SYNTHESIS AND BIOLOGICAL EVALUATION OF SUBSTITUTED TETRAHYDRO-1H-QUINO[7,8-B][1,4]BENZODIAZEPINE-3-CARBOXYLIC DERIVATIVES

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Abstract

This research paper aims the preparation of substituted tetrahydroquino[7,8-*b*] [1,4]benzodiazepine-3-carboxylic acids **4a**, **4b** and **4c**. Reaction of 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-1,4-dihydroquinoline-3-carboxylic acid (**6**) with each of 2-amino-5-methylbenzoic acid (**5a**), 2-amino-5-flourobenzoic acid (**5b**), and 2-amino-5-nitrobenzoic acid (**5c**) yielded 8-nitro-7-substituted anilino-1,4-dihydroquinoline-3-carboxylic acids **7** (**a-c**) with low yields. Reduction of **7** with sodium dithionite or stannous chloride resulted in the production of 8-amino-7-substituted aniline-1,4-dihydro-quinoline-3-carboxylic acids **10** (**a-c**). Polyphosphoric acid (PPA) catalyzed thermal lactamization of **10** resulted in the production of **4 (a-c)**. All intermediates and target compounds were characterized using elemental analysis, NMR, IR and MS spectral data. The prepared targets and the intermediates have shown interesting antibacterial activity mainly against Gram positive strains. In particular, the reduced intermediates **10 (a-c)** showed good activity against standard *S. aureus* (MIC = 0.05 - 0.19 µg/mL). Intermediates **10** (**a-c**) have also shown reasonable activity against resistant gram positive strains. The targets **4b** and 4**c** have comparable activity to the reference against standard gram positive strains.

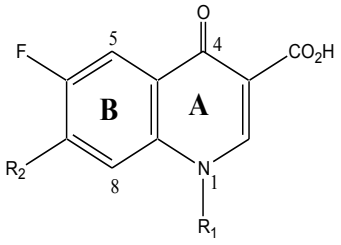
Rezumat

Prezentul studiu a avut ca scop, obținerea unor noi derivați ai acidului tetrahidrochino [7,8-b][1,4]-benzodiazepin 3-carboxilic. Toți produșii intermediari și finali au fost caracterizați folosind analiza elementală, rezonanța magnetică nucleară, studii spectrometrice de masă și în infraroșu. Acești compuși au fost evaluați și din punct de vedere al activității antimicrobiene asupra unor tulpini Gram pozitive, demonstrând rezultate promițătoare.

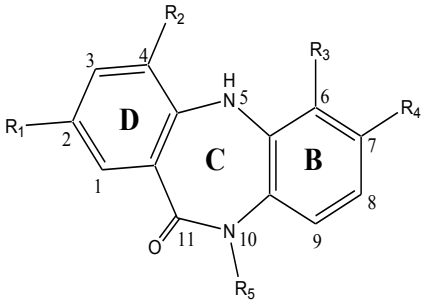
Keywords: 8-nitro-4-oxoquinoline-3-carboxylic acid; 5-fluoro-2-aminobenzoic acid; 5-methyl-2-aminobenzoic acid; tetrahydroquino[7,8-*b*][1,4]benzodiazepine; antibacterial activity.

Introduction

Norfloxacin (**1**) and related fluoroquinolones are synthetic antibacterial agents [2, 4, 8, 11, 14, 16, 18, 21]. Addition of 6-fluoro to the parent 1,4-dihydroquinoline-3-carboxylic acid has revolutionized the clinical use of this nucleus. On the other hand, 5,10-dihydro-11*H*-dibenzo[*b*,*e*][1,4]diazepine-11-ones (e.g. **2a**, Figure 1), were prepared and reported to display different biological activities [1, 3, 5-7, 10, 12, 13, 15, 17, 19, 20]. Some substituted derivatives, such as the natural antibiotic diazepinomicin (**2b**, Figure 1), have been isolated as dibenzodiazepine alkaloids from natural sources [7]. Other derivatives such as clobenzepam (**2c**, Figure 1), and related drugs (e.g. dibenzepine, propizepine, pirenzepine) are successful antidepressant agents [6, 9, 20]. Some of these derivatives were reported to exhibit antimicrobial activity [3, 10, 13], oxytocin and vasopressin antagonist activity [1], antiarrhythmic activity [19], hypoglycemic activity [17], and antitumor activity [5, 12, 15].



1 (R_1 = Et; R_2 = 1-piperazinyl)

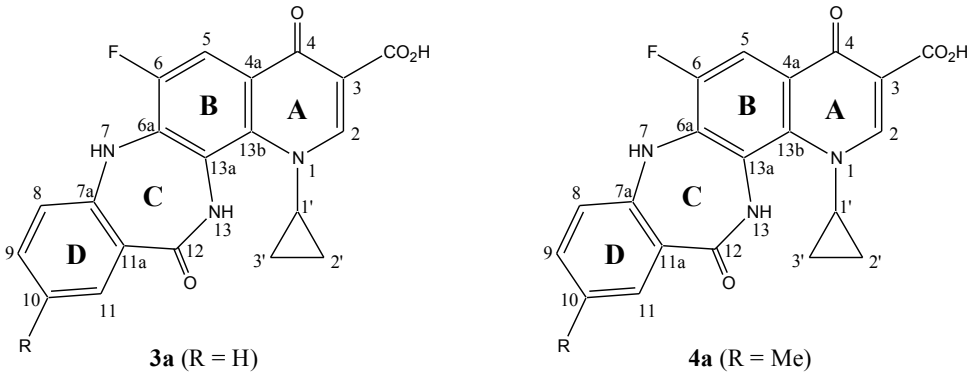


2a ($R_1 = R_2 = R_3 = R_4 = R_5 = H$)
2b ($R_1 = R_2 = R_3 = OH$; $R_4 = H$, R_5 = farnesyl)
2c ($R_1 = R_2 = R_3 = H$; $R_4 = Cl$; $R_5 = CH_2CH_2NMe_2$)

Figure 1
Structures of norfloxacin (**1**), 5,10-dihydro-11H-dibenzo[b,e][1,4]diazepine- 11-one (**2a**), diazepinomicin (**2b**), clobenzepam (**2c**)

Owing to the potential biological interest in these heterocyclic compounds **1** and **2**, a previous research of our group was carried out for synthesis and characterization of heterocyclic system incorporating 4-oxopyridine nucleus condensed to dibenzo[*b*,*e*][1,4]diazepinone to form compounds **3** (**a**,**b**) [3] (Figure 2). This new hybrid system has shown interesting antibacterial activity that guided the present research. As a continuation, this research addresses the preparation of new heterocyclic compounds with the same nucleus **4** (**a**-**c**) (Figure 2). Such novel tetracyclic

systems **4 (a-c)** might exhibit interesting bio-properties such as antimicrobial and/or antitumor activity.



3a (R = H)
3b (R = SO_3H)

4a (R = Me)
4b (R = F)
4c (R = NH_2)

Figure 2
Structures of targets 4,12-dioxo-tetrahydroquino[7,8-*b*][1,4]benzodiazepine-3-carboxylic acid derivatives **3a**, **b** and **4 (a-c)**

Materials and Methods

2-Amino-5-methylbenzoic acid, 2-amino-5-fluorobenzoic acid, and 2-amino-5-nitrobenzoic acid were purchased from Acros, Belgium. Sodium dithionite was purchased from Merck, and $SnCl_2$ from BDH. Melting points (uncorrected) were determined in open capillaries on a Stuart scientific electro-thermal melting point apparatus. Infrared (IR) spectra were recorded with Avatar Thermo Nicolet Impact 400 FT-IR spectrophotometer. Samples were prepared as potassium bromide discs. ^{1}H- and ^{13}C-NMR spectra were measured on a Varian 300 MHz spectrometer and a Bruker UltraShield-300 MHz instrument. Chemical shifts are given in δ (ppm) using tetramethylsilane (Me_4Si) as internal reference and DMSO-d_6 as solvent.

High resolution mass spectra (HRMS) were measured in negative ion mode by electrospray ionization (ESI) technique on a Bruker APEX-2 instrument. The samples were dissolved in acetone, diluted in spray solution (methanol:water:ammonia, in the ratio 1:1:1, v/v/v) and infused using a syringe pump with a flow rate of 2 mm^3/min. External calibration was conducted using arginine cluster in a mass range *m/z* = 175-871.

Elemental analyses were performed on a Euro Vector Elemental Analyzer (EA 3000A-Italy). Thin layer chromatography (TLC) was performed on 10 x 10 cm^2 aluminum plates pre-coated with fluorescent

silica gel GF_{254} (ALBET, Germany). Mobile phase mixtures were chloroform:methanol:formic acid (95:4:1).

Chemistry

7-Chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(6)**

This compound was prepared from 2,4-dichloro-5-fluoro-3-nitrobenzoic acid and ethyl 3-(*N,N*-dimethylamino)-acrylate, according to literature procedure [2].

7-[(2-Carboxy-4-methylphenyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **(7a)**

A stirred mixture of 2-amino-5-methylbenzoic acid (**5a**) (1.4 g, 9.3 mmol), synthon **6** (1.0 g, 3 mmol) and sodium hydrogen carbonate (1.5 g, 18 mmol) in 80 % aqueous ethanol (140 mL) was heated at 80-90°C for 10 days under reflux conditions. The mixture was extracted with dichloromethane (2 x 50 mL) to remove starting material. The aqueous layer was cooled, its pH adjusted to 6-7 by addition of 3.5N HCl and re-extracted with CH_2Cl_2 (2x50 mL). Further acidification of the leftover aqueous layer to pH 2, yielded a yellowish precipitate. The product was then separated by column chromatography, dried and re-crystallized from a mixture of chloroform and ethanol (1:1, v/v), to give the title compound as dark yellow solid.

7-[(2-Carboxy-4-fluorophenyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(7b)**

A stirred mixture of 2-amino-5-flourobenzoic acid (**5b**) (1.42 g, 9 mmol), synthon **6** (1.0 g, 3 mmol) and sodium hydrogen carbonate (1.5 g, 18 mmol) in 50 % aqueous ethanol (140 mL) was heated at 70-75°C for 8-10 days under reflux conditions. Work-up was carried out as described for **7a** to yield the title compound as dark yellow solid.

7-[(2-Carboxy-4-nitrophenyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(7c)**

A stirred mixture of 2-amino-5-nitrobenzoic acid (**5c**) (1.64 g, 9 mmol), synthon **6** (1.0 g, 3 mmol) and sodium hydrogen carbonate (1.5 g, 18 mmol) in 50 % aqueous ethanol (140 mL) was heated at 90-100°C for 7 days under reflux conditions. The work was carried out as described for **7a** to yield the title compound as green yellow solid.

8-Amino-7-[(2-carboxy-4-methylphenyl)-amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(10a)**

To a stirred solution of compound **7a** (0.45 g, 1 mmol) and potassium carbonate (0.98 g, 7 mmol) in 30 mL water was added dropwise an aqueous solution of sodium dithionite (0.88 g, 5 mmol) in water (10 mL). The reaction mixture was then stirred at room temperature (rt) for 60 min.

Thereafter, the pH of the solution was adjusted to about 4 and the precipitated product was collected by filtration, washed with water, air-dried and re-crystallized from acetone and ethanol (1:1, v/v) producing faint yellow crystals of **10a.**

8-Amino-7-[(2-carboxy-4-fluorophenyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(10b)**

To a stirred solution of compound **7b** (0.45 g, 1 mmol) and potassium carbonate (0.98 g, 7 mmol) in 20 mL water was added dropwise an aqueous solution of sodium dithionite (0.87 g, 5 mmol) in water (10 mL). The reaction mixture was further stirred at room temperature for 40 min. The work was carried out as described for **10a**. Crystallization has furnished yellow crystals of **10b**.

8-Amino-7-[(4-amino-2-carboxyphenyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(10c)**

To a stirred solution of compound **7c** (0.48 g, 1 mmol) and potassium carbonate (0.98 g, 7 mmol) in 20 mL water was added dropwise an aqueous solution of sodium dithionite (0.88 g, 5 mmol) in water (10 mL). The reaction mixture was further stirred at room temperature for 120 min. The work was carried out as described for **10a**. Crystallization has furnished greenish yellow crystals of **10c**. This procedure gave low yield and two products (mono reduced).

1-Cyclopropyl-6-fluoro-4,12-dioxo-10-methyl-4,7,12,13-tetrahydro-1H-quino [7,8-b][1,4]benzodiazepine-3-carboxylic acid **(4a)**

A stirred solution of compound **10a** (0.21 g, 0.5 mmol) and PPA (10 mL) was heated under reflux conditions (150-160°C) for 3 h. The resulting mixture was then cooled to 50°C, and poured onto cold water (100 mL) with vigorous stirring. The precipitated greenish solid was collected by suction filtration, washed with water (2 x 10 mL) and dried.

1-Cyclopropyl-6,10-difluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino [7,8-b][1,4]benzodiazepine-3-carboxylic acid **(4b)**

A stirred solution of compound **10b** (0.21 g, 0.5 mmol) and PPA (10 mL) was heated under reflux conditions (150-160°C) for 2 h. The work was carried out as described for **4a**. The reaction furnished a yellow greenish solid.

10-Amino-1-cyclopropyl-6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino [7,8-b][1,4]benzodiazepine-3-carboxylic acid **(4c)**

A stirred solution of compound **10c** (0.21 g, 0.5 mmol) and PPA (10 mL) was heated under reflux conditions (150-160°C) for 1 h. The work was carried out as described for **4a**. The precipitated yellowish green solid was then treated with aq. $NaHCO_3$ to convert the system into free base and was

then separated, collected by suction filtration, washed with water (2 x 10 mL) and dried.

1-Cyclopropyl-6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino[7,8-b] [1,4]benzodiazepine-3-carboxylic acid **(3a)** [3]

A stirred solution of compound *8-Amino-7-[(2-carboxy-4-phenyl) amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (0.2 g, 0.5 mmol) and PPA (10 mL) was heated under reflux conditions (150-160°C) for 3 h. The work was carried out as described for **4a**. This compound was prepared for biological testing [3].

1-Cyclopropyl-6-fluoro-4-oxo-12-hydroxy-4, 7-dihydro-1H-quino[7, 8b][1,4] benzodiazepine-3-carboxylic acid **(3c)**

A stirred solution of *8-Amino-7-[(2-carboxy-phenyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid* (0.2 g, 0.5 mmol) and PPA (10 mL) was heated under reflux conditions (150-160°C) for 3 h. The resulting mixture was then cooled to 50°C, and poured onto cold water (60 mL) with vigorous stirring. pH was adjusted to 7.0-7.5 by adding NaOH solution (40%). The precipitated yellowish green solid product was collected by suction filtration, washed with water (2 x 10 mL) and dried.

1-Cyclopropyl-6-fluoro-4-oxo-12-hydroxy-10-methyl-4, 7-dihydro-1H-quino [7, 8b][1,4]benzodiazepine-3-carboxylic acid **(11a)**

A stirred solution of **10a** (0.21 g, 0.5 mmol) and PPA (10 mL) was heated under reflux conditions (150-160°C) for 3 h. The resulting mixture was then cooled to 50°C, and poured onto cold water (80 mL) with vigorous stirring. pH was adjusted to 7.0-7.5 by adding NaOH solution (40%). The precipitated yellowish green solid product was collected by suction filtration, washed with water (2 x 10 mL) and dried. Compound **11b** and **11c** were prepared similarly.

In vitro Antibacterial Testing: Broth Micro-dilution Method

The minimum inhibitory concentration (MIC) was determined according to the Broth microdilution susceptibility assay which was originally described by the National Committee of Clinical Laboratory Standards (NCCLS) (NCCLS, 2005) currently known as Clinical and Laboratory Standard Institute (CLSI), with some modifications. MIC test was performed using two fold broth dilution method in 96 well microtitre plates. Nutrient agar and Nutrient broth were obtained from Himedia, Mumbai, India. 0.5 McFarland suspension was prepared by adding 0.5 mL of $BaCl_2$ (1.175% w/v $BaCl_2$. $2H_2O$) to 99.5 mL of 0.36N H_2SO_4 (1.0% v/v). Sterilization of materials and equipments was carried out using Raypa steam sterilizer Autoclave. Microbiology samples were incubated at 37°C

using a WTC binder incubator. 96-Flat bottom microplates were used in the conduction of broth dilution test. ELx 800 UV universal microplate reader, Biotek instrument was used to determine the turbidity in the wells. Ciprofloxacin free base was used as reference. The bacterial strains used were *Staphylococcus aureus* ATCC 6538p, resistant *Staphylococcus aureus* ATCC *43300, Escherichia coli* ATCC 8731 *and clinical* resistant strains of *Escherichia coli* 1058. Bacterial suspensions were prepared in sterilized distilled water, in a concentration around $1x10^7$ cfu/mL, which was standardized according to 0.5 McFarland suspension as described by the Clinical and Laboratories Standards Institute (CLSI, 2007).

The minimum inhibitory concentrations (MICs, μg/mL) of test compounds were determined by broth dilution method, screening different concentrations in the range 100–0.048 μg/mL. The MIC is defined as the lowest concentration of the tested compound showing no growth.

A stock solution of each tested compound was prepared in DMSO (100 μg/mL). The MIC test was performed in 96 flat bottom microtiter plates, 100 μL of previously prepared and sterilized broth (prepared by dissolving 1.3 g of dry preparation in 100 mL of distilled water) was added in each well, with an exception to the first well where 100 μL of double strength, sterilized broth was added (prepared by dissolving 1.3 g of dry preparation in 50 mL of distilled water) in order to maintain the consistency of the broth along the plate after the addition of the tested compound. An equivalent volume of 50 μg/mL of each compound was added to the first well, mixed with the broth, followed by two fold serial dilutions onto successive wells across the plate to end up with 11 successive two fold dilutions for each of the tested compounds. Then 10 μL of bacterial suspension was used to inoculate each well. Control tests for each experiment were performed. Positive growth control was performed by adding one drop of each micro-organism suspensions to four wells in each plate of the culture medium without the test compound. Negative growth control was also performed using four un-inoculated wells of medium without the test compound. Plates were incubated at 37°C for 24 h, and were checked for turbidity. Two fold serial dilutions were carried out in a similar manner for DMSO (20% v/v in water) to test its antibacterial activity. Ciprofloxacin standard was tested also as reference compound. The turbidity was determined visually and using a micro-plate reader.

Evaluation of the anticonvulsant activity (subcutaneous Pentylenetetrazole (PTZ) seizure threshold test)

Male albino mice weighing 22-30 g, maintained under a controlled temperature (25°C) with 45% humidity at the Al-Ahliyya Amman

University animal care center were used as experimental animals. The animals had free access to food and water except when removed from their cages for the experimental procedures. Group 1 served as control and received an equivalent amount of 0.9% sodium chloride solution, group 2 received phenytoin (25 mg/kg body weight i.p) and served as reference standard. For preliminary screening, two compounds (**3a** and **4a**) were injected intraperitoneally (i.p.) in groups of 4 animals at doses of 30 mg/kg body weight (suspended in 0.5% methylcellulose / water mixture) and gross behavior was monitored. Forty five min later, PTZ was injected subcutaneously at a dose of 80 mg/kg body weight dissolved in 0.9% sodium chloride solution.

The animals were then observed for 1 h for convulsions. For the experimental compounds, protection against convulsions was defined as the failure to observe an episode of clonic spasm of at least 5 seconds duration during this time period.

Results and Discussion

The targets 4,12-dioxo-tetrahydroquino[7,8-*b*][1,4]benzodiazepine-3-carboxylic acid derivatives (**4a-c**) were prepared *via* direct reaction of 2-amino-5-methylbenzoic acid (**5a**), 2-amino-5-fluorobenzoic acid (**5b**), and 2-amino-5-nitrobenzoic acid (**5c**) with 7-chloro-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**6**) in 50% aqueous ethanol containing sodium bicarbonate (Figure 3).

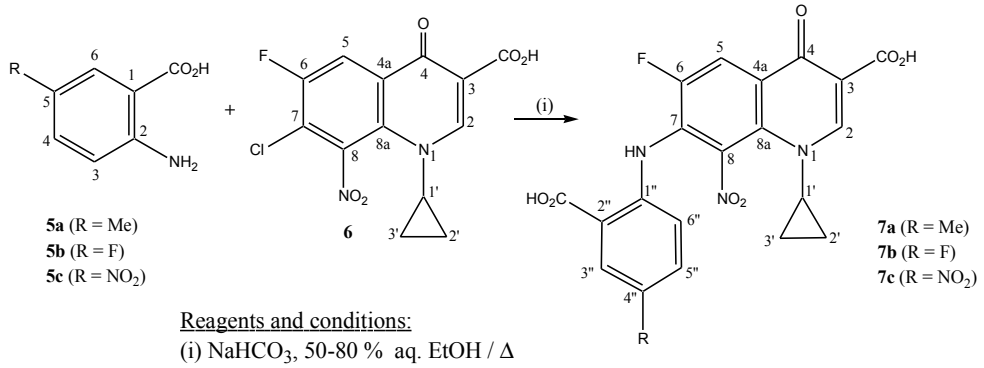


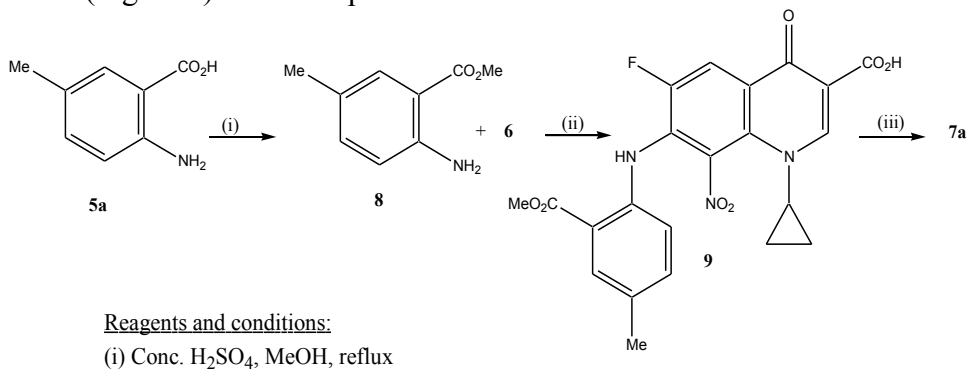
Figure 3
Synthesis of **7a-c**

The primary amino group of 2-amino-5-fluorobenzoic acid and 2-amino-5-methylbenzoate acts as a nucleophile that binds to the C-7 of the quinolone nucleus *via* a regiospecific nucleophilic aromatic substitution

(addition-elimination) reaction. This mode of S_N-Ar substitution reaction is assumed to be facilitated by the presence of the electron-withdrawing nitro group at C-8 of synthon **6**, together with the keto group and fluorine atom at positions 4 and 6, respectively.

However, this procedure allowed the obtaining of low yield of the intermediates **7** and this was due to low nucleophilicity of the aromatic amine and bad solubility in aq. solution. Although ethanol was increases up to 80 %, the yield did not improve significantly and the experimental procedure was not reproducible. Only one or two attempts gave the products **7 (a-c)** followed by column chromatography. Different attempts were carried out involving the use of DMSO/pyridine, DMF/K_2CO_3 yielding mainly the 1-cycloprpyl-6-fluoro-7-hydroxy-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid and 1-cycloprpyl-7-ethoxy-6-flouro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid side products [2]. The products **7** (**a**-**c**) were collected upon column chromatography for further reduction. Although low amounts of **7** were collected, enough was used to carry out the next two steps.

To solve this problem, an alternative procedure was adopted to prepare **7a** as a model. The ester 5-methyl-2-aminobenzoate (**8**) was prepared to solve solubility problem, giving higher yields upon coupling with **6** (Figure 4). Further optimization is still needed.

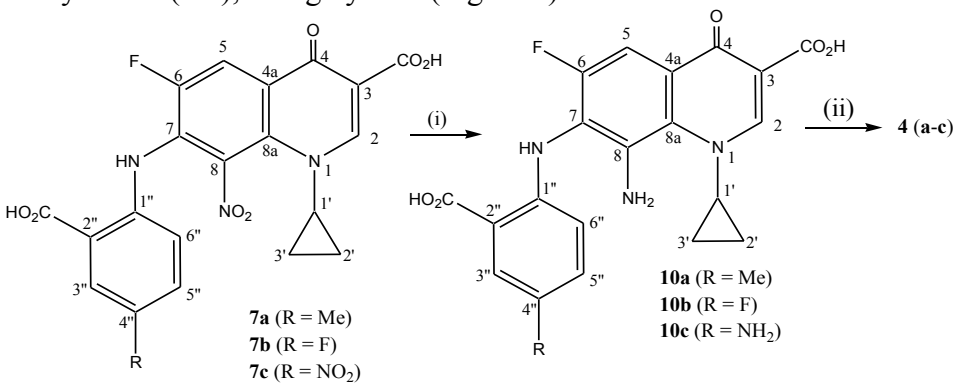


Reagents and conditions:
(i) Conc. H_2SO_4, MeOH, reflux
(ii) $NaHCO_3$, 50 % aq. EtOH / Δ
(iii) conc.H_2SO_4, abs. EtOH / Δ

Figure 4
Synthesis of **7a**

Reduction of the 8-nitro derivatives **7** (**a**-**c**) with sodium dithionite in aqueous potassium carbonate furnished the respective 8-amino intermediates **10** (**a**-**b**) and the diamine **10c.** Alternatively, this step was carried out using $SnCl_2$ in HCl and reflux overnight producing high amounts. The latter compounds **10** (**a**-**c**) underwent thermal cyclization

upon heating with polyphosphoric acid (PPA) for 2-4 h to afford the tetracyclic 4,12-dioxo-tetrahydroquino[7,8-*b*][1,4]benzodiazepine-3-carboxylic acid system **4** (**a-c**), in high yields (Figure 5).



Reagents and conditions:

(i) aq. K_2CO_3, $Na_2S_2O_4$ / 20 ^oC or $SnCl_2$ in HCl

(ii) PPA / 150-160 ^oC , 3 h

Figure 5

Synthesis of **4a**, **b** and **c**

Lactamization to prepare **4** (**a-c**) with PPA followed by NaOH workup, has led to the formation of compounds **11** (**a-c**) (Figure 6). Although **3a** was prepared for biological screening and comparison, it showed the same phenomenon giving rise to **3c**.

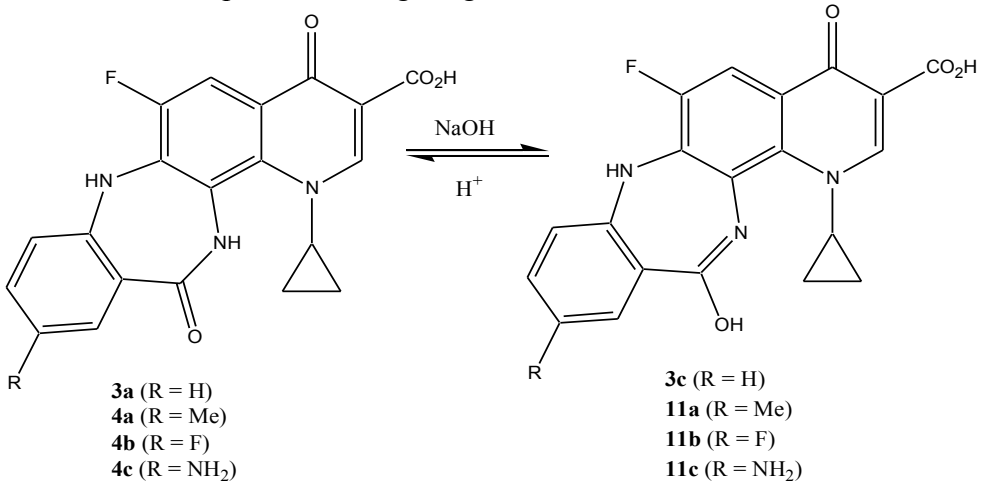


Figure 6

Enol formation of (**3c**, **11a-c**)

The identification of the prepared intermediates and target compounds was based on elemental analysis, IR, MS, ^{1}H- and ^{13}C-NMR spectral data, given in the Experimental part. These spectral data were all consistent with the proposed structures. Signal assignments to the various proton and carbons were mostly determined following DEPT and 2D (COSY, HMQC and HMBC) experiments. It was clearly apparent that H-5 in all intermediates and targets which resonate at around 8.0 ppm (d, $^{3}J_{H\text{-}F} \approx$ 11-13 Hz), showed consistent splitting patterns in all compounds and intermediates due to coupling with the vicinal fluorine atom. It was revealed from the new broad signal at around 9 to 10 ppm, assigned for the NH at C-7 in nitro intermediates **7** (**a**-**c**) that the aniline side chain was successfully introduced in **7** (**a**-**c**). This was confirmed by fluorescent yellow-orange color. Appearance of singlet broad peak (2H) for NH_2 down field shifted at around 6.0 ppm was indicative for the formation of the 8-amino derivative **10a-c**. In case of the target compounds **4a**, **b**, and **c**, a singlet peak for the amide –N(13)H was observed at around 10 ppm indicating that lactamization has taken place.

For compounds **4a-c**, long-range correlations are observed between H-2 and each of C-4, C-13b and CO_2H. Corresponding long-range correlations are also observed between H-5 and its neighbor carbons C-4, C-13b and C-6a. The skeletal carbons of the fused benzene ring (**B**) are recognizable by their signal splitting arising from coupling with fluorine atom (different value of *J* for each carbon) and from long-range coupling with neighboring protons. For compound **3c**, disappearance of amide –N(13)H which is usually observed at around 10 ppm and appearance of new peak for OH was indicative of formation. Furthermore, a distinctive proof was provided by disappearance of the C-12 carbonyl signal (167 ppm) and appearance of new peak for N=C_{12}-OH at 154 ppm.

7-[(2-Carboxy-4-methylphenyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **(7a)**

Yield 20%; mp 300-315°C (decomposition); R_f value = 0.64; IR (KBr)/cm: 3445, 3071, 2928, 2361, 1701, 1616, 1589, 1543, 1505, 1300, 1223, 1157, 1050, 890, 800, 760 cm^{-1}; ^{1}H- NMR (300 MHz, DMSO-d_6): δ 0.91, 1.03 (2m, 4H, H_2-2′/H_2-3′), 2.35(s, 3H, CH_3), 3.67 (m, 1H, H-1′), 6.78 (dd, *J* = 7.5, 7.3 Hz, 1H, H-6”), 7.40 (d, *J* = 7.5 Hz, 1H, H-5”), 7.90 (s, 1H, H-3”), 8.32 (d, $^{3}J_{H\text{-}F}$ = 10.5 Hz, 1H, H-5), 8.91 (s, 1H, H-2), 10.55 (br s, 1H, N*H*-Ar), 13-14.45 (2br/s, 2H, Ar-CO_2H & C(3)-CO_2H overlapping); ^{13}C-NMR: δ 10.2 (C-2′/C-3′), 21.2 (Ar-CH_3), 40.4 (C-1′), 109.1 (C-3), 116.2 (d, $^{2}J_{C\text{-}F}$ = 22 Hz, C-5), 116.5 (d, C-4a), 117.3 (d, *J* = 5.1 Hz, C-6”), 124.1 (d, $^{3}J_{C\text{-}F}$ = 7.0 Hz, C-8), 127.1 (C-4”), 131.0 (d, $^{2}J_{C\text{-}F}$ = 17.3 Hz, C-7), 131.4 (C-

5"), 133.1 (C-8a), 134.5 (C-3"), 138.4 (C-2"), 144.5 (d, *J* = 2.0 Hz, C-1"), 152.7 (C-2), 153.3 (d, $^{1}J_{C\text{-}F}$ = 249 Hz, C-6), 164.9 (C(3)-*C*O_2H), 170.4 (Ar-*C*O_2H), 176.1 (d, $^{4}J_{C\text{-}F}$ = 1.8 Hz, C-4); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{21}H_{16}FN_3O_7$ $[M+H]^+$: 442.10505, found: 442.10500; Anal. calcd. for $C_{21}H_{16}FN_3O_7$ (441.37): C, 57.15; H, 3.65; N, 9.52. Found: C, 56.98; H, 3.43; N, 9.50.

7-[(2-Carboxy-4-fluorophenyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(7b)**

Yield 11%; mp 298-300°C (decomposition); R_f value = 0.62; IR (KBr)/cm: 3502, 3450, 2995, 2890, 1675, 1430, 1436, 1309, 1050, 955, 709 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) 1.04, 1.12 (2m, 4H, H2' /H3'), 3.76 (m, 1H, H-1'), 6.95 (dd, *J* = 7.90, 6.60 Hz, 1H, H-6"), 7.48 (dd, *J* = 7.22, 11.85 Hz, 1H, H-5"), 7.65 (d, *J* = 11.2.0 Hz, 1H, H-3"), 8.33(d, $^{3}J_{H\text{-}F}$ = 11.50 Hz, 1H, H-5), 8.89 (s, 1H, H-2), 9.83 (br s, 1H, N*H*), 13.20 -15.7 (2 br s, 2H, 2COO*H*); ^{13}C-NMR (75 MHz, DMSO-d_6): 10.92 (C-2' / C-3'), 41.02 (C-1'), 109.75 (C-3), 115.86 (d, $^{2}J_{C\text{-}F}$ = 22.0 Hz, C-5), 116.56 (d, $^{3}J_{C\text{-}F}$ = 9.20 Hz, C-6"), 117.39 (C-4a), 123.24 (d, $^{3}J_{C\text{-}F}$ = 7.30 Hz, C-8), 131.45 (d, $^{2}J_{C\text{-}F}$ = 23.10 Hz, C-3"), 134.43 (d, $^{2}J_{C\text{-}F}$ = 23.0 Hz, C-5"), 137.33 (d, *J* = 2.60 Hz, C-8a), 138.50 (d, $^{2}J_{C\text{-}F}$ = 20.0 Hz, C-7), 143.55 (d,C-2"), 149.65 (C-1"), 153.22 (C-2), 153.66 (d, $^{1}J_{C\text{-}F}$ = 254.0 Hz, C-6), 162.88 (d, $^{1}J_{C\text{-}F}$ = 247.0 Hz, C-4"), 164.35 (C(3)*C*OOH), 171.78 (C(2")*C*OOH), 176.89 (C-4); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{20}H_{13}F_2N_3O_7$ $[M]^+$: 445.07216, found: 445.07325; Anal. calcd. for $C_{20}H_{13}F_2N_3O_7$ (445.33): C, 53.94; H, 2.94; N, 9.44. Found: C, 53.84; H, 3.13; N, 9.51.

7-[(2-Carboxy-4-nitrophenyl)amino]-1-cyclopropyl-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(7c)**

Yield 31%; mp > 305°C (decomposition); R_f value = 0.68; IR (KBr) cm^{-1}: *v* 3457, 3055, 2874, 2360, 2342, 1748, 1670, 1616, 1543, 1451, 1254, 1095, 806, 764; ^{1}H-NMR (300 MHz, DMSO-d_6) 9.87, 1.12 (2m, 4H, H2' /H3'), 3.78 (m, 1H, H-1'), 6.95 (dd, *J* = 7.80, 6.65 Hz, 1H, H-6"), 8.08 (d, *J* = 7.20 Hz, 1H, H-5"), 8.30 (d, $^{3}J_{H\text{-}F}$ = 11.00 Hz, 1H, H-5), 8.75 (br s, 1H, H-3"), 8.89 (s, 1H, H-2), 10.88 (br s, 1H, N*H*), 13.20 -15.8 (2 br s, 2H, 2COO*H*); ^{13}C-NMR (75 MHz, DMSO-d_6): 10.58 (C-2' / C-3'), 39.74 (C-1'), 108.90 (C-3), 115.85 (d, $^{2}J_{C\text{-}F}$ = 21.0 Hz, 1H, C-5), 119.32 (d, C-6"), 121.55 (d, $^{4}J_{C\text{-}F}$ = 2.10 Hz, C-8a), 124.65 (d, $^{2}J_{C\text{-}F}$ = 19.0 Hz, C-7), 125.54 (C-4a), 129.24 (C-8), 129.95 (C-5"), 132.61 (C-3"), 136.87 (C-2"), 145.12 (C-4"),147.72 (C-1"), 152.21 (d, $^{1}J_{C\text{-}F}$ = 245 Hz, C-6), 153.47 (C-2), 166.22 (C(3)*C*OOH), 173.15 (Ar*C*OOH), 176.01 (C-4); HRMS ((-ve)-ESI): *m/z* calcd. for $C_{20}H_{13}FN_4O_9$ $[M-H]^-$: 471.05883, found: 471.06355; Anal. calcd.

for $C_{20}H_{13}FN_4O_9$ (472.34): C, 50.86; H, 2.77; N, 11.86. Found: C, 50.34; H, 2.87; N, 11.58.

8-Amino-7-[(2-carboxy-4-methylphenyl)-amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid **(10a)**

Yield 76%; mp = 289-292°C, R_f value in system (**1**) = 0.52; IR (KBr) cm^{-1}: 3488, 3392, 2924, 2366, 1719, 1673, 1591, 1551, 1502, 1450, 1326, 1243, 1155, 1083, 1044; ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 1.19 (m, 4H, H_2-2′/H_2-3′), 2.34(s, 3H, CH_3), 4.45 (m, 1H, H.1′), 5.98 (br s, 2H, NH_2), 6.54 (d, J = 9.0 Hz, 1H, H-6″), 7.29 (d, J = 8.5 Hz, 1H, H-5″), 7.83 (s, 1H, H-3″), 7.38 (d, $^3J_{H\text{-}F}$ = 10.0 Hz, 1H, H-5), 8.68 (s, 1H, H-2), 8.95 (br s, 1H, N*H*-Ar, exch.), 14.32 (br s, 1H, C(3)-CO_2H), 15.05 (br s, 1H, C(2)-CO_2H, overlapping); ^{13}C-NMR: δ 10.33 (C-2′/C-3′),20.9 (Ar-CH_3), 39.4 (C-1′), 100.1 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, C-5), 108.7 (C-3), 114.2 (C-6"), 118.8 (d, $^2J_{C\text{-}F}$ = 16.5 Hz, C-7), 123.1 (C-8), 123.2 (C-4"), 124.0 (d, $^3J_{C\text{-}F}$ = 6.8 Hz, C-4a), 127.7 (C-8a), 131.4 (C-5"), 133.9 (C-3"), 138.4 (d, J = 3.2 Hz, C-2"), 146.6 (C-1"), 151.6 (C-2), 157.2 (d, $^1J_{C\text{-}F}$ = 244 Hz, C-6), 166.1 (C(3)-CO_2H), 170.8 (C-(2")-CO_2H), 177.4 (d, $^4J_{C\text{-}F}$ = 3.1 Hz, C-4); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{21}H_{19}FN_3O_5$ $[M+H]^+$: 412.13088, found: 412.12124; Anal. calcd. for $C_{21}H_{18}FN_3O_5$ (397.36): C, 61.31; H, 4.41; N, 10.21. Found: C, 61.53; H, 4.74; N, 10.35.

8-Amino-7-[(2-carboxy-4-fluorophenyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(10b)**

Yield 81%; mp 293–295°C; R_f value = 0.50; IR (KBr)/cm: 3478, 3390, 2915, 2354, 1712, 1671, 1587, 1551, 1495, 1455, 1312, 1026, 957, 702, 671 cm^{-1}; ^{1}H- NMR (300 MHz, DMSO-d_6): 0.98, 1.01 (2m, 4H, H2' /H3'), 4.56 (m, 1H, H-1'), 5.24 (br s, 2H, NH_2), 6.20 (m, 1H, H-6"), 6.89 (dd, J = 8.10, 6.78 Hz, 1H, H-5"), 7.45 (d, J = 8.0 Hz, 1H, H-3"), 7.68 (d, $^3J_{H\text{-}F}$ = 10.40 Hz, 1H, H-5), 8.49 (br s, 1H, N*H*), 8.65 (s, 1H, H-2), 11.52 - 13.45 (2 br s, 2H, 2COO*H,* exch.); ^{13}C-NMR (75 MHz, DMSO- d_6): 10.90 (C-2' / C-3'), 41.01 (C-1'), 109.55 (C-3), 115.86 (d, $^2J_{C\text{-}F}$ = 22.0 Hz, C-5), 116.56 (d, $^3J_{C\text{-}F}$ = 8.8 Hz, C-6"), 121.44 (d, $^2J_{C\text{-}F}$ = 17.5 Hz, C-7), 122.21 (d, $^3J_{C\text{-}F}$ = 7.6 Hz, C-8), 127.52 (C-4a), 128.43 (d, $^2J_{C\text{-}F}$ = 22.0 Hz, C-5"), 131.62 (d, $^2J_{C\text{-}F}$ = 23.10 Hz, C-3"), 134.25 (d, $^4J_{C\text{-}F}$ = 2.40 Hz, C-8a), 142.55 (d,C-2"), 149.64 (C-1"), 153.33 (C-2), 153.66 (d, $^1J_{C\text{-}F}$ = 255.0 Hz, C-6), 157.85 (d, $^1J_{C\text{-}F}$ = 243.0 Hz, C-4"), 165.35 (C(3)*C*OOH), 170.78 (C(2")*C*OOH), 174.57 (C-4); HRMS ((-ve)-ESI): *m/z* calcd. for $C_{20}H_{14}F_2N_3O_5$ $[M-H]^-$: 414.09015, found: 414. 09021; Anal. calcd. for $C_{20}H_{15}F_2N_3O_5$ (415.35): C, 57.83; H, 3.64; N, 10.12. Found: C, 57.43; H, 3.41; N, 10.31.

8-Amino-7-[(4-amino-2-carboxyphenyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(10c)**

Yield 93%; mp > 305°C (decomposition); R_f value = 0.42; IR (KBr) cm^{1}: 3422, 3337, 3055, 1717, 1609, 1543, 1501, 1454, 1389, 1339,, 1250, 1161,1084, 806, 752; ^{1}H-NMR (300 MHz, DMSO-d_6): δ 1.22 (m, 4H, H_2-2′/H_2-3′), 4.56 (m, 1H, H-1′), 5.91-5.98 (2br s, 4H, $2NH_2$ overlapping), 6.42 (d, J = 8.3 Hz, 1H, H-6″), 6.80 (d, J = 8.2 Hz, 1H, H-5″), 7.38 (d, $^{3}J_{H\text{-}F}$ = 11.2 Hz, 1H, H-5), 7.95 (s, 1H, H-3″), 8.78 (s, 1H, H-2), 9.77 (br s, 1H, N*H*-Ar, exch.), 14.0-15.5 (2br s, 1H, C(3)-CO_2H and 1H, C(2)-CO_2H, overlapping); ^{13}C-NMR: δ 10.63 (C-2′/C-3′), 39.94 (C-1′), 98.07 (d, $^{2}J_{C\text{-}F}$ = 22.0 Hz, C-5), 106.77(C-3), 113.64 (C-6"), 117.93 (C-5"), 118.96 (d, $^{2}J_{C\text{-}F}$ = 17.5 Hz, C-7), 125.21 (C-8), 126.21 (d, $^{3}J_{C\text{-}F}$ = 4.7 Hz, C-4a), 127.67 (C-8a), 131.94 (C-3"), 140.42 (d, J = 3.4 Hz, C-2"), 147.67 (C-1"), 151.23 (C-2), 150.24 (C-4"), 153.12 (d, $^{1}J_{C\text{-}F}$ = 252 Hz, C-6), 166.22 (C(3)-CO_2H), 170.66 (C-(2")-CO_2H), 177.28 (d, $^{4}J_{C\text{-}F}$ = 1.1 Hz, C-4); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{20}H_{18}FN_4O_5$ $[M+H]^+$: 413.12612, found: 413.12514; Anal. calcd. for $C_{20}H_{17}FN_4O_5$ (397.36): C, 58.25; H, 4.16; N, 13.59. Found: C, 58.53; H, 4.24; N, 13.45.

1-Cyclopropyl-6-fluoro-4,12-dioxo-10-methyl-4,7,12,13-tetrahydro-1H-quino[7,8-b][1,4]benzodiazepine-3-carboxylic acid **(4a)**

Yield 93%; mp 326–328°C (decomp); R_f value = 0.59; IR (KBr) cm^{-1}: 3435, 2996, 2910, 2580, 2315, 2222, 2098, 1998, 1658br, 1445, 1412, 1322, 1027, 950, 902, 702, 671; ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 0.87, 1.07 (2m, 4H, H_2-2′/ H_2-3′), 2.42(s, 3H, CH_3), 4.31 (m, 1H, H-1′), 7.23 (d, J = 7.7 Hz, 1H, H-8), 7.44 (d, J = 7.2, Hz, 1H, H-9), 7.68 (d, J = 0.8 Hz, 1H, H-11), 7.83 (d, $^{3}J_{H\text{-}F}$ = 11.1 Hz, 1H, H-5), 8.71 (d, J = 2.5 Hz, 1H, N(7)-*H*) and 8.73 (s, 1H, H-2), 10.10 (br s, 1H, N(13)-*H*)), 15.10 (br s, 1H, CO_2H); ^{13}C-NMR: δ 9.9 (C-2′/C-3′), 20.5 (CH_3), 39.2 (C-1′), 108.0 (d, $^{2}J_{C\text{-}F}$ = 22.0 Hz, C-5), 108.8 (C-3), 120.7 (d, $^{3}J_{C\text{-}F}$ = 4.1 Hz, C-13a), 122.2 (d, $^{3}J_{C\text{-}F}$ = 7.3 Hz,C-4a), 122.8 (C-13b), 124.2 (C-8), 131.3 (C-9), 132.22(C-10), 133.9 (C-11), 134.5 (C-11a), 141.3 (d, $^{2}J_{C\text{-}F}$ = 14.9 Hz, C-6a), 149.5 (C-7a), 151.1 (d, $^{1}J_{C\text{-}F}$ = 254 Hz, C-6), 152.4 (C-2), 166.2 (C(3)-CO_2H), 168.8 (C-12), 177.6 (d, $^{4}J_{C\text{-}F}$ = 1.7 Hz, C-4); HRMS ((-ve)-ESI): *m/z* calcd. for $C_{21}H_{15}FN_3O_4$ $[M-H]^-$: 392.10466, found: 392.10442; Anal. calcd. for $C_{21}H_{16}FN_3O_4$ (393.36): C, 64.12; H, 4.10; N, 10.68. Found: C, 63.97; H, 4.05; N, 10.96.

1-Cyclopropyl-6,10-difluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino [7,8-b][1,4]benzodiazepine-3-carboxylic acid **(4b)**

Yield 95%; mp 318–320°C (decomp); R_f value = 0.62; IR (KBr) /cm: 3433, 2994, 2909, 1651, 1435, 1408, 1312, 1057, 1026, 957, 903 cm^{-1}; ^{1}H-NMR: (300 MHz, DMSO-d_6): δ 0.87, 1.09 (2m, 4H, H_2-2′/ H_2-3′), 4.46

(m, 1H, H.1′), 7.20 (dd, *J* = 7.5 Hz, 1H, H-8), 7.54 (dd, *J* = 7.9, 9.8, Hz, 1H, H-9), 7.81-7.82 (m, 2H, H-11 and H-5), 8.65 (brs, 1H, N(7)-*H*), 8.81 (s, 1H, H-2), 9.98 (br s, 1H, N(13)-*H*)), 14.10-15.2 (br s, 1H, CO_2H); ^{13}C-NMR: δ 10.1 (C-2′/C-3′), 40.4 (C-1′), 107.6 (d, $^2J_{C\text{-}F}$ = 21.5 Hz, C-5), 108.3 (C-3), 120.8 (d, $^3J_{C\text{-}F}$ = 3.4 Hz, C-13a), 121.2 (d, $^3J_{C\text{-}F}$ = 6.6 Hz,C-8), 122.3 (d, $^3J_{C\text{-}F}$ = 6.5 Hz,C-4a), 125.7 (C-13b), 131.3 (d, $^2J_{C\text{-}F}$ = 18.4 Hz, C-9), 133.9 (d, $^2J_{C\text{-}F}$ = 19.8 Hz, C-11), 135.1 (d, $^3J_{C\text{-}F}$ = 9.4 Hz, C-11a), 141.3 (d, $^2J_{C\text{-}F}$ = 16.5 Hz, C-6a), 145.5 (C-7a), 148.9 (d, $^1J_{C\text{-}F}$ = 247 Hz, C-10), 151.8 (d, $^1J_{C\text{-}F}$ = 255 Hz, C-6), 152.4 (C-2), 166.0 (C(3)-*C*O_2H), 167.7 (C-12), 177.1 (d, $^4J_{C\text{-}F}$ = 2.4 Hz, C-4); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{20}H_{13}F_2N_3NaO_4$ $[M\text{-}Na]^+$: 420.07718, found: 420.07721; Anal. calcd. for $C_{20}H_{13}F_2N_3O_4$ (397.33): C, 60.46; H, 3.30; N, 10.58. Found: C, 60.02; H, 3.15; N, 10.66.

10-Amino-1-cyclopropyl-6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino [7,8b][1,4]-benzodiazepine-3-carboxylic acid **(4c)**

Yield 60%; mp 326–328°C (decomp); R_f value = 0.61; IR (KBr) cm^1: 3422, 3337, 3055, 2993, 1717, 1657, 1609, 1543, 1501, 1454, 1389, 1339,, 1250, 1161,1084, 806, 752; 300 MHz, DMSO-d_6):δ 0.85, 1.10 (2m, 4H, H_2-2′/ H_2-3′), 4.35 (m, 1H, H.1′), 6.01 (br/s, 2H, NH_2), 7.25 (d, *J* = 8.1 Hz, 1H, H-8), 7.66 (d, *J* = 6.6 Hz, 1H, H-9), 7.93 (d, $^3J_{H\text{-}F}$ = 10.1 Hz, 1H, H-5), 7.94 (d, *J* = 2.2 Hz, 1H, H-11), 8.78 (br s, 1H, N(7)-*H*), 8.92 (s, 1H, H-2), 10.12 (s, 1H, N(13)-*H*)), 14.4-15.50 (br s, 1H, CO_2H); ^{13}C-NMR: δ 10.2 (C-2′/C-3′), 38.7 (C-1′), 107.62 (C-3), 108.12 (d, $^2J_{C\text{-}F}$ = 22.0 Hz, C-5), 120.20 (d, $^3J_{C\text{-}F}$ = 3.5 Hz, C-13a), 121.32 (C-8), 122.62 (d, $^3J_{C\text{-}F}$ = 8.2 Hz,C-4a), 123.37 (C-10), 131.32 (C-9), 131.2 (C-11), 131.8 (C-13b), 140.4 (d, $^2J_{C\text{-}F}$ = 16.2 Hz, C-6a), 143.5(C-11a), 149.3 (C-7a), 151.3 (d, $^1J_{C\text{-}F}$ = 246 Hz, C-6), 152.23 (C-2), 166.2 (C(3)-*C*O_2H), 167.8 (C-12), 176.6 (d, $^4J_{C\text{-}F}$ = 3.0 Hz, C-4); HRMS ((-ve)-ESI): *m/z* calcd. for $C_{20}H_{15}FN_4NaO_4$ $[M+Na]^+$: 417.09750, found: 417.09451.

1-Cyclopropyl-6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino[7,8-b] [1,4] benzodiazepine -3-carboxylic acid **(3a) [**3]

Yield ≈ 0.19 g (95%); mp = 325–326°C (decomposition); R_f value in system (**1**) = 0.63.

1-Cyclopropyl-6-fluoro-4-oxo-12-hydroxy-4, 7-dihydro-1H-quino[7, 8b][1,4] benzodiazepine-3-carboxylic acid **(3c)**

Yield 90%; mp = 313–318°C (decomposition); R_f value in system (**1**) = 0.68; ^{1}H-NMR (300 MHz, DMSO-d_6): 0.99, 1.16 (2m, 4H, H-2′/ H-3′), 4.79 (m, 1H, H.1′), 4.20 – 4.80 (br s, 1H, O*H*), 7.28 -7.42 (2m, 6H, 4ArH + N(7)*H* +H-5), 8.51 (s, 1H, H-2), 14.0 -15.0 (br s, 1H, CO_2H exchangeable); ^{13}C-NMR (75 MHz, DMSO-d_6 (Depth)): 9.53 (C-2′/C-3′), 40.18 (C-1′), 115.27 (d, $^2J_{C\text{-}F}$ = 22.0 Hz, C-5), 126.38 (CH-Ar), 127.01 (CH-

Ar), 127.30 (CH-Ar), 128.48 (CH-Ar), 145.76 (C-2), 154.70 (N=*C*-OH), 165.52 (C(3)-*C*O_2H), 175.62 (C-4); HRMS ((+ve)-ESI): *m/z* calculated for $C_{20}H_{14}FN_3O_4$[M-H]$^+$: 402.08660, found: 402.08608; *EA* calculated for $C_{20}H_{14}FN_3O_4$ (379.34): C, 63.32; H, 3.72; N, 11.08. Found: C, 63.45; H, 3.65; N, 10.96.

1-Cyclopropyl-6-fluoro-4-oxo-12-hydroxy-10-methyl-4, 7-dihydro-1H-quino [7, 8b][1,4] benzodiazepine-3-carboxylic acid **(11a)**

Yield 85%; mp 314–318°C (decomposition); R_f value in system (1) = 0.69; ^{1}H-NMR (300 MHz, DMSO-d_6): 0.98, 1.14 (2m, 4H, H-2′/ H-3′), 2.34(s, 3H, CH_3), 4.72 (m, 1H, H.1′), 4.70 (br/s, 1H, O*H*), 7.20 -7.51 (2 br/m, 5H, 3ArH, N(7)*H,* H-5), 8.62 (s, 1H, H-2), 14.5 -15.3 (br s, 1H, CO_2H); ^{13}C-NMR (75 MHz, DMSO-d_6 (Depth)): 9.81 (C-2′/C-3′), 21.2 (CH_3), 40.12 (C-1′), 112.33 (d, $^2J_{C\text{-}F}$ = 22.0 Hz, C-5), 124.5 (C-8), 128.11 (C-9), 129.5 (C-11), 149.56 (C-2), 154.70 (N=C_{12}-OH), 166.22 (C(3)-*C*O_2H), 176.10 (C-4); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{21}H_{15}FN_3O_4$ [M+Na]$^+$: 416.10225, found: 416.10412; Anal. calcd. for $C_{21}H_{16}FN_3O_4$ (393.36): C, 64.12; H, 4.10; N, 10.68. Found: C, 64.01; H, 4.21; N, 10.32.

1-Cyclopropyl-6,10-difluoro-4-oxo-12-hydroxy-4, 7-dihydro-1H-quino[7, 8b] [1,4] benzodiazepine-3-carboxylic acid (**11b**)

Yield 85%; mp 310–315°C (decomposition); R_f value in system (1) = 0.65; ^{1}H-NMR (300 MHz, DMSO-d_6): 0.98, 1.14 (2m, 4H, H-2′/ H-3′), 4.70 (m, 1H, H.1′), 4.74 (br/s, 1H, O*H*), 6.58 -7.10 (2 br/m, 5H, 3ArH, N(7)*H,* H-5), 8.20 (s, 1H, H-2), 14.5 -15.3 (br s, 1H, CO_2H); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{20}H_{13}F_2N_3O_4$ [M+Na]$^+$: 420.32020, found: 420.30412; Anal. calcd. for $C_{20}H_{13}F_2N_3O_4$ (397.09): C, 60.46; H, 3.30; N, 10.58. Found: C, 60.41; H, 3.31; N, 10.59.

10-Amino-1-cyclopropyl-6-fluoro-4-oxo-12-hydroxy-4, 7-dihydro-1H-quino [7, 8b][1,4] benzodiazepine-3-carboxylic acid (**11c)**

Yield 85%; mp 310–315°C (decomposition); R_f value in system (1) = 0.68; ^{1}H-NMR (300 MHz, DMSO-d_6): 0.95, 1.12 (2m, 4H, H-2′/ H-3′), 4.70-4.74 (m/br, 3H, H.1′ and NH_2), 4.78 (br/s, 1H, O*H*), 6.85 -7.21 (br/m, 5H, 3ArH, N(7)*H,* H-5), 8.25 (s, 1H, H-2), 14.0 -15.1 (br s, 1H, CO_2H); HRMS ((+ve)-ESI): *m/z* calcd. for $C_{20}H_{15}FN_4O_4$ [M+Na]$^+$: 417.09695, found: 417.08101; Anal. calcd. for $C_{20}H_{15}FN_4O_4$ (394.11): C, 60.91; H, 3.83; N, 14.21. Found: C, 60.89; H, 3.82; N, 14.20.

Antibacterial activity

The *in vitro* antibacterial activity of all intermediates and targeted products was evaluated against a variety of standard and resistant Gram positive and Gram negative bacterial strains using the minimum inhibitory concentration (MIC) approach (Table I). The prepared targets **4** (**a-c**) and

the intermediates **7**(**a-c**)/ **10**(**a-c**) have shown strong antibacterial activity mainly against Gram positive standard strains (Table I), with minimal or no activity against standard Gram negative bacteria. Almost none of the tested compounds have shown any activity against gram negative resistant strains. The reduced derivatives **10** (**a-c**) were the most active against standard gram positive *S. aureus* with activity ranging from 0.05 to 0.19 µg/mL and they also exhibited good activity against resistant gram positive *S. aureus*. The targets **4** (**a-c**) have shown lesser activity against gram positive standard strains compared to nitro and reduced intermediates 10 (a-c) but still comparable to the reference ciprofloxacin.

Table I

MICs (μg/mL) for compounds **7 (a-c), 10 (a-c), 3a, 4 (a-c)** against Gram positive and Gram negative bacterial strains

Compound No.	*S. aureus* ATCC 6538P	*S. aureus* ATCC 43300 (MRSA)	*E.coli* ATCC 8739	*E.coli* ATCC 1058 (resistant)
7a	1.56	ND	>50	ND
7b	6.25	ND	ND	ND
7c	12.5	ND	ND	ND
10a	0.19	3.13	25	ND
10b	0.10	12.5	12.5	>50
10c	0.05	6.25	>12.5	>25
3a	6.25	ND	ND	ND
4a	3.13	ND	>50	ND
4b	1.56	ND	25	ND
4c	1.56	25	25	ND
Ciprofloxacin	1.56	ND	0.39	ND

* ND: Not detected (> 100 µg/mL)

It is generally assumed that the more lipophilic quinolones can penetrate better the lipophilic cell membrane of Gram positive bacteria, while less lipophilic compounds are more liable to penetrate the cell wall of Gram negative bacteria. These compounds exhibited similar patterns to previously reported derivative **3a** [3] and their activities are in correlation with this theory since they are lipophilic.

Anticonvulsant activity (Pentylenetetrazole (PTZ) seizure threshold test)

Anticonvulsant activity of two targets; **3a** (1-Cyclopropyl-6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino [7,8-b][1,4]benzodiazepine-3-carboxylic acid**)**, and **4a (**1-Cyclopropyl-6-fluoro-4,12-dioxo-10-methyl-4,7,12,13-tetra-hydro-1H-quino[7,8-b][1,4]-benzodiazepine-3-carboxylic acid) was carried out using pentylenetetrazole (PTZ) seizure threshold test. This test is based on producing convulsions or seizures in animals by a chemical agent and assesses the protection effect to any potential compounds within one hour compared to a phenytoin treated group. None of the tested compounds **3a**,

4a produced anticonvulsant activity. Both compounds did not provide protection against convulsions and episodes of clonic spasm were observed constantly. The animals of the standard groups were protected with phenytoin.

Conclusions

In conclusion, based on the structures **4** and **10,** we synthesized 1-cyclopropyl-6-fluoro-4,12-dioxo-10-substituted-4,7,12,13-tetrahydro-1H-quino [7,8-b][1,4]benzodiazepine-3-carboxylic acids and 8-Amino-7-[(2-carboxy-4-substituted-phenyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acids and tried to find more potent compounds as antibacterial agents. As a result, we obtained several compounds with enhanced biological profiles, of which compounds **10a-c** and **4b, c** exhibited the most promising activities. These compounds are now undergoing further biological tests including *in vivo* evaluation to be selected as candidates for further clinical trials. None of the compounds produces any effect on the central nervous system.

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References

1. Albright J.D., Sum F.W., Tricyclic benzazepine oxytocin and vasopressin antagonists. U.S. Pat. 1999; 5869483 A.
2. Al-Hiari Y.M., Al-Mazari I.S., Shakya A.K., Darwish R.M., Abu-Dahab R.M., Synthesis and antibacterial properties of new 8-nitro fluoroquinolone derivatives. *Molecules*. 2007; 12: 1240-1258.
3. Al-Hiari Y.M., Abu-Dahab R., El-Abadelah M.M., Heterocycles [h]-fused onto 4-oxoquinolone-3-carboxylic acid, part VIII. Convenient synthesis and antimicrobial properties of substituted hexahydro[1,4]diazepino[2,3-h]quinoline-9-carboxylic acid and its tetrahydroquino[7,8-b]benzodiazepine analogue. *Molecules*. 2008; 13: 2880-2893.
4. Al-Hiari Y.M., Shakya A.K., Alzweiri M.H., Al-Qirim T.M., Shattat G.F., El-Abadleh M.M., Synthesis and antibacterial properties of new N4- acetylated hexahydro-2,7-dioxopyrido[2,3-f]quinoxaline-8-carboxylic acids. *J Enz Inhib Med Chem.* 2011; 26: 649-665.
5. Bachmann B.O., Mcalpine J.B., Zazopoulos E., Farnet C.M., Piraee M., Methods for de novo biosynthesis of farnesyl dibenzodiazepinone, ECO-04601, in Micromonospora and its use as antitumor, antibacterial, and anti-inflammatory agent. S. African Pat. ZA 2006; 004214 A.
6. Beccalli E.M., Broggini G., Paladino G., Zoni C., Palladium-mediated approach to dibenzo[b,e][1,4]diazepines and benzopyrido-analogues, An efficient synthesis of tarpane. *Tetrahedron*. 2005; 61: 61-68.

7. Charan R.D., Schlingmann G., Janso J., Bernan V., Feng X., Carter G.T., Diazepinomicin, a new antimicrobial alkaloid from a marine Micromonospora sp. *J Nat Prod*. 2004; 67: 1431-1433.
8. Da Silva A.D., DeAlmeida M.V., DeSouza M.V.N., Couri M.R.C., Biological activity and synthetic methodologies for the preparation of fluoroquinolones, a class of potent antibacterial agents. *Curr Med Chem*. 2003; 10: 21-39.
9. Ek F., Olsson R., Ohlsson J., Amino-substituted diaryl[a,d]cycloheptene analogs as muscarinic agonists, their preparation and use in the treatment of neuropsychiatric disorders. PCT Int. Appl. 2005; WO 063254 A2.
10. Farnet C.M., Dimitriadou V., Bachmann B.O., Preparation of farnesyl dibenzodiazepinones, their production with microorganisms, and their use as antitumor, antibacterial, and antiinflammatory agents, U.S. Pat. Appl. 2005; US 107363 A1.
11. Ramesh S., Lokesh B., Jyoti W., Pandurang G., Substituent selection for design and synthesis of antimicrobial 1,3 oxazines: a topliss modified approach. *Farmacia*. 2012; 60(1): 32-39.
12. Gourdeau H., Ranger M., Berger F., Simard B., I.V. administration of farnesyl dibenzodiazepinone for treatment of cancer. U.S. Pat. Appl. 2006; 270662 A1.
13. Igarashi Y., Miyanaga S., Onaka H., Takeshita M., Furumai T., Revision of the structure assigned to the antibiotic BU-4664L from Micromonopora. *J Antibiotics*. 2005; 58: 350-352.
14. Li Q., Mitscher L.A., Shen L.L., The 2-pyridone antibacterial agents: Bacterial topoisomerase inhibitors. *Med Res Rev*. 1993; 20: 231-293.
15. McAlpine J.B., Banskota A.H., Aouidate M., Preparation of dibenzodiazepinone analogs in anticancer pharmaceutical compositions. U.S. Pat. Appl. 2008; 161291 A1.
16. Okada T., Ezumi K., Yamakawa M., Sato H., Tsuji T., Tsushima T., Motokawa K., Komatsu Y., Quantitative structure-activity relationships of antibacterial agents, 7-heterocyclic amine substituted 1-cyclopropyl-6,8-difluoro-4-oxoquinoline-3-carboxylic acids. *Chem Pharm Bull*. (Japan). 1993; 41: 126-131.
17. Olsen U.B., Piperidinecarboxylic acid derivatives for reducing blood glucose levels. PCT Int. Appl. 1997; WO 9722338 A1.
18. Petersen U., Bartel S., Bremm K.D., Himmler T., Krebs A., Schenke T., The synthesis and biological properties of 6-fluoroquinolone carboxylic acids. *Bull Soc Chim Belg*. 1996; 105: 683-699.
19. Poppe H., Kaverina N.V., Lyskovzev V.V., Egerland U., Sauer W., Lichoscherstow A., Ruger Carla C., Skoldinow A., New 5-aminoacyl-5,10-dihydro-11H-dibenzo [b,e][1,4] diazepine-11-ones with antiarrhythmic activity. *Pharmazie*. 1997; 52: 821-830.
20. Lupaşcu D., Tuchiluş C., Lupuşoru C.E., Ghiciuc C., Şutu M., Neagu A., Profire L., Synthesis and biological evaluation of some new rutin semisynthetic derivatives as antibacterial agents. *Farmacia*. 2012; 60(4): 556-564.
21. Oniga O., Ndongo J.T., Moldovan C., Tiperciuc B., Oniga S., Pîrnău A., Vlase L., Verité P., Synthesis and antimicrobial activity of some new 2-hydrazone-thiazoline-4-ones. *Farmacia*. 2012; 60(6): 785-797.

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