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# ANTIMICROBIAL SCREENING OF NOVEL N-4-FLUOROPHENYLQUINO-[7,8-b][1,4]-BENZODIAZEPIN-3-CARBOXYLIC ACID DERIVATIVES

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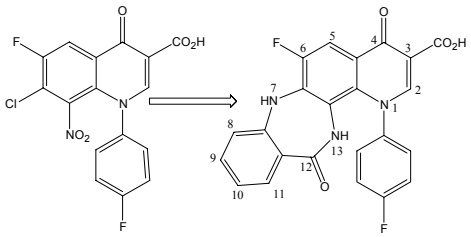
New 6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1*H*-quino-[7,8-b][1,4]-benzodiazepine-3-carboxylic acid derivatives with N-4-fluorophenyl substitution were prepared and characterized for the first time. *In-vitro* antimicrobial screening of targets and related intermediates revealed that the quino[7,8-b]benzodiazepines targets **11a-c** have shown good antibacterial activity against gram positive strains whereas other intermediates (**9** and **10**) are having stronger and broader spectrum of activity. Compounds **10 a, b** and **c** were most active against standard and resistant gram positive strain and standard gram negative strain. In particular compound **10a** was 4 fold stronger than reference drug (ciprofloxacin) against standard *S. aureus* and exhibited comparable activity to reference against resistant gram positive strain with MIC values of 0.37 and 23.4 μg/ml, respectively. It was also as active as reference against both gram negative *E. coli* and *P. aeruginosa* strains with MIC values of 0.18 and 23.4 μg/mL, respectively. Compounds **9a-c** showed the best antifungal activity against *C. albicans* and *C. glabrata*.



## INTRODUCTION

Quinolones represent a successful class of broad-spectrum anti-microbial agents used in the prevention and treatment of a variety of infections.[1-6] Nowadays, fluoroquinolones (*e.g.* Ciprofloxacin **1**, Fig. 1) became the most frequently used anti-microbial agents worldwide.[7] The dibenzo-[b,e][1,4]diazepine (Fig. 1) and related derivatives, 5, 10-dihydro-11H-dibenzo [b,e][1,4]diazepine-11-ones (**2a**, Fig. 1), were prepared[8,9] and reported to display different biological activities.[10,11] Other derivatives such as clobenzepam (**2b**, Fig. 1), and related drugs (*e.g.* dibenzepine, propizepine, pirenzepine) are successful antidepressant agents.[12,13] Some of these derivatives were reported to exhibit muscarine receptor antagonist activity,[8] antimicrobial activity,[14,15] oxytocin and vasopressin antagonist activity,[10,16] anti-arrhythmic activity,[17,18] hypoglycemic activity,[19] analgesic and anti-inflammatory activity[20] and antitumor activity.[21] Owing to the potential biological interest in these heterocyclic compounds, our research group[22] has previously prepared a new heterocyclic system incorporating 4-oxopyridine nucleus condensed to the dibenzo-[b,e][1,4]-diazepinone to form a tetra-heterocyclic derivative (**3**, Fig. 1). This new hybrid system has shown interesting antibacterial activity that guided this research. As a continuation, this research addresses the preparation of new heterocyclic systems of the same nucleus 3 with new substitutions on $N_1$ such as *p*-fluorophenyl. $N_1$-cyclopropyl and $N_1$-ethyl are also prepared for biological screening.[22,23] The new $N^1$ substitutions are justified since they are reported in clinical fluoroquinolone drugs (N-cyclopropyl *e.g.* Ciprofloxacin, N-ethyl e.g. Norfloxacin and N-*p*-fluorophenyl *e.g.* Difloxacin) and consequently might modify the activity of our targets.

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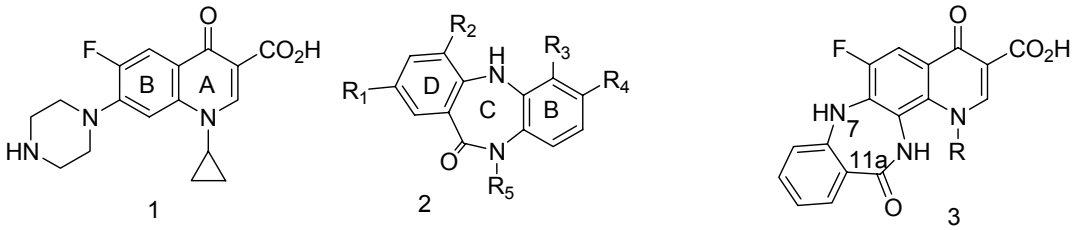



2a (R1 = R2 = R3 =R4 = R5 = H)
2b (R1 = R2 = R3 = H; R4 = Cl; R5 = $CH_2CH_2NMe_2$)

Fig. 1 – Structure of Ciprofloxacin (**1**), Dibenzodiazepine (**2a**), clonazepam (**2b**) and 1-substituted-6-fluoro-4,12-dioxo-4,7,12,13-tetrahydro-1H-quino[7,8-b][1,4]benzodiazepine-3-carboxylic acid derivatives (**3**).

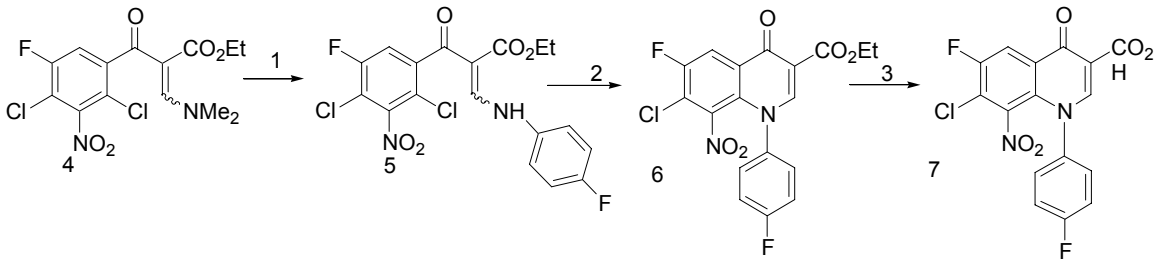
## RESULTS AND DISCUSSION

The reaction of 2-aminobenzoic acid **8** with each of **7a**; which was prepared according to reported method, (Scheme 1);[22,23] provided the nitro derivative **9a**, Scheme 2.

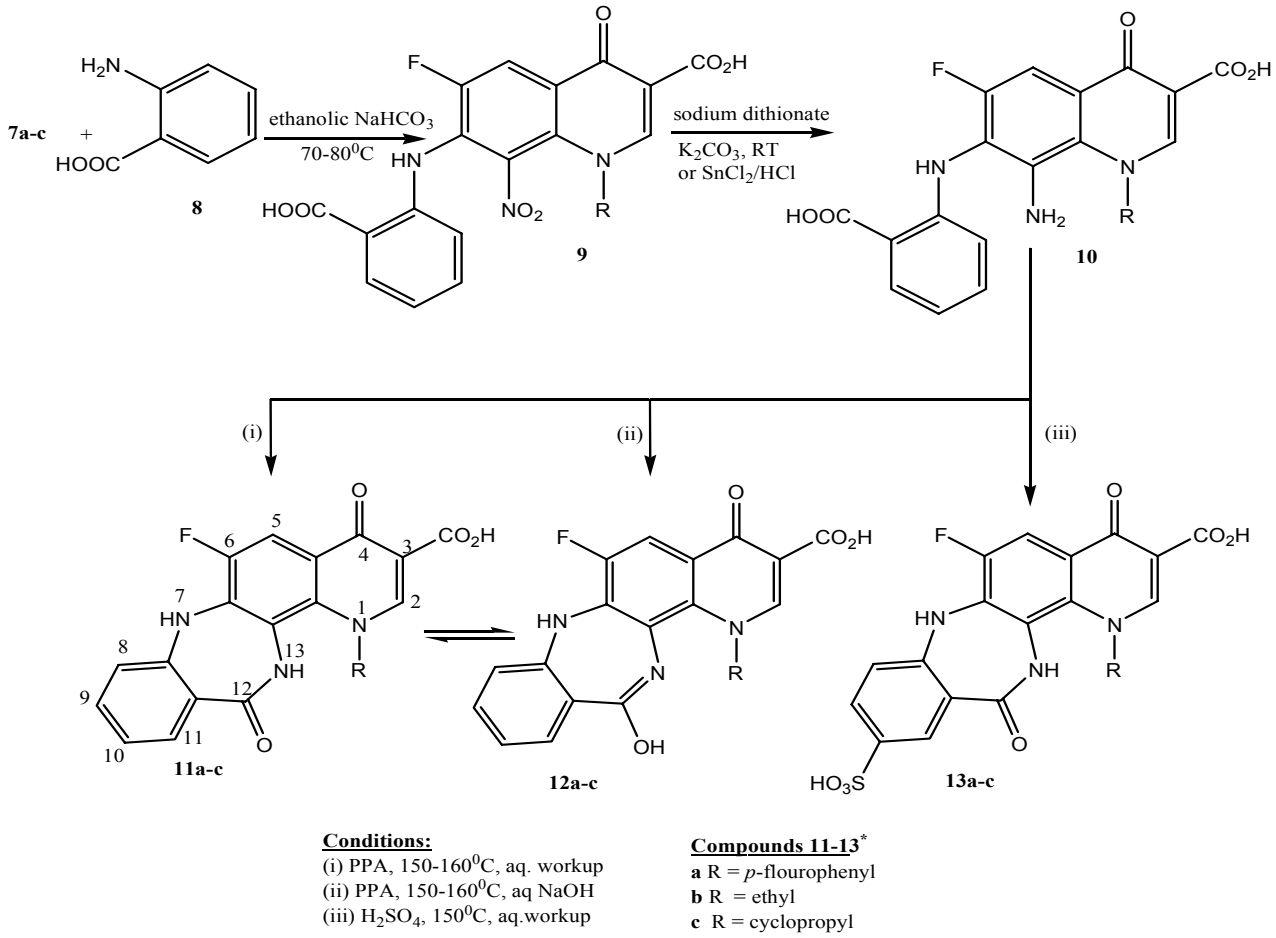
Next, **9a** was reduced successfully with aqueous basic sodium dithionite to 8-amino intermediates **10a** with high yield (Scheme 2). Subsequently **10a** was cyclized to quino-benzodiazepine target (**11a)** with polyphosphoric acid (PPA) in sand bath for 2-4 hrs. Workup with aqueous NaOH gave the tautameric derivatives **12a**. The cyclization step was also carried out with concentrated $H_2SO_4$ at 150°C for 2-4 hours, giving rise to the dibenzodiazepine-10-sulphonic acid **13a**. All intermediates **5-10a** and final targets **11-13a** were identified and fully characterized by spectroscopic techniques; following DEPT and 2D (COSY, HMQC and HMBC) experiments.

*In vitro* anti-microbial screening was performed for pure intermediates and final targets **9-11a-c** against standard *E. coli* (ATCC8739), and against standard *S. aureus* (ATCC6538) using NCCLS broth microdilution reference method to determine minimum inhibitory concentration MIC (μg/ml, Table 1).

Table 1 illustrates that the nitro derivatives **9a-c**, the amino derivatives **10a-c** and the targets **11a-c** exhibited more activity against gram positive bacteria, mainly against standard *S. aureus* strains, in comparison to gram negative strains. The amino derivatives **10a-c** have shown the best activity against standard *S. aureus* strains with MIC values of 0.37, 2.93 and 2.93 μg/mL respectively. Although the targets **11a-c** have almost lost gram negative activity against *E-coli* strain, the amino derivatives **10a-c** exhibited the best activity against standard *E-coli* strain with MIC value of 0.18, 23.4 and 11.7 μg/mL respectively. Compound **10a** with *p*-fluorophenyl at $N^1$ has showed comparable activity to reference drug also against standard strain of *P. aeruginosa* with MIC value of 23.4 μg/mL. Surprisingly, the nitro compounds **9b** and **9c** have shown the best activity against standard *P. aeruginosa* strain with MIC value of 5.86 and 1.46 μg/ml respectively. Some of the amino derivatives (**10a**, **10c**) and targets **11b** and **11c** exhibited moderate activity against resistant *S. aureus* strain. Compound **10a** was the best with MIC value of 23.4 μg/mL. None of the compounds tested have shown any activity against gram negative resistant strains tested (not included in Table 1). Some of the nitro derivatives and the amino intermediates have shown reasonable antifungal activity against *Candida* strains tested. The targets quino[7,8-b][1,4]benzodiazepines **11a-c** have almost lost antifungal activity. Compounds **9b**, **9c**, and **10c** have shown best anticandida activity, mainly against *C. albicans* ATCC 10231 with MIC values of 1.46, 0.73 and 5.86 μg/mL respectively. The minimum inhibitory concentration of compound **9a, b** and **c** against *C. glabrata* were 11.7, 23.4 and 47.0 μg/mL respectively. Compound **11c** of the targets has also exhibited similar antifungal activity to miconazole reference against *Candida albicans* strains with MIC values of 1.46 μg/mL. It can be concluded that the targets quino-[7,8-b][1,4]-benzodiazepines **11a-c** have good activity against standard gram positive strains with no activity against gram negative nor both resistant strains. Compounds having 8-amino substituent (**10a-c**) exhibited the best activity against standard gram positive strains, resistant gram positive strains and standard gram negative strains with broad spectrum anticandida activity.



Scheme 1 – Synthesis of 1-(4-fluorophenyl)-7-chloro-6-fluoro-8-nitro-4-oxo-1,4-dihydroquinolin-3-carboxylic acid (7). *Reagents and conditions*: (1) methanol, *p*-fluoroaniline, reflux; (2) DMF, $K_2CO_3$; (3) Abs. ethanol, $H_2SO_4$.




*11b [23], 11c, 13c [22]

Scheme 2 – Preparation of 7-(carboxy phenyl amino)-fluoroquinolones (**9a-c, 10a-c**), Quino-benzo-diazepino targets (**11a-c**), their tautameric (**12a-c**) and sulfonated compounds (**13a-c**).

*Table 1*

Mean Minimum inhibitory concentration (μg/ml) of synthesized compounds

| Compd. | *BS* | *SA* | *SR* | *PA* | *EC* | *CA* | *CR* | *CT* | *CK* | *CG* | *Clog P* |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Ciprofloxacin** | 0.36 | 5.85 | 23.4 | 23.4 | 0.18 | - | - | - | - | - | - |
| **Miconazole** | - | - | - | - | - | 1.46 | 2.93 | 0.73 | 2.93 | 2.93 | 1.86 |
| **9a** | 23.4 | 5.86 | ND | 47.0 | 23.4 | ND | 93.8 | ND | ND | 11.7 | 5.82 |
| **9b** | 11.7 | 47.0 | 93.8 | 5.86 | ND | 1.46 | ND | ND | ND | 23.4 | 4.34 |
| **9c** | 5.86 | 0.73 | ND | 1.46 | ND | 0.73 | 47.0 | 47.0 | ND | 47.0 | 4.39 |
| **10a** | 11.70 | 0.37 | 23.4 | 23.4 | 0.183 | ND | ND | ND | ND | ND | 4.52 |
| **10b** | 47.0 | 2.93 | ND | 47.0 | 23.4 | 47.0 | ND | ND | ND | ND | 3.03 |

*Table 1 (continued)*

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **10c** | 11.7 | 2.93 | 47 | 11.7 | 11.7 | 5.86 | 94 | 5.86 | - | 5.86 | 3.09 |
| **11a** | ND | 93.8 | ND | ND | ND | ND | ND | ND | ND | ND | 3.97 |
| **11b** | 11.7 | 11.7 | 47 | ND | ND | ND | ND | ND | ND | ND | 2.48 |
| **11c** | 2.93 | 5.86 | 47 | ND | ND | 1.46 | ND | ND | ND | ND | 2.53 |
| **13a** | 1.46 | 11.7 | ND | ND | ND | ND | ND | ND | ND | ND | - |

* *BS-B.subtilis* (ATCC6633); SA-*S. aureus* (ATCC6538); SR-*S. aureus* (resistant); PA-*P.aeruginosa* (ATCC25923); EC-*E.coli* (ATCC8739); CA-*C.albicans* (ATCC10231); CR- *C.albican* (resistant); CT- *C. tropicalis* (ATCC3267); CK- *C. krusei* (ATCC 6258), CG-*C. Glabrata* (ATCC 1615). ND- Not detected > 125μg/ml; [*] *ClogP* value was calculated using ChemDraw Ultra V.11.

It is well acknowledged that the nature of substituent at C-7 of fluoroquinolone system has significant impact on the spectrum and extent of antibacterial activity.[25] It is well established also that more lipophilic quinolones should have enhanced ability to penetrate the lipophilic membrane of gram positive bacteria explaining better trends against these gram positive strains.[26] The high *ClogP* values of our intermediates and targets (>2.5) do explain the general trends against standard and resistant gram positive bacteria. The nitro derivatives **9a** and **9c** have shown stronger activity than the reference ciprofloxacin against *S. aureus*, whereas all amino derivatives **10a-c** were 2-3 folds stronger than reference against *S. aureus* strain and comparable against resistant gram positive strain. It was clear that introduction of more hydrophilic group such 8-amino in **10a-c** has optimized the lipophilicity/hydrophilicity balance (*ClogP* values around 3-4) allowing better penetration and activity against gram positive strains, with also better penetration of more hydrophilic membrane of gram negative bacteria. The intermediate *ClogP* values of **10a-c** have enhanced gram negative activity of this group leading to improved activity. Compound **10a**, with N-*p*-fluorophenyl, was comparable in its activity to ciprofloxacin against both gram negative strains tested *E. coli* and *P. aeruginosa* with MIC values of 0.18 and 23.4 μg/mL respectively. It was noticed that higher *ClogP* values of **9a-c** enhanced activity against *P. aeruginosa* mainly, whereas lower values in targets **11a-c** lead to complete loss of gram negative activity. Considering the fact that reduction of the nitro group at C-8 into amino (**10a-c**) has increased the antibacterial activity against both Gram positive and Gram negative bacteria (**10a-c**), this would suggest that the site of action of compounds **9-11** is possibly common in both types of bacteria and eliminate the effect of the outer layers or membranes in both bacterial strains. This might suggest that mechanism of action of compounds prepared in this work is similar to other known fluoro quinolones which were reported to have their activity on DNA enzymes.

## EXPERIMENTAL

All chemicals, reagents and solvents were of analytical/ synthetic grade were purchased from Sigma-Aldrich and Acros, Belgium, and used directly without further purification. Nuclear magnetic resonance spectra (NMR) were recorded on Bruker, Avance DPX-300 spectrometer. Infra red (IR) spectra were recorded using Shimadzu 8400F FT-IR spectrophotometer (KBr discs). Melting points (MP) were determined in open capillaries on a Stuart scientific electro-thermal melting point apparatus, and are uncorrected. High-resolution mass spectra (HRMS) were measured in positive or negative ion mode using electrospray ion trap (ESI) technique by collision-induced dissociation on a Bruker APEX-4 (7 Tesla) instrument. Microanalyses were performed using EuroVector Elemental Analyser, model (EA3000 A), Jordan University. Mobile phase mixtures for TLC were: System (1): Chloroform: methanol: formic acid ($CHCl_3$: MeOH: FA) (94: 5: 1); System (2): $CHCl_3$: MeOH: FA (90: 10: 1); System (3): Hexane: Ethyl acetate (50:50); System (4): System (1): system (3) (50: 50).

***Ethyl 2-(2,4-dichloro-5-fluoro-3-nitrobenzoyl)-3-(4-fluoro-phenyl)-acrylate*** **(5a):** A stirred solution of ethyl-3-(*N,N*-dimethyl-amino)-2- (2,4-dichloro-5-fluoro-3-nitro-benzoyl) acrylate[4] (**4**, 13.5 g, 36 mmol) in 50 mL of 80% methanol in dichloromethane was treated drop-wise with (6.4 g, 57.6 mmol) of 4-fluoroaniline in ice bath for 1 hr. A white precipitate started to form in the first 10 minutes of the reaction, at the end of the reaction, the precipitate was filtered, washed with few milliliters of diethyl ether and kept for the next step since it was one spot (pure). Yield ≈ 13.5 g (84 %); $R_f$value in system (3) = 0.91; mp = 136-138 °C (decomposition); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 0.98 (t, $J$ = 7.2 Hz, 3H, $OCH_2CH_3$), 4.05 (q, $J$ = 7.2 Hz, 2H, $OCH_2CH_3$), 7.17 (d, d, $J$ = 8.9, 11.18 Hz, 2H, H-3"/H-5"), 7.25 (d, d, d, $J$ = 5.2, 7.92, 9.6 Hz, 3H; H-2"/H-6" and H-6'), 8.68 (*Z*/*E*, $J$ = 14.2 Hz, 1H, *N*-C(3)-*H*), 11.45, 11.51 (*Z*/*E*, $J$ = 18 Hz, 1H, *N*-H - exchangeable); $^{13}$C NMR (300MHz, $CDCl_3$)-Dept: 13.30 ($CH_3$), 60.58 ($CH_2$), 116.31 (d, $^2J_{C\text{-}F}$ = 23.23 Hz, C-6'), 117.04 (d, $^2J_{C\text{-}F}$ = 23.1 Hz, C-3"/C-5"), 119.78 (d, $^3J_{C\text{-}F}$ = 8.4 Hz, C-2"/C-6"), 153.79 (C-3"); IR (NaCl): *v* 3750, 2850, 1732, 1632, 1635, 1381, 1327 cm$^{-1}$; HRMS (ESI, +ve): m/z $[M+H]^+$ 429.022 $C_{18}H_{13}Cl_2F_2N_2O_5$ requires 429.317; *EA* calculated for $C_{18}H_{12}Cl_2F_2N_2O_5$ (428.01): C, 50.37; H, 2.82; N, 6.53; found: C, 50.21; H, 2.56; N, 6.44.

***Ethyl 7-chloro-1-(4-fluoro-phenyl)-6-fluoro-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylate*** **(6a):** The cyclization process of the resulted (**5a**, 12 g, 27.02 mmol) was carried out using potassium carbonate (11.7 g, 85 mmol) in dimethyl-formamide (DMF, 50 ml), the mixture was heated at

70 °C with continuous stirring for 1 hour. The reaction mixture was then poured onto crushed ice (250 g) with vigorous stirring for 15 min. Further washing gave gummy yellowish white layer which was filtered by suction filtration and left to dry in dark. Yield ≈ 10.4 g (95.4 %); $R_f$ value in system (**3**) = 0.58; mp = 180-186 °C (decomposition); $^1$H NMR (300 MHz, $CDCl_3$): $\delta$ 1.39 ((t, $J$ = 7.2 Hz, and 2.78 (d, $J$ = 1.75 Hz, 3H, $CH_3$, rotomers)), 4.38 (2q, , $J$ = 7.1 Hz, 2H, O$CH_2CH_3$, rotomers), 7.21 (d, d, , $J$ = 8.2, 11.8 Hz, H-3'/H-5'), 7.37 (d, d, $J$ = 7.7, 4.5 Hz, H-2'/H-6'), 8.32 (d, $^3J_{H\text{-}F}$ = 11.7 Hz, 0.3 H, H-5) and 8.45 (d, $^3J_{H\text{-}F}$ = 8.23, 0.7 H, H-5, rotomers), 8.36 (br s, 1H, H-2); $^{13}$C NMR (300MHz, $CDCl_3$): 14.33, 43.6 ($CH_3$, rotomers), 61.45, 61.66 ($CH_2$, rotomers), 112.12 (C-3), 115.90, 116.68 (2d, $^2J_{C\text{-}F}$ = 22.5, 23.25 Hz, C-5, rotomers), 117.04 (d, $^2J_{C\text{-}F}$ = 23.33 Hz, C-3' / C-5'), 122.12 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, C-7), 128.21 (d, $^2J_{C\text{-}F}$ = 6.60 Hz, C-4a), 129.84 (d, $^3J_{C\text{-}F\ 4'}$ = 9.22 Hz, C-2' / C-6'), 135.19 (d, $^3J_{C\text{-}F}$ = 2.40Hz, C-8a), 135.67 (d, C-8), 151.90, 151.91 (C-2, rotomers), 152.4 (d, $^1J_{C\text{-}F}$ = 205.6 Hz, C-6), 161.78, 163.92 (C(3)-$COOC_2H_5$, rotomers), 163.36 (d, $^1J_{C\text{-}F}$ = 237.22 Hz, C-4'), 165.48 (C-1'), 164.94, 165.14 (C-4, rotomers); IR (NaCl): $\nu$ 3909, 3425, 2283, 1635, 1489, 1381, 1327, 1142 cm$^{-1}$; HRMS (ESI, +ve): m/z $[M^+ + Na]^+$ 431.02 $C_{18}H_{11}ClF_2N_2O_5Na$ requires 431.02168; *EA* calculated for $C_{18}H_{11}ClF_2N_2O_5$(408.03): C, 52.89; H, 2.71; N, 6.85; found: C, 52.76; H, 2.57; N, 6.79.

***7-Chloro-6-fluoro-1-(4-fluoro-phenyl)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid*** **(7a):** A vigorously stirred suspension of 7-Chloro-6-fluoro-1-(4-fluoro phenyl) – 8-nitro – 4 – oxo - 1,4-dihydro quinoline-3-carboxylate (**6a**, 8.0 g, 19.0 mmol) in 150 mL mixture of (11.2 N HCl and 96% Ethanol (7:3) was heated at 75-80 °C under reflux conditions for 48 h. Thereafter, the reaction mixture was cooled, poured onto crushed ice 150 g and the resulting heavy off white precipitate was collected, washed with cold water (2 x 20 ml), dried and recrystallized from a mixture of chloroform and methanol (70 ml, 1:1) Yield 7.5 g (99 %); $R_f$ value in system (1) = 0.75 and in system (4) = 0.40; mp = 195-200°C (decomposition);$^1$H NMR (300 MHz, DMSO-$d_6$):7.39 (d, d, $J$ = 8.10, 8.40 Hz, 2H, H-3' / H-5'), 7.75 (d, d, $J$ = 4.50, 7.50 Hz, 2H, H-2' / H-6'), 8.55 (d, $^3J_{H\text{-}F}$ = 8.50 Hz, 1H, H-5), 8.61 (s, 1H, H-2), 13.78 (br s, 1H, COO*H*); $^{13}$C NMR (300MHz, DMSO- *d6*): 109.93 (C-3), 115.02 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, C-5), 116.75 (d, $^2J_{C\text{-}F\ 4'}$ = 23.3 Hz, C-3' / C-5'), 122.08 (d, $^2J_{C\text{-}F}$ = 24.0 Hz, C-7), 128.21 (d, $^2J_{C\text{-}F}$ = 6.60 Hz, C-4a), 129.99 (d, $^4J_{C\text{-}F}$ = 2.40Hz, C-8a), 131.25 (d, $^3J_{C\text{-}F\ 4'}$ = 9.50 Hz, 2CH, C-2' / C-6'), 135.94 (d, $^3J_{C\text{-}F}$ = 2.40Hz, C-8), 141.22 (C-1'), 153.52 (C-2), 154.58 (d, $^1J_{C\text{-}F}$ = 250 Hz, C-6), 163.33 (d, $^1J_{C\text{-}F\ 4'}$ = 247.0 Hz, C-4'), 164.69 (C (3)-COOH), 175.24 (d, $^4J_{C\text{-}F}$ = 2.0 Hz, C-4); IR (KBr): $\nu$ 3399, 3075, 2893, 1724, 1613, 1555, 1505, 1458, 1385, 1223, 1157, 860, 1092, 806 cm$^{-1}$; HRMS (ESI, +ve): m/z $[M]^+$ 380.0012 $C_{16}H_7ClF_2N_2O_5$ requires 380.00898; *EA* calculated for $C_{16}H_7ClF_2N_2O_5$: (380.68): C, 50.48; H, 1.85; N, 7.36. Found: C, 50.61; H, 1.92; N, 7.43.

***7-(2-Carboxy–phenylamino)-6-fluoro-1-(4-fluoro-phenyl)-8-nitro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid*** **(9a).** A stirred mixture of 2-Aminobenzoic acid (1.25 g, 9 mmol), **7a** (1.0 g, 2.63 mmol) and sodium hydrogen carbonate (1.5 g, 18 mmol) in 70 % aqueous ethanol (140 ml) was heated at 70-75 °C for 6-7 days under reflux conditions. The mixture was extracted with dichloromethane (2 x 50 mL). The aqueous layer was cooled, its pH adjusted to 6-7 by addition of 3.5N HCl and re-extracted with $CH_2Cl_2$ (50 mL). Further acidification of the leftover aqueous layer to pH = 1-2 gave the title compound as dark brown solid which was collected by filtration, washed with cold water (2 x 10 mL), dried and re-crystallized from a mixture of chloroform and ethanol (1:1, v/v), to give the title compound as dark brown solid. Yield ≈ 1.1g (87%); MP = 252-254°C (decomposition); $R_f$ value in system (**1**) = 0.68, $R_f$value in system (**2**) = 0.75; $^1$H NMR (300 MHz, DMSO-$d_6$): 6.86 (dd, $J$ = 7.20, 7.30 Hz, 1H, H-6''), 7.03 (dd, $J$ = 7.20, 7.80 Hz, 1H, H-4''), 7.43 (m, 3H, ArH), 7.68 (m, 1H, ArH), 7.77 (m, 1H, ArH), 7.91 (d, $J$ = 7.80 Hz, 1H, ArH), 8.42 (d, $^3J_{H\text{-}F}$ = 11.10 Hz, H-5), 8.65 ( s, 1H, H-2), 10.20 (br s, 1H, N*H*), 13.50 – 15.40 (2 br s, C(3)COO*H* and C(2'')COO*H*); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 109.73 (C-3), 115.25 (d, $^2J_{C\text{-}F}$ = 21.60 Hz, C-5), 115.41 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, C-3'), 116.57 (C-6''), 115.39 (C-4a), 116.96 (d, $^2J_{C\text{-}F}$ = 23.0 Hz, C-5'). 121.70 (C-4''), 123.80 (d, $^3J_{C\text{-}F}$ = 7.20 Hz, C-8), 129.80 (d, $^3J_{C\text{-}F}$ = 9.20 Hz, C-6'), 130.11 (d, $^3J_{C\text{-}F}$ = 9.10 Hz, C-2''), 131.59 (C-3''), 133.38 (C-1'), 134.43 (C-5''), 137.28 (d, $J$ = 2.60 Hz, C-8a), 137.50 (d, $^2J_{C\text{-}F}$ = 18.0 Hz, C-7), 143.78 (C-2''), 149.65 (C-1''), 153.05 (C-2), 153.62 (d, $^1J_{C\text{-}F}$ = 254.0 Hz, C-6), 162.81 (d, $^1J_{C\text{-}F}$ = 247.0 Hz, C-4'), 165.03 (C(3)*C*OOH), 170.04 (C(2'')*C*OOH), 175.88 (C-4); IR:$\nu$ 3445, 3071, 2928, 2361, 1701, 1616, 1589, 1543, 1505, 1300, 1223, 1157, 1050, 890, 800, 760 cm$^1$; HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{12}F_2N_3O_7$ $[M\text{-}H]^-$:480.06433, found: 480.06488; *EA* calculated for $C_{23}H_{13}F2N_3O_7$ (481.36), C, 57.39; H, 2.72; N, 8.73; Found: C, 57.34, H, 2.43; N, 8.54.

***8-Amino-7-(2-carboxy-phenylamino)-6-fluoro-1-(4-fluoro-phenyl)-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid*** **(10a):** To a stirred solution of compound **9a** (0.50 g, 1 mmol) and potassium carbonate (0.96 g, 7 mmol) in 20 mL water was added drop-wise an aqueous solution of sodium dithionite (0.87 g, 5 mmol) in water (5 mL). The reaction mixture was further stirred at RT for 30 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 **10b** Yield ≈ 0.40 g (89%); MP = 240-245°C (decomposition), $R_f$ value in system (**1**) = 0.60, $R_f$ value in system (**2**) = 0.58; $^1$H NMR (300 MHz, DMSO-$d_6$): 4.55 (br s, 2H, N*H2*), 6.34 (d, $J$ = 8.40 Hz, 1H, H-6''), 6.79 (dd, $J$ = 7.50, 7.50Hz, 1H, H-4''), 7.34 (dd, $J$ = 7.50, 8.10 Hz, 1H, H-3''), 7.48 (m, 3H, H-5 + H-2' +H-6'), 7.82 (m, 3H, H-5'' + H-3' +H-5'), 8.57 (s, 1H, H-2), 9.18 (br s, 1H, N*H*), 14.25 - 15.5 (2 br s overlapping, 2H, C(3)COO*H* +C(2'')COOH); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 98.67 (d, $^2J_{C\text{-}F}$ = 22.5 Hz, C-5), 107.21 (C-3), 113.22 (C-1'), 113.86 (Ar C-H), 117.03 (Ar C-H), 117.33 (Ar C-H), 118.13 (Ar C-H), 119.09 (d, $^2J_{C\text{-}F}$ = 17.33 Hz, C-7), 120.82 (C-8), 125.87 (C-8a), 126.36 (d, C-4a), 128.98 (Ar C-H), 129.10 (Ar C-H), 131.87 (Ar C-H), 134.60 (Ar C-H), 139.38 (d, C-2''), 147.65 (C-1''), 151.58 (C-2), 157.82 (d, $^1J_{C\text{-}F}$= 240.0 Hz, C-6), 164.51 (d, $J$= 252.0 Hz, C-4'), 165.98 (C(3)*C*OOH), 170.44 (C(2'')*C*OOH), 177.79 (C-4); IR:$\nu$ 3487, 3360, 3074, 1721, 1682, 1586, 1501, 1454, 1416, 1319, 1223, 1157, 984, 860, 800, 765 cm$^{-1}$; HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{14}F2N_3O_5$ $[M\text{-}H]^-$:450.09015, found: 450.09232; *EA* calculated for $C_{23}H_{14}F2N_3O_5$ (451.38) C, 61.20; H, 3.35; N, 9.31; Found C, 61.60; H, 3.12; N, 9.55.

***6-Fluoro-1-(4-fluoro-phenyl)-4, 12-dioxo-4, 7, 12, 13-tetrahydro-1H-quino [7,8-b][1,4]benzo diazepine-3-carboxylic acid*** **(11a):** A stirred solution of compound **10a** (0.2 g, 0.44 mmol) and polyphosphoric acid PPA (10 mL) was heated under reflux conditions or in sand bath (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. The precipitated yellowish green solid product was collected by suction filtration, washed with water (2 x 10 ml) and dried. Yield ≈ 0.18 g (94%); MP = 318-322 °C (decomposition); $R_f$

value system (**1**) = 0.65; $^1$H NMR (300 MHz, DMSO-$d_6$):7.51 -7.72 (m, 5H, ArH), 7.81 -7.85 (m, 3H, ArH), 8.12 (d, $^3J_{H-F}$=11.50 Hz, H-5), 8.56 (s, 1H, N (7) *H*), 8.78 (s, 1H, H-2), 10.21 (br s, 1H, N (13) *H*), 14.01 (br s, 1H, COO*H*); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 110.51 (C-3), 118.02 (d, $^3J_{C-F}$ = 14.10 Hz, C-4a), 118.10 (d, $^2J_{C-F}$ = 24.2 Hz, C-5), 120.30 (d, $^3J_{C-F}$ = 3.8 Hz, C-13a), 127.21 (CH-Ar), 128.57 (CH-Ar), 129.11 (CH-Ar), 129.31 (2CH-Ar, C-3',C-5'), 129.45 (2CH-Ar, C-2', C-6'), 129.55 (CH-Ar), 135.31 (C-13b), 138.42 (C7a), 140.92 (d, $^2J_{C-F}$ = 16.50 Hz, C-6a), 146.78 (C-1″), 151.20 (C-2), 151.22 (C-11a), 153.52 (d, $^1J_{C-F}$ = 250.0 Hz, C-6), 163.24 (d, $^1J_{C-F}$ = 244.0 Hz, C-4″), 166.10 (C(3)*C*OOH), 168.18 (C-12), 178.42 (C-4); IR: *v* 3433, 2994, 2909, 2585, 2315, 2222, 2099, 1659, 1435, 1412, 1312, 1026, 957, 702, 671 cm$^{-1}$; HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{12}F2N_3O_4$ [M-H]$^-$ : 432.07959, found: 432.07721; *EA* calculated for $C_{23}H_{12}F2N_3O_4$, (433.36), C, 63.74; H, 3.02; N, 9.70; found C, 63.42; H, 3.22; N, 10.05.

***1-(4-fluoro phenyl) - 6-fluoro- 4-oxo -12 – hydroxyl-4,7-dihydro-1H-quino [7,8-b] [1,4]benzodiazepine -3-carboxylic acid* (12a).** A stirred solution of compound **10a** (0.2 g, 0.5 mmol) and polyphosphoric acid 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. Then pH was adjusted to 7.0-7.5 by adding NaOH solution (40%) then product precipitate; the precipitated yellowish green solid product was collected by suction filtration, washed with water (2 x 10 mL) and dried. Yield ≈ 0.18 g (98%); MP = 311-314 °C (decomposition); $R_f$ value in system (**1**) = 0.68;$R_f$ value in system (**2**) = 0.70; $^1$H NMR (300 MHz, DMSO-$d_6$):4.20 (br s, 1H, O*H*, exch.), 7.47 -7.70 (m, 5H, ArH), 7.75 -7.83 (m, 3H, ArH), 7.90 (d, $^3J_{H-F}$=11.40 Hz, H-5), 8.43 (s, 1H, N(7) *H*), 8.56 (br s, 1H, H-2), 13.92 (br s, 1H, COO*H*); $^{13}$C NMR (75 MHz, DMSO-$d_6$): 105.21 (d, $^2J_{C-F}$ = 20.0 Hz, C-6a), 107.44 (C-3), 117.31 (d, $^3J_{C-F}$ = 13.80 Hz, C-4a), 117.93 (d, $^2J_{C-F}$ = 23.0 Hz, C-5), 119.81 (d, $^3J_{C-F}$ = 6.70 Hz, C-13a), 128.01 (CH-Ar), 128.12 (CH-Ar), 128.56 (CH-Ar), 129.29 (2CH-Ar), 129.42 (2CH-Ar), 129.54 (CH-Ar),134.39 (C-13b), 137.31 (C-7a), 145.45 (C-1″), 151.21 (C-2), 151.29 (C-11a), 153.10 (N=C$_{12}$-OH), 155.27 (d, $^1J_{C-F}$ = 251.0 Hz, C-6), 163.22 (d, $^1J_{C-F}$ = 244.0 Hz, C-4′), 165.87 (C(3)*C*OOH), 177.37 (C-4); IR: *v* 3433, 2994, 2909, 2585, 2315, 2222, 2099, 1659, 1435, 1412, 1312, 1026, 957, 702, 671 cm$^{-1}$; HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{12}F2N_3O_4$ [M-H]$^-$ : 432.07959, found: 432.07721; *EA* calculated for $C_{23}H_{13}F_2N_3O_4$, (433.36), C, 63.74; H, 3.02; N, 9.70; found C, 63.42; H, 3.22; N, 10.05.

***1-p-fluorophenyl-6-fluoro-4,12-dioxo-10-sulfo-4,7,12,13-tetrahydro-1H-quino[7,8-b][1,4]benzo-diazepine-3-carb-oxylic acid* (13a):** A stirred solution of compound **10a** (0.2 g, 0.44 mmol) and conc. Sulphuric acid (15 mL) was heated under reflux conditions (100 °C) for 3 h. The resulting mixture was poured onto water (60 mL) with vigorous stirring. The precipitated solid product was collected by suction filtration, washed with water (2 x 10 mL) and dried to furnish green-brownish solid product. Yield ≈ 0.19 (86%); MP = >300 °C; $R_f$ value in System (**4**) = 0.61; $^1$H-NMR (300 MHz, DMSO-$d_6$): 6.92 -7.78 (m, 4H, ArH), 7.85 -7.91 (m, 3H, ArH), 8.09 (d, $^3J_{H-F}$ = 11.0 Hz, 1H, H-5), 8.65 (br s, 1H, N(7)-*H*), 8.82 ( br s, 1H, H-2), 10.22 (br s, 1H, N(13)-*H*)), 14.01-15.50 (br s, 2H, $CO_2H$ + $SO_3H$); IR: *v* 3438, 2995, 2915, 1656, 1441, 1412, 1070, 957, 905 cm$^{-1}$; HRMS (ESI, -ve): *m/z* calculated for $C_{23}H_{13}F_2N_3O_7S$ [M]$^+$ : 513.04423, found 513.04410.

***In-vitro* antibacterial activity**

Antibacterial screening was carried out using broth dilution method according to reported protocols.[22-24] The reference drugs used were Ciprofloxacin (Al-Hikma Pharmaceutical, Jordan) and Miconazole (Dar-Aldawa Pharmaceutical, Jordan). The microorganisms used were *Pseudomonas aeruginosa* ATCC25923, *Escherichia coli* ATCC8739, *Staphylococcus aureus ATCC6538*, and *Bacillus subtilis* ATCC6633. The fungal strains were *Candida albicans* ATCC10231, *Candida glabrata* ATCC1615, *Candida tropicalis* ATCC3267 and *Candida krusei* ATCC 6258. Resistant strains of *E. coli, S. aureus and C. albicans* were isolated from hospitalized patients from the Jordan University Hospital and their identity confirmed by biochemical tests. Stock solution concentrations of all compounds and reference drugs were prepared in DMSO (1 mg/ml). The working solutions (500-0.244 µg/ml) of tested compounds were prepared. In all assays, positive growth controls and negative controls were prepared. Negative control for DMSO was carried out to check its activity. MICs were expressed as the average of two successive concentrations of the antimicrobial agent showing no growth and growth, respectively. The microorganism's growth was detected as turbidity, using a microtitre plate reader (at 630 nm) relative to an un-inoculated well. MIC determination was carried out in duplicate.

## CONCLUSIONS

In this work, we report the synthesis and antibacterial properties of 1-substituted-8-amino-1,4-dihydroquinoline derivatives and 1H-quino-[7,8-b][1,4]-benzo diazepine-3-carboxylic acid derivatives. The structures of the compound products were established with spectroscopic data of proton and carbon-13 NMR and mass. The synthesized compounds exhibited appreciable antibacterial and antifungal activity.

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