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Synthesis of novel ethyl 1-ethyl-6-fluoro-7-(fatty amido)-1, 4-dihydro-4-oxoquinoline-3-carboxylate derivatives and their biological evaluation

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ABSTRACT

A series of novel ethyl 1-ethyl-6-fluoro-7-(fatty amido)-1,4-dihydro-4-oxoquinoline-3-carboxylate derivatives were prepared through multistep synthesis. The key step in the synthesis was to obtain the C-7 fatty amide derivative. The azide was selectively formed at C-7 position using sodium azide at 60 °C. Subsequently, the azide was reduced under mild conditions using zinc and ammonium chloride to form the corresponding amine. The synthesized derivatives were further subjected to biological evaluation studies like cytotoxicity against a panel of cancer cell lines such as DU145, A549, SKOV3, MCF7 and normal lung cells, IMR-90 as well as with antimicrobial and antioxidant activities. It was observed that the carboxylated quinolone derivatives with hexanoic (**8a**), octanoic (**8b**), lauric (**8d**) and myristic (**8e**) moieties exhibited promising cytotoxicity against all the tested cancer cell lines. The results also suggested that hexanoic acid-based fatty amide carboxylated quinolone derivative (**8a**) exhibited promising activity against both bacterial and fungal strains and significant antibacterial activity was observed against *Staphylococcus aureus* MTCC 96 (MIC value of 3.9 μg/mL). The compound **8a** also showed excellent anti-biofilm activity against *Staphylococcus aureus* MTCC 96 and *Bacillus subtilis* MTCC 121 with MIC values of 2.1 and 4.6 μg/mL, respectively.

In the recent years, antimicrobial resistance has gained a renewed interest from a clinical perspective and has raised a serious public health attention due to the incidence of various drug-resistant microbial infections. The primary reason for antimicrobial resistance is the wide usage or misuse of the currently available antimicrobial agents by the medical practitioners.[1,2] In view of the increased threat from these drug-resistant Gram-positive and -negative bacterial strains and also *Candida* strains, there is a continuous demand to identify new antimicrobial agents.

Quinolone as a privileged scaffold represents one of the most important structural unit prevalent in various naturally occurring and bioactive compounds. Quinolones are presently used as antibacterial agents for the treatment of many systemic infections and functionally exert their effect by inhibition of two type II bacterial topoisomerases, namely DNA gyrase and topoisomerase IV.[3,4a,b] In addition, Fluoroquinolones (FQs) belong to the class of quinolone compounds with a broad spectrum of antibacterial activity.[5–7] FQs are presently used for the treatment of various bacterial infections of the respiratory, gastrointestinal and urinary tract, as well as sexually transmitted diseases and chronic osteomyelitis.[8–11] In the recent years, non-classical anti-tumor activity of FQ derivatives has been described.[12–14]

They also display anti-proliferative activity in some tumor cells such as breast cancer cells,[15] bladder transitional cell carcinoma,[16–18] non-small cell lung carcinoma,[19] prostate carcinoma[20,21] and colorectal carcinoma.[22] One of the quinolone derivatives, ciprofloxacin (CP) displays anti-proliferative and apoptosis-inducing activities both on prostate and bladder cancer cells.[23,24,20,21] From a structure–activity relationship (SAR) perspective on different quinolone derivatives, it was noticed that different substituents (however, these substituents are not fatty acyl moieties) at C-7 position of the FQ that contribute to their antimicrobial activities.[25–27] Another study suggested that by increasing the bulkiness of the C-7 substituent resulted in the enhancement of antibacterial activities.[28] Further, the side chains at the C-7

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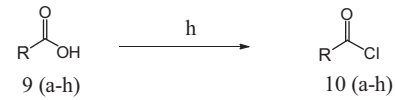
position of the quinolones may interact with the target enzyme.[29–31] In addition, many fatty acids[32] and fatty amides[33] are known to exhibit antimicrobial and cytotoxic activities. Long alkyl/alkenyl chains exhibited antimicrobial activity and some fatty acid derivatives were also known to possess antitumor and anti-depressant activities.[34–36] Some reports exist on the heterocyclic and fatty acid-based hybrids exhibiting biological activities.[37,38] However, there is paucity of information on the synthesis and biological evaluation of carboxylated quinolone derivatives with C-7 fatty amide substitution. Considering these facts, the present study was undertaken to replace the C–N bond in quinolones with different fatty amides and further evaluated their cytotoxicity, antioxidant and antimicrobial activities.

The synthesis of target compounds (**8a**–**h**) is described in Schemes 1 and 2. Carboxylated fatty amide quinolone derivatives were obtained using a synthetic route comprising of 8 steps. The starting material, 3,4-difluoro nitro benzene (**1**) was converted to 3,4-difluorobenzenamine (**2**) by a facile method in which iron and ammonium chloride were taken in methanol and water at RT and the contents were further refluxed at 90 °C. Then 3,4-difluorobenzenamine (**2**) was treated with diethyl ethoxymethyl enemalonate to yield diethyl 2-((3,4-difluorophenylamino) methylene)malonate (**3**). The compound **3** was thermally cyclized at 255 °C for 6 h to give ethyl 6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**4**) where the product was obtained by filtration of the reaction mixture at room temperature.[39] Ethyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**4**) was directly treated with iodoethane in the presence of potassium carbonate at 85 °C to form ethyl-1-ethyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**5**).[40]

In this method the key step was the formation of azide at C-7 position selectively by the reaction of ethyl-1-ethyl-6,7-difluoro-1,4-dihydro-4-oxoquinoline-3-carboxylate (**5**) with sodium azide at 60 °C in dimethyl formamide to yield compound **6**.[41] The compound **6** was subjected to a simple catalytic reduction in which zinc and ammonium chloride mixture was used as catalyst to yield compound **7**.[42] In all the above conversions, moderate to good yields were obtained (33–77%).

On the other hand, fatty acids (**10a**–**h**) were converted to fatty acid chlorides by oxalyl chloride in the presence of catalytic amount of dimethyl formamide in dichloromethane at 0 °C for 3 h (Scheme 2). The fatty acyl chlorides so formed were treated with compound **7** in the presence of triethyl amine in dichloromethane to form the corresponding target compounds (**8a**–**h**), which were purified by column chromatography.

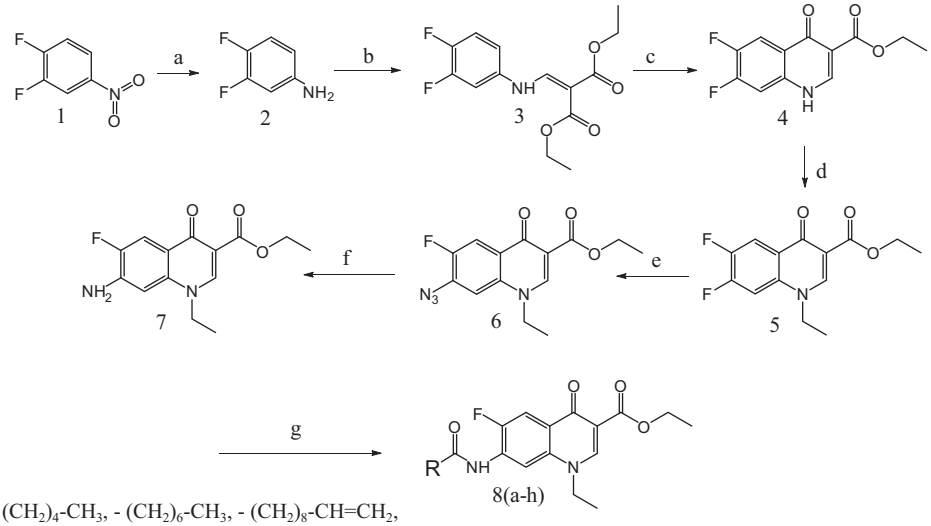


R = - $(CH_2)_4$-CH_3, - $(CH_2)_6$-CH_3, - $(CH_2)_8$-CH=CH_2,
- $(CH_2)_{10}$-CH_3, - $(CH_2)_{12}$- CH_3, - $(CH_2)_{14}$-CH_3,
- $(CH_2)_7$-CH=CH_2-$(CH_2)_7$-CH_3, - $(CH_2)_{16}$- CH_3.

Scheme 2. Synthesis of fatty acid chlorides. Reagents and conditions: (h) $COCl_2$, cat. DMF, DCM, 3 h.

All the synthesized compounds (**8c**–**h**) were screened against a panel of four cancer cell lines such as DU145, A549, SKOV3, MCF7 and IMR-90 (normal lung cell line) and Doxorubicin was used as a standard positive control. The cytotoxicity results for all the synthesized compounds are depicted in Table 1.

Most of the compounds showed significant cytotoxic effect. Among the tested compounds, the compounds **8a**, **8b**, **8d** and **8e** exhibited promising cytotoxicity against all the cancer cell lines. Further, these promising compounds were screened against IMR-90 (Normal lung cell line). The selectivity index (SI) was also determined using the following formula: SI = IC_{50} of pure compound in a normal cell line/IC_{50} of the same pure compound in cancer cell line, where IC_{50} is the concentration required to inhibit 50% of the cell population. High SI value (>2) of a compound gives a selective toxicity towards cancer cells, while the compound with SI value <2 is considered to give general toxicity in which it also can cause cytotoxicity in normal cells.[43] The compounds **8a**, **8b**, **8d** and **8e** comprising of 6 (hexanoyl), 8 (octanoyl), 12 (dodecanoyl) and 14 (tetradecanoyl) carbon chain lengths, respectively, containing amide linkage at the C-7 position exhibited promising cytotoxicity against all the tested cancer cell lines. This observation corroborates with some of the earlier studies which suggests that ω-hydroxy fatty acid analogs and 2,6-diisopropylphenol fatty acid



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- $(CH_2)_{10}$-CH_3, - $(CH_2)_{12}$- CH_3, - $(CH_2)_{14}$-CH_3,
- $(CH_2)_7$-CH=CH_2-$(CH_2)_7$-CH_3, - $(CH_2)_{16}$- CH_3.

Scheme 1. Synthesis of carboxylated fatty amide quinolones. Reagents and conditions: (a) Fe, NH_4Cl, H_2O, MeOH, 90 °C, 12 h; (b) diethyl ethoxymethyl enemalonate, 120 °C, 4 h; (c) diphenyl ether, 255 °C, 6 h; (d) K_2CO_3, C_2H_5-I, DMF, 85 °C, 16 h; (e) NaN_3, DMF, 60 °C, 12 h; (f) Zn, NH_4Cl, EtOH, H_2O, 90 °C, 6 h; (g) acid chloride, triethyl amine, DCM, RT, 12 h.