'RESEARCH JOURNEY' International E- Research Journal
Impact Factor - (SJIF) – 6.625 (2019), (CIF ) - 3.452(2015), (GIF)–0.676 (2013)
Vol. 6, Issue 3
Peer Reiewed Journal
E-ISSN : 2348-7143
July-Aug-Sept. 2019

# Synthesis and Evaluation of Various N-substituted Ciprofloxacin Derivatives for Preparation of Specific $^{18}$F-Radiolabel Compound for PET

**Sachin U. Kalme**
Assistant Professor
S.S.J.E.S., Arts, Commerce and Science College, Gangakhed.
Corresponding Author email ID: kalmesachin@gmail.com

**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, bacterial infection, bacterial infection in radiopharmaceutical, Positron emission tomography (PET), Radiofluorination, fluorine-18.

## 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 picomolar 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, 18F

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.

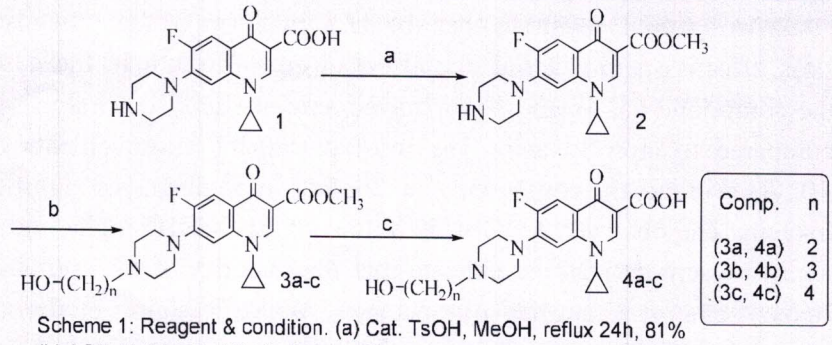
**Experiments Procedure:**

General: Reagent and solvent were purchased from Sigma-Aldrich Korea and used without further purification. Flash column chromatography was carried out over silica gel (60 (0.040-0.063 mm) Merck KGaA). Measurement of mass spectra (MS) and high resolution MS (HRMS) were performed with JEOL JMS SX-102A mass spectrometer. Nuclear magnetic resonance (NMR) spectra were measured DEAL (600MHz) spectrometer. The chemical shift are expressed in part per million (δ value) relative to residual solvent such as chloroform (δ = 7.26) as an internal standard.

The high performance liquid chromatography (HPLC) was carried out using WinScan instrument with Chemquiest software. The radio HPLC system was a BioScan instrument equipped with a γ detector (BioScan flow count). A semipreparative column Phenomenex Luna C18 column (50 X 4.6 mm, 3 μm; flow rate 4mL/min.) was used for the final purification of the compound [$^{18}$F]6. The following mobile phase system were used both for analytical and preparative HPLC. Solvent A, 10mmol phosphoric acid (88%); Solvent B Ethanol (12%) at 4mL/min. The desired peak was elevated at 16 min. [$^{18}$F]Fluoride was produced by cyclotron (Scanditronix MC40) using the 18O (p,n)$^{18}$F nuclear reaction with 19 MeV proton irradiation of an enriched [$^{18}$O]$H_2O$ target.

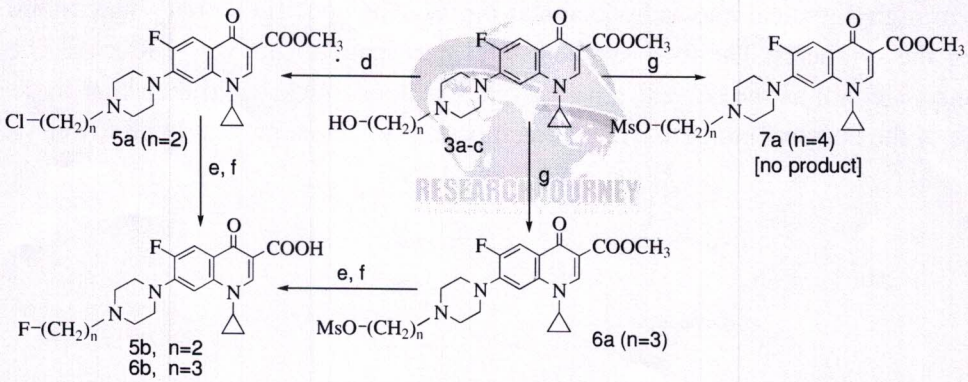
Biochemical studies: DNA supercoiling activity was assayed with relaxed pHOT-1 DNA as a substrate (TopoGEN, Inc., FL, and USA) according to the manufacturer's protocol. The standard reaction mixture (20 μL) contained 35 mM Tris-Cl, pH 7.5, 24 mM KCl, 4 mM MgCl2, 2 mM dithiothreitol, 1.8 mM spermidine, 1 mM ATP, 6.5% glycerol, 0.1 mg/mL BSA, 167 mg/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%.

Results 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.




Scheme 1: Reagent & condition. (a) Cat. TsOH, MeOH, reflux 24h, 81% (b) ACN, $K_2CO_3$, Br-$(CH_2)_n$-OH, 100 °C, 16 h, (c) LiOH, MeOH:$H_2O$ (4:1), AcOH, RT, 16 h.

The derivatives 4a-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. In the case of fluoroalkyne derivatives of ciprofloxacin, it was prepared by the conversion of alcohol (compounds 3a and 3b) to chloroethyl and methaneesulfonylpropyl derivatives, which was nucleophilc fluorinated using protic solvent, followed by hydrolysis using LiOH to give compounds 5a and 5b as shown in scheme 2.



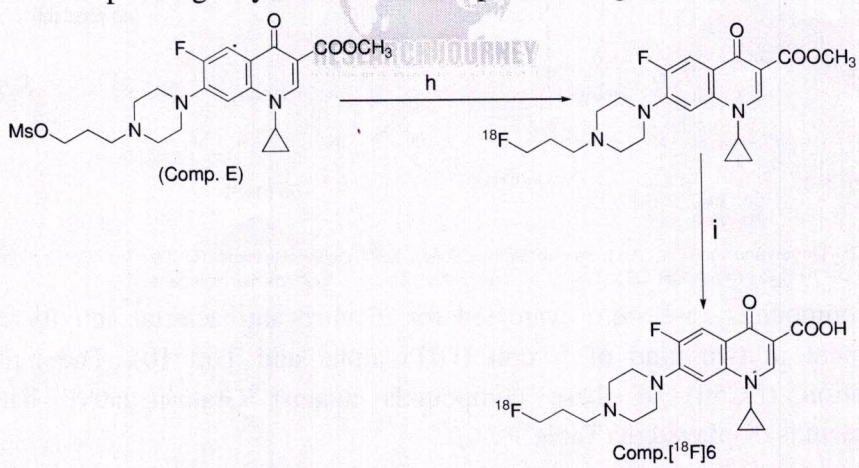

Scheme 2: Reagen and condition. (d) p-toluenesulfonylchloride, $Et_3N$, dichloromethane, RT, 20 h (e) TBAF hydride,t-amyl alcohol 80 °C, 6 h, (f) LiOH, MeOH:$H_2O$ (4:1), AcOH, RT, 16 h, (g) methanesulfonylchloride, $Et_3N$, dichloromethane, RT, 2 h.

Compounds 3a-e were evaluated for in vitro antibacterial activity using optical density measurement in two kind of E coli (DH5 alpha and Top 10). The half maximal effective concentration (EC50) of these compounds against Gram-negative bacteria compared to ciprofloxacin is displayed in (Table 1).

| TABLE 1 | LOG $EC_{50}$ ng/mL) | | TABLE 2 | Compound | LOG $EC_{50}$ ng/mL) |
|---|---|---|---|---|---|
| Compound | DH5 alpha | Top 10 | | Ciprofloxacin | 3.009e-006 |
| Ciprofloxacin | -7.346 | -8.594 | | 4a | 1.21e-005 |
| 4a | -6.780 | -9.189 | | 4b | 1.655e-005 |
| 4b | -6.637 | -8.830 | | 4c | 9.163e-006 |
| 4c | -6.408 | 8.692 | | | |
| 5b | -6.273 | -7.301 | | | |
| 6b | -6.607 | -8.936 | | | |

The data in Table 1 illustrate that the LOGEC50 values of N-substituted ciprofloxacin derivatives less effective antibacterial activity than ciprofloxacin in the case of the Ecoli DH5 alpha. But the compound 4a, 4b and 6b 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 LOGEC50 values of Ecoli DH5 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 (4a, 5b and 6b) for inhibition of the enzymes that are targeted by the ciprofloxacin. (Table 2). The observed data show that N-substituted ciprofloxacin compounds should be weaker DNA gyrase compare to ciprofloxacin. The measured EC50 values of 6b displayed far greater activities than compound 4a and 5b. It is of the note that the LOGEC50 values of compound 6b 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.

Radiosynthesis of [$^{18}$F]6. [$^{18}$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 3). 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 radiolbeling 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.




Reagent and condition. (h) [$^{18}$F] fluoride, TBABA, t-amyl alcohol, 100 °C, (i) LiOH, MeOH/$H_2O$ (4:1), AcOH, 100 °C.

The overall decay-corrected radiochemical yield was approximately 40%. Specific activity at the end of synthesis was calculated by relating radioactivity to the mass associated with the UV absorbance (215 nm) peak of cold compound. Specific radioactivity of [$^{18}$F]**6** (149 GBq/μmol) was obtained on an HPLC column.

**Conclusion:**

We successfully synthesized N-substituted Ciprofloxacin compound and identified their antibiotic activity with Ciprofloxacin and we developed a sensitive $^{18}$F- rediolabelded compound

[$^{18}$F]6 with high radiochemical yield and a specific activity, which suitable as a bacterial-specific infection imaging tracer for future PET imaging study.

**References.**

1. Yoshikazu Asahina, Każuhiko Iwase, Fujio Iinuma, Masaki Hosaka, and Takayoshi Ishizaki, J. Med. Chem. 2005, 48, 3194-3202
2. Alireza Foroumadi, Saeed Emami, Massood Mehni, Mohammad Hassan Moshafi and Abbas Shafiee, Bioorganic & Medicinal Chemistry Letter 15 (2005) 4536-4539.
3. Alireza Foroumadi, Mehdi Oboudiat, Saeed Emami, Alireza Karimollah, Lotfollah Saghaee, Mohammad Hassan Moshafi and Abbas Shafiee, Bioorganic & Medicinal Chemistry Letter 14 (2006) 3421-3427.
4. Yun Chai, Zhi-Long Wan, Bo Wang, Hui-Yuan Guo, Ming-Liang Liu, European Journal Chemistry 44 (2009) 4063-4069.
5. Varvara Pokrovskaya, Valery Belakhov, Mariana Hainrichson, Sima Yaron, and Timor Baasov. J. Med. Chem. 2009, 52, 2243-2254
6. D. Bouzard, P. Di Cesare, M. Essiz, J. P. Jacquet, J. R. Kiechel, P. Remuzon, A. Weber, T. Oki, M. Masuyoshi, R. E. Kessler, J Fung-Tomc, and Desiderio. J. Med. Chem. 1990, 33, 1344-1352.
7. Zrinka Banic Tomisic, Nedjeljko Kulundzic, Mirjana Bukvic Krajacic, Aleksandar Visnjevac, Biserka Kojic-Prodic. Journal of Molecular Structure 611 (2002) 73-81.
8. Brijesh Kumar Srivastava, Manish Solanki, Bhupendra Mishra, Rina Soni, Sanjaya Jayadev, Darshan Valani, Mukul Jain, and Pankaj R. Patel. Bioorganic & Medicinal Chemistry Letter 17 (2007) 1924-1929.
9. Alireza Foroumadi, Shahla Mansouri, Zahra Kiani, Afsaneh Rahmani. European Journal Chemistry 38 (2003) 851-854.
10. Martin Brunner, Oliver Langer, Georg Dobrozemsky, Ulrich Muller, Markus Zeitlinger, Markus Mitterhauser, Wolfgang Wadsak, Robert Dudczak, Kurt Kletter, and Markus Muller. Antimicrobial Agents and Chemotherapy, Oct. 2004, p. 3850-3857.
11. Rien H. Siaens, Msc; Huub J. Rennen, PhD; Boerman, PhD; Rudi Dierckx, MD; and Guido Slegers, PhD. J Nucl Med 2004; 45: 2088-2094
12. Sang Ju Lee, Seung Ju Oh, Dae Yoon Chi, Byoung Se Lee, Jin Sook Ryu, and Dae Hyuk Moon. J. Label Compd. Radiopharm 2008, 51 80-82
13. Nadezhda German, Peng Wei, Glenn W Kaatz, Robert J. Kerns. European Journal of Medicinal Chemistry 43 (2008) 2453-2463.
14. Dharmarajan Sriram, Perumal Yogeeswari, Jafar Sadik Basha, Deshpande R. Radha and Valakunja Nagaraja. Bioorganic & Medicinal Chemistry 13 (2005) 5774-5778
15. Zhenfa Zhang, Aizhen Yu and Weicheng Zhou. Bioorganic & Medicinal Chemistry 15 (2007) 7274-7280.
16. Seed Emami, Alireza Foroumadi, Nasrin Samadi, Mohammad A. Faramarzi, and Saeed Rajabalian. Arch. Pharm. Chem. Life Sci. 2009, 342, 405-411.
17. Dong Zhou, Hsiaoju Lee, Justin M. Rothfuss, Delphine L. Chen, Datta E. Ponde, Michael J. Welch, and Robert H. Mach. J. Med. Chem. 26 March 2009