Fluoroquinolone derivatives: New *N*-substituted Ciprofloxacin derivatives synthesis and evaluation of a specific ^{18}F- Radiolabelled compound for PET Study

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Abstract: A series of new N-substituted Ciprofloxacin derivatives were designed and synthesized. Their antibacterial activities were determined against Gram-negative microorganism. Specifically, N-substituted in a fluoroquinolone moiety (FQ) connected by various linker was synthesized according to their structure activity relationship studies. Selected *N*-substituted Ciprofloxacin derivatives showed DNA gyrase inhabitation compared to that Ciprofloxacin. We described a new ^{18}F-labeled *N*-substituted Ciprofloxacin derivative ([^{18}F] 6) which gives a specific activity compared to Ciprofloxacin and it was produced easily with high radiochemical yield using tertiary alcohol as a reaction media for nucleophilic fluorination. ([^{18}F] 6) is applicable properties for future imaging of bacterial infection with PET.

Keywords. Ciprofloxacin, Fluoroquinolene, Radiofluorination, Fluorine-18, Positron emission tomography (PET).

I. INTRODUCTION

Compounds Ciprofloxacin is one of the most potent fluoroquinolone antibiotics agents and it has been show their broad antibacterial spectrum both to Gram positive and Gram negative bacteria. Thus, recent development of a new fluoroquinolone that can provide improved Gram-positive Gram negative antibacterial activity is clinically used for the treatment of various infection diseases 4. Fluoroquinolone bactericidal activity is caused by the inhibition of two bacterial enzymes; DNA gyrase and topoisomerase IV. While the interaction of the C7-substituent fluoroquinolone with the enzyme plays a supporting role 3. A number of fluoroquinolone are synthesized according to their structure-activity relationship (SAR) studies. N-substituted Fluoroquinolone plays important role in the antibacterial activity of the Fluoroquinolone and alkyl group such as ethyl, propyl and butyl have been regard as suitable N-substituent 1.

The nature of substituent at C-7 or N- position has a great impact of potency, spectrum, solubility and pharmacokinetics. From these data the C-7 or N position in lead structure offers a potential site for structural modifications 2, thereby providing an excellent not only in generating a library of potential fluoroquinolones molecule but also in targeting the potential precursor for radiolabeling. We synthesized a number of molecules with modification at the C-7 or N- position of the Ciprofloxacin compound and evaluated their antibacterial activity in vitro. However, our idea was to concentrate on the synthesis of fluorinated analogues and compare their specific activity against radiolabeled Ciprofloxacin compound.

PET is being used more frequently in clinical and research studies because of its high sensitivity, good spatial resolution, and ease in accurate quantification. Additionally, PET possesses sensitivity in the lower Pico molar range but requires the drug of interest to be radiolabeled with appropriate positron-emitting radioisotope, such as carbon-11 (^{11}C; half-life, 20.4min.) or fluorine-18 (^{18}F; half-life, 110min.). Owing to its longer physical half-life, ^{18}F preferred for imaging for bacterial infection analysis since it allow longer durations 6. A number of these compounds after radiolabeled, we succeeded in the synthesis of a first in this series fluorine-18 labeled N-substituted Ciprofloxacin derivatives model compound ([^{18}F] 6) with high radiochemical yield which gives good specific activity than [^{18}F]Ciprofloxacin for applicable to imaging of bacterial infection for PET study.

II. EXPIRMENTAL

General. Reagents and solvents were purchased from Sigma-Aldrich and used without further purification. Flash column chromatography was carried out over silica gel. Measurement of mass spectra (MS) and high resolution MS (HRMS) were performed with JEOL Ltd JMS-700 Mstation mass spectrometer. Nuclear magnetic resonance (NMR) spectra were measured on a JEOL Ltd. (JNM-ECA600) 600 MHz spectrometer. The chemical shift (δ value) are expressed in part per million (ppm) relative to residual solvent such as chloroform (δ = 7.26) as an internal standard.

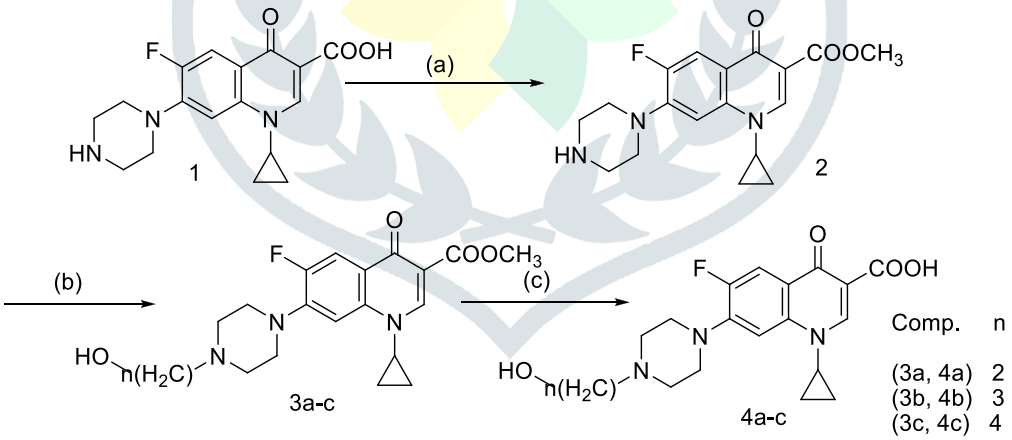
The analytic reversed-phase high performance liquid chromatography (RP-HPLC) method was performed with spectra system SCM100 degasser, P4000 pump, and UV/vis 3000 detector (Thermo Scientific, Waltham, MA) and a γ detector (BioScan flow count). The absorbance was monitored at 251 nm and a column (250 × 4.6 mm) was used. ChromQuest 4.2 software was used to record chromatograms. The flow was 4 mL/min, with the mobile phase varying from 88% solvent A (10 mmol phosphoric acid) and 12% solvent B (Ethanol) to 40% solvent B at 10 min, 60% solvent B at 15 min, and 90% solvent B at 20 min was used for the final purification of the compound [^{18}F]6, and the desired peak was elevated at 10.6 min. [^{18}F]Fluoride was produced by our site cyclotron (KIRAMS 13 MeV, South Korea) using the ^{18}O(p,n)18F nuclear reaction with 19 MeV proton irradiation of an enriched [^{18}O]H_2O target.

Biochemical studies:DNA supercoiling activity was assayed with relaxed pHOT-1 DNA as a substrate (TopoGEN, Inc., FL, USA) according to the manufacturer's protocol. The standard reaction mixture (20 µL) contained 35 mM Tris-Cl, pH 7.5, 24 mM KCl, 4 mM MgCl2, 2 mM dithiothreitol, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, 0.1 mg/mL BSA, 167 ng/µL relaxed pHOT-1, and E. coli DNA gyrase. The reaction mixture was incubated at 37 °C for 1 hr and then was terminated by addition of a stop buffer (5% Sarkosyl, 0.125% bromophenol blue, 25% glycerol) and chloroform/isoamyl alcohol (24:1) mixture. After a brief vortex, the blue aqua phase was analyzed by electrophoresis in 0.8% agarose. The IC50 was defined as the drug concentration that reduced the enzymatic activity observed with drug-free controls by 50%.

III. RESULT AND DISCUSSION

In order to coupling via amidation of the piperazinyl ring, ciprofloxacin was esterified using Cat. TsOH mediated for methylation to give the methyl ester of ciprofloxacin (comp.2) in good yield. Several SAR studies of fluoroquinolones have demonstrated a high tolerance for structure variations at the 7-position of the phenyl ring, including alkylation at the terminal nitrogen of the piperazine moiety. On the basis of this information, we chose to modify Ciprofloxacin at the terminal nitrogen of the piperazine moiety with various linkers connecting an alkyl groups.The derivatives 3a-c were prepared by direct coupling of the commercial available bromohydroxyalkyl group with compound 2 under reflux and base conditions (K_2CO_3, CH_3CN), followed by purification of intimidate ester and hydrolysis by LiOH is described in Scheme 1.

Scheme 1:



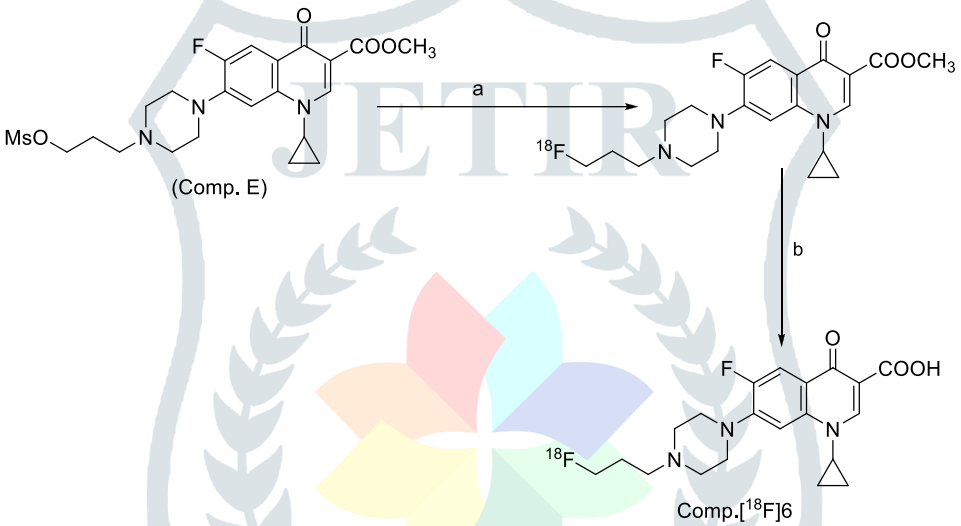
Reagent and Condition: Synthesis of N-substituted ciprofloxacin derivatives 4a-c. Reagent and condition (a) Cat. TsOH, MeOH, reflux 24 h, 81% (b) ACN, K2CO3, Br (CH2)n-OH, 100 oC 16h, (c) LiOH, MeOH:H2O (4:1), AcOH, RT, 16 h

In case of the fluoroalkyne derivatives of ciprofloxacin (compounds 5a and 5b) were prepared by coupling of methyl ester ciprofloxacin (comp. 2) with bromohydroxy alkane to give alcohol (compounds 3a and 3b). It was converted to chloroethyl and methaneesulfonylpropyl derivatives to give compounds 5a and 5b. Compounds 3a-e were evaluated for in vitro antibacterial activity using optical density measurement in two kind of E coli (DH_5 alpha and Top 10). The half maximal effective concentration (EC_{50}) of these compounds against Gram-negative bacteria compared to ciprofloxacin DH_5 alpha. But the compound 3a, 3b and 3d displayed similar (Ecoli DH5 alpha) antibacterial activity efficiency compared to ciprofloxacin. The most prominent improvement was observed against Ecoli TOP 10. On average, the compounds 3a, 3b, 3c and 3d displayed significantly better potency than ciprofloxacin. The observed EC_{50} values of Ecoli DH_5 alpha and Ecoli TOP 10 of N-substituted ciprofloxacin derivatives indicate that the majority of the compounds are more active than ciprofloxacin against Gram-negative

bacteria. While retaining moderate DNA supercoiling activity promoted us to further investigate the inhibition activity of DNA gyrase.

For this purpose, we tested selected N-substituted ciprofloxacin compounds (3a, 3b and 3d) for inhibition of the enzymes that are targeted by the ciprofloxacin.. The observed data show that N-substituted ciprofloxacin compounds should be weaker DNA gyrase compare to ciprofloxacin. The measured EC_{50} values of 3d displayed far greater activities than compound 3a and 3b. It is of the note that the EC_{50} values of compound 3d determined to be similar to ciprofloxacin for the inhibition of DNA gyrase. These data clearly confirm our designing principle of N-substituted ciprofloxacin derivatives is applicable for the radiosynthesis. Radiolabeling of [^{18}F]6 was synthesized via a nucleophilic substitution of the mesylate precursor (4b) with [^{18}F]fluoride using tertiary alcohol as a reaction media, followed by protonation with LiOH (Scheme 4). However, after reversed phase HPLC purification, [^{18}F]6 was obtained in high chemical and radiochemical purity. The specific activity was > 300 uCi/umol at the end of the synthesis. The total synthesis and purification time was 180 min. The radiolabelling using t-amyl alcohol as the solvent gave a higher radiochemical yield than that in acetonitrile and DMF, use of the corresponding tosylate and chlorate precursors gave a much lower yield.

Scheme 2:



Reagent and condition: (a) [^{18}F]fluoride, TBABA, *t*-amyl alcohol, 100 ^{o}C. (b) LiOH, MeOH/H_2O(4:1), AcOH, 100 ^{o}C

IV. CONCLUSION

We succeeded in the design, synthesis, and evaluation of in vitro antibacterial activity on the N-alkylated ciprofloxacin derivatives 4a-c, 6c, and 6d, with ciprofloxacin. On the basis of the in vitro antibacterial activity analysis, as well as analysis of the DNA gyrase inhibitory ability of 4a, 6c, and 6d, and the conformation analysis of 6d, it can be conclude that the conformation of 6d is mostly likely equivalent to the active ciprofloxacin. Radiosynthesis of [^{18}F]6 was accomplished in two step approach by radiofluorination of *N*-mesylate ciprofloxacin precursor 5b, followed by the hydrolysis using LiOH reagents. The radiochemical synthesis was achieved in high chemical yield and in a specific radioactivity, was conformed after co-chromatography of the radiolabeled and non-radiolabeled compound.

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