m/z* = 385 (M^+), 312 (M^+ – *tert*-BuO), 285.– Anal. ($C_{21}H_{17}F_2NO_4$).

*2,3-Difluoro-5,8,13-trihydro-5-oxoquino[1,2-a][3,1]benzoxazine-6-carboxylic Acid (**10**)*

A solution of **8** (200 mg, 0.52 mmole) in TFA (3 ml) was stirred at room temperature for 1.5 h. The reaction mixture was poured into an ice-water mixture (100 ml), causing precipitation of a crude product which was collected on a filter, washed with ethanol, and dried *in vacuo* to give 140 mg of analytically pure **10** as a white solid: 82% yield; mp 214–217 °C.– ^1H NMR ($CDCl_3$ + TFA-*d*): δ = 5.2–5.9 (br. d, 2 H, CH_2), 7.61 (m, 4 H, aromatic H) 7.90 (dd, $J_{H\text{-}F(1)}$ = 10.6 Hz, $J_{H\text{-}F(2)}$ = 6.2 Hz, 1 H, aromatic H_1), 8.28 (dd, $J_{H\text{-}F(1)}$ = $J_{H\text{-}F(2)}$ = 8.7 Hz, 1 H, aromatic H_4).– MS; *m/z* = 329 (M^+), 285 (M^+ – CO_2).– Anal. ($C_{17}H_9F_2NO_4$).

*3-Fluoro-2-(piperazin-1-yl)-5,8,13-trihydro-5-oxoquino[1,2-a]-[3,1]benzoxazine-6-carboxylic Acid, Trifluoroacetic Acid Salt (**3a**)*

A mixture of **8** (500 mg, 1.3 mmole) and piperazine (450 mg, 5.2 mmole) in 1-methyl-2-pyrrolidinone (1 ml) and pyridine (2 ml) was stirred at room temperature for 24 h. Dichloromethane (50 ml) was added to the reaction mixture. The diluted reaction mixture was washed with water (30 ml × 4), dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. The oily residue was purified by column chromatography (silica gel), eluting with 7% methanol in chloroform to give **9a** (400 mg, 68% yield). The product was dissolved in a mixture of dichloromethane (10 ml) and TFA (2 ml), and the resulting solution was stirred at room temp. overnight. Addition of ether (60 ml) to the reaction mixture caused precipitation of a solid material which was collected on a filter, washed with ether, and dried under vacuum to give 400 mg of **3a**: 60% yield from **8**; mp 190 °C (dec.).– IR (KBr): 3450 (OH), 1727 (C=O), 1701 (C=O), 1657 (C=O) cm^{-1}.– ^1H NMR (TFA-*d*): δ = 3.70–3.90 (m, 8 H, H of piperazine), 7.73 (m, 5 H, aromatic H), 8.24 (d, $J_{H\text{-}F}$ = 12.4 Hz, 1 H, aromatic H_4).– MS (FAB); *m/z* = 532 (M^+ + Na).– Anal. ($C_{21}H_{19}FN_3O_4$·TFA·$2H_2O$).

*3-Fluoro-2-(4-methylpiperazin-1-yl)-5,8,13-trihydro-5-oxoquino[1,2-a]-[3,1]benzoxazine-6-carboxylic Acid, Trifluoroacetic Acid Salt (**3b**)*

Compound **3b** was prepared from **8** (500 mg, 1.3 mmole) and 1-methylpiperazine (1.04 g, 5.2 mmole) following the method used for the preparation of **3a**: 57% (390 mg) yield, mp 180 °C (dec.).– IR (KBr): 3450 (OH), 1716 (C=O), 1705 (C=O), 1690 (C=O) cm^{-1}.– ^1H NMR (TFA-*d*): δ = 3.16 (s, 3H, N-CH_3), 3.40–4.40 (m, 8 H, H of piperazine), 5.66 (s, 1 H, OCH_2), 5.85 (s, 1 H, OCH_2), 7.77 (m, 5 H, aromatic H), 8.26 (d, $J_{H\text{-}F}$ = 12.4 Hz, 1 H, aromatic H_4).– MS (FAB); *m/z* = 524 (MH^+).– Anal. ($C_{22}H_{20}FN_3O_4$·TFA·$2H_2O$).

Geometry Optimizations of **1**, **2**, *and* **3** were performed with the semiempirical methods PM3 [14] available in the MOPAC 93 program [15]. MOPAC 93 was run using default settings on a Silicon Graphics INDY R4000 workstation.

In vitro Antibacterial Activity was tested according to the literature method using ofloxacin as a reference [11].

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