Research Article

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DESIGN, SYNTHESIS, ANTI-VIRAL ACTIVITY AND CYTOTOXICITY STUDIES OF SOME NOVEL N-SUBSTITUTED PIPERAZINYL FLUOROQUINOLONE DERIVATIVES

[*,1]Neetha Thankachen, [1]Periyasamy Selvam,[1]Christophe Pannecouque, [2]E De Clercq

[*1]Devaki Amma Memorial College of Pharmacy, Chelembra, Malappuram, Kerala, India – 673 634.

[2] Rega Institute for Medical Research, Kotholieke University, Leuven, Minderbroedersstraat, B-3000 Leuven, Belgium.

Abstract

A series of novel N-substituted piperazinyl fluoroquinolones were synthesized and screened for their antiviral activity and cytotoxicity. Twenty two derivatives of known fluoroquinolones (Ciprofloxacin, Norfloxacin, Sparfloxacin and Gatifloxacin) were synthesized by modifying the N^4-hydrogen of piperazine in fluoroquinolone with Mannich reaction. The purity of the compounds was ascertained by consistency in the R_f value as well as melting point determination and were characterized by means of their spectral analysis (IR and ^{1}H NMR). The anti-HIV activities of the derivatives were screened against replication of HIV-1(III B) and HIV-2(ROD) in MT-4 cells. The synthesized compounds were tested for antiviral activity against HeLa cells (VSV and RSV) HEL cells (HSV-1 and HSV-2) CRFK cells (Feline Corona and Feline Herpes Virus) and Vero cells (Para influenza-3, Reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus). Among the compounds, compound SF-2A4PT and GF-2A4PT exhibited anti-viral activity against Vesicular Stomatitis virus in HeLa cells at the concentration of 7µg/ml and 12µg/ml respectively, where as their cytotoxicity was found to be more than 100µg/ml. Compound SF-2A4PT also inhibit the replication of Respiratory Syncytial Virus (RSV) at the concentration of 45µg/ml and its cytotoxicity was found to be more than 100µg/ml. *In–vitro* cytotoxicity studies of the synthesized compounds were determined by using MTT assay in Human liver cancer cells (Hep G2 cells). All the tested compounds exhibited significant cytotoxicity. Hence these merits further investigation to screen its anti cancer activity using *in-vitro* and *in-vivo* models.

Keywords: Mannich reaction, Fluoroquinolones, MT-4 Cells, HeLa cells, HEL Cells, Vero cells, MTT assay.

Introduction

Quinolones are considered as a big family of multi-faceted drugs; their chemical synthesis is flexible and can be easily adapted to prepare new congeners with rationally devised structures. Quinolone derivatives have been shown to inhibit HIV-1 replication in do novo- and chronically infected cells[1]. A few works are available in the literature for Fluoroquinolone derivatives with different substitutions. SAR studies revealed that very fine changes in the main skeleton, as well as the fusion of rings will substantially affect the

Author for Correspondence:
Neetha Thankachen,
Devaki Amma Memorial College of Pharmacy,
Chelembra, Malappuram, Kerala, India – 673 634.
Email: neethathankachen@gmail.com

pharmacological activity profiles of these compounds. Among the various fluoroquinolones reported, N-1 substituted fluoroquinolones and N-substituted piperazinyl fluoroquinolones exhibit interesting pharmacological activities. N-substituted piperazinyl fluoroquinolones are considered a central scaffold to build chemical libraries with promising bioactivity potential and have been reported to display biological activities like antibacterial[2,3], antifungal[4], anticonvulsant, anti-HIV[5,6], antitubercular[7], antiplatelet, anti-viral[8], and anticancer[9,10] activities.

In spite of a large number of fluoroquinolones that have been synthesised and studied for various pharmacological activities, the anti viral and anti cancer activities of fluoroquinolones are selectively less explored. Studies revealed that antibacterial fluoroquinolones can exhibit antiviral activity as well. In the SAR study, the aryl substituents on the piperazine nitrogen were found to play an important role for the anti-HIV-I activity. Recently, newer arylpiperazinyl fluoroquinolones were synthesized and studied for their anti- HIV activity[11-17]. The present work deals with the synthesis of Mannich bases of fluoroquinolones with different heterocyclic primary amines, formaldehyde and to study their important pharmacological properties (antiviral activities and cytotoxicity) that are selectively less explored.

Methods

The purity of the newly synthesized compounds were checked by Thin Layer Chromatography (TLC) using silica gel-G as stationary phase and the spot was visualized by Iodine vapour. The melting point of synthesized compounds was determined by open ended capillary tube method on a Thomas Hoover melting point apparatus and the values are found and uncorrected. The structures of the synthesized compounds were elucidated by Fourier Transformed-Infra Red (FT-IR) spectrophotometer by using KBr pellet. IR values are measured in cm^{-1} in the range of 4000 to 400 cm^{-1}. The measurements were conducted on Bruker ATR, ZnSe - FTIR (Al-Shifa College of Pharmacy); JASCO 4100 (Calicut university) ; Perkin Elmer Spectrum AXI (CSIR, Chennai). Proton NMR of the synthesised compounds was recorded on solutions in dimethyl sulfoxide DMSO-d_6 on Bruker Ultra Shield DPX 400 at IISe Bengaluru. Tetra Methyl Silane (TMS) was used as internal standard. Chemical shifts were reported in δ (ppm).

General Procedure For Synthesis

0.01 mole of active hydrogen compound (2-amino - 4-phenyl thiazole, Sulphaguanidine, Sulphanilic acid, 2-amino benzthiazole, 2-amino pyridine, Trimethoprim, Lamotrigine, p-amino acetophenone, p-nitro aniline) was dissolved in 5ml of methanol in a 100ml beaker. The P^H of reaction mixture was adjusted to 3.5-4.0 with concentrated hydrochloric acid. The beaker was kept in perfect ice cold condition on a magnetic stirrer. 1.0 ml of formaldehyde solution 37% w/v was added slowly with constant stirring. This was followed by addition of equimolar concentration of fluoroquinolone (0.01mol) (Ciprofloxacin, Norfloxacin, Sparfloxacin, Gatifloxacin) in small installments with constant stirring at efficient ice cooling. The reaction mixture was cooled well and stirred for 3 hours on a magnetic stirrer.

After 3 hours the reaction mixture was transferred into a 250 ml round bottom flask and placed on a water bath for refluxing. The reflux time varied with different active hydrogen compound used. The reaction mixture was kept at 0^0C over night in refrigerator. The Mannich product thus obtained was filtered and purified by washing twice with diethyl ether and then with acetone and then recrystallized from ethanol. (Scheme1, Table 01 & 02). The physical constants of the synthesised compounds are presented in table 03.

Spectral Data

CF-2A4PT: IR (KBr) NH-3520, OH-3357, C=O-1714, C=N-1624, C=C-1495, Ar-H-696, CF-557. PMR (DMSO-d_6) 9.34-(s, 1H, NH), 8.66-(s, 1H ,COOH), 7.95-(d, 1H, quinine CH), 7.45-7.65-(m, 6H, Ar-H), 4.49-(s, 2H, N-CH_2-N), 3.82-3.86-(m, 8H, Piperazinyl), 3.55-(m, 4H, Piperazinyl), 1.12-1.19-(m, 3H, Cyclopropyl). **CF-SG:** IR (KBr) NH-3410, C=N-1589, C=C-1540, SO_2-1I22, Ar-H-750, CF-553. PMR (DMSO-d_6) 8.8-(s, 1H, NH), 8.6-(s, 1H, COOH), 7.5-8.0-(m, Cyclopropyl). **CF-SA:** IR (KBr) NH-3535, C=O-1714, C=C-1642, SO_2-1178, Ar-H-696, CF-589. **CF-2ABT:** IR (KBr) NH-3520, C=O-1720, C=N-1624, C-Nstr.-1482, Ar-H-696, CF-574. **CF-TRM:** IR (KBr) NH-3534, C=0-1720, C=N-1625, C=C-1589, C-O-C-1124, Ar-H-690, CF-535. PMR (DMSO-d_6) 9.53-(s, b, 2H, NH_2), 8.673-(s, 1H, COOH), 7.96-(d, 1H, Quinone -CH), 7.57-7.75-

(m, 2H, Ar-H), 7.45-(s, 1H, Pyrimidinyl), 6.613-(s, 2H, Benzyl), 3.87-(s, 2H, N-CH_2-N), 3.62-(s, 2H, Piperazinyl), 3.58-(m, 4H, Piperazinyl), 3.42-(s, 9H, 3×OCH_3), 1.16-1.2-(m, 3H, Cyclopropyl). **CF-LTG:** IR (KBr) NH-3520, C=O-1724, C=N-1624, CNstr.-1464, Ar-H-624, CF-571. PMR (DMSO-d_6) 9.6-(b, 2H, NH_2-Pyrimidinyl), 9.182-(s, IH, CH-NH), 8.66-(s, 1H, COOH), 7.95-(d, 1H, Quinone-CH), 7.83-(s, IH, Pyrimidinyl), 7.15-7.5-(m, 5H, Ar-H), 3.87-(s, 2H, N-CH_2-N), 3.45-(b, 4H, Piperazinyl), 1.15-1.19-(m, 3H, Cyclopropyl). **CF-PAA:** IR (KBr) NH-3525, C=O-1714, C=C-1614, CNstr.-1464, Ar-H-642, CF-571. **CF-PNA:** IR (KBr) NH-3510, C=O-1732, C=C-1642, CNstr.-1492, Ar-H-714, CF-571. **NF-2A4PT:** IR (KBr) NH-3428, OH-2939, C-Acyl-2723, C=O-1722, C=N-1626, C=C-1475, Ar-H-748, CF-670. **NF-SG:** IR (KBr) NH-3419, OH-2935, C-Acyl-2724, C=O-1722, C=N-1627, C=C-1477, SO_2-1133, Ar-H-748, CF-567. **NF-SA:** IR (KBr) NH-3404, OH-2931, C-Acyl-2723, C=O-1719, C=N-1627, C=C-1480, SO_2-1165, Ar-H-685, CF-567. PMR (DMSO-d_6) 9.16-(b, 1H, NH-CH_2), 8.96-(s, 1H, COOH), 7.96-(d, 1H, CH), 7.1-7.8-(m, 6H, Ar-H), 5.3-(s, 2H, N-CH_2-N), 4.6-(q, 2H, CH_2), 3.54-(s, 4H, Piperazinyl), 1.4-(t, 3H, CH_3). **NF-TRM:** IR (KBr) NH-3408, OH-2934, C-Acyl-2408, C=O-1721, C=N-1627, C=C-1475, C-O-C-1093, Ar-H-695, CF-566. **NF-LTG:** IR (KBr) NH-3308, OH-3095, C-Acyl-2934, C=O-1717, C=N-1633, C=C-1483, Ar-H-749, CF-571. **NF-PAA:** IR (KBr) NH-3423, OH-2936, C-Acyl-2724, C=O-1721, C=N-1626, C=C-1476, Ar-H-748, CF-567. **NF-PNA:** IR (KBr) NH-3407, OH-2927, C-Acyl-2720, C=O-1717, C=N-1627, C=C-1482, Ar-H-748, CF-568. **SF-2A4PT:** IR (KBr) NH-3412, OH-3271, C=O-1709, C=N-1634, C=C-1564, Ar-H-658,708, CF-549. PMR (DMSO-d_6) 9.34-(b, 1H, NH), 8.53-(s, 1H, COOH), 7.39-7.69-(m, 5H, Ar-H), 4.015-(s, 2H, N-CH_2-N), 3.17-3.23-(s, 4H, Piperazinyl), 1.26-(s, 6H, 2 X CH_3), 1.22-(s, 3H, CH_3), 1.11-1.13-(m, 3H, Cyclopropyl). **SF-SG:** IR (KBr) NH-3412, C=O-1708, C=N-1641, C=C-1546, Ar-H-673, CF-549. PMR (DMSO-d_6) 9.22-(b, 1H, NH-guanidine), 8.71-(b, 1H, NH-CH_2), 8.53-(s, 1H, COOH)7.1-7.4-(m, 4H, Ar-H), 4.016-(s, 2H, CH_2-NH), 3.39-3.53-(m, 4H, Piperazinyl), 3.23-(m, 2H, Piperazinyl), 1.25-(s, 3H, CH_3), 1.24-(s, 3H, CH_3), 1.10-(s, 3H, CH-Cyclopropyl). **SF-SA:** IR (KBr) C=O-1708, C=N-1641, C=C-1546, SO_2-1180, Ar-H-673, CF-548. **SF-TRM:** IR (KBr) NH_2-3450, C=O-1708, C=N-1639, C=C-1560, C-O-C-1128, Ar-H-639, CF-522. **SF-2AP:** IR (KBr) NH-3565, C=O-1707, C=N-1644, C=C-1514, Ar-H-664, CF-538. PMR (DMSO-d_6) 8.66-(b, 1H, NH-CH_2), 8.53-(s, 1H, COOH), 7.2-7.4-(m, 4H, Ar-H), 4.01-(s, 2H, CH_2-NH), 3.39-3.5-(m, 4H, Piperazinyl), 3.18-3.21-(m, 2H, Piperazinyl), 1.25-(d, 6H, 2×CH_3), 1.11-(m, 3H, Cyclopropyl). **GF-2A4PT:** IR (KBr) C=O-1707, C=N-1645, C=C-1547, C-O-C-1083, Ar-H-677, CF-537. **GF-SG:** IR (KBr) C=O-1714, C=N-1654, C=C-1540, C-O-C-1092, Ar-H-664, CF-541.

Anti-HIV activity

The synthesised compounds were screened for anti-HIV activity against HIV-1 (III B) and HIV-2 (ROD) in acutely infected MT-4 cells[18,19,20] and cytotoxicity of compounds were also tested with mock-infected MT-4 cells[21] (Adult-C-type T-Leukemia cells). The MT-4 cells were grown in RPM1-1640 DM ("Dutch Modification") medium (Flow Laboratories, Irvine, Scotland), supplemented with 10% (v/v) heat-inactivated fetal calf serum (FCS) and 20µg/ml gentamycin (E. Merck, Darmstadt, F.R.G.). The cells were maintained at 37°C in a humidified atmosphere of 5% CO_2 in air. Inhibitory effect of test compounds on HIV replications was determined by inhibition of virus- induced cytopathic effect in MT-4 cells and was estimated by MTT assay. The MTT assay was based on the reduction of the yellow colored 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) by mitochondrial dehydrogenase of metabolically active cells to a blue formazan which can be measured spectrophotometrically. The absorbances were read at two wavelengths (540, 690 nm). The absorbance measured at 690 nm was automatically subtracted from the absorbance at 540 nm, so as to eliminate the effects of non-specific absorption. A blank was also carried out directly on the micro titer plates with all reagents except the MT-4 cells. IC_{50} - the effective concentration of compound, achieving 50% protection of MT-4 cells against the cytopathic effect of HIV was calculated. The 50% cytotoxic concentration (CC_{50}) was also calculated which was defined as the concentration of the compound that reduced the absorbance (OD_{540}) of the Mock-infected control sample by 50%. The maximum percentage protection achieved by the compounds in HIV-infected cells was calculated by the following formula:

$$\frac{(ODT)HIV-(ODC)HIV}{(ODT)mock-(ODC)mock}$$

Cytotoxicity of test compounds against mock-infected MT-4 cells was also assessed by the MTT method. Compounds were evaluated for their inhibitory effect on the replication of HIV in human MT-4 cells. The anti-HIV and cytotoxicity data are presented in table 04.

Antiviral activity

Anti-viral activity and cytotoxicity of the synthesised compounds were determined by an in-vitro cell culture technique[22]. The anti-viral assays were based on inhibition of virus-induced cytopathicity of HeLa cells (VSV and RSV), HEL cells (HSV-1 and HSV-2), CRFK cells (Feline Corona and Feline Herpes Virus), Vero cells (Parainfluenza-3, Reovirus-1, Sindbis virus, Coxsackie virus, B4 and Punta Toro Virus). Briefly, confluent cell culture in 96-well microtiter plates were inoculated with 100 $CCID_{50}$ of virus, one $CCID_{50}$ being the virus dose required to infect 50% of the cell cultures. After 1 hr. of virus adsorption period, residual virus was removed, and the cell cultures were incubated in the presence of varying concentrations (400, 200 and 100 µg/ml) of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were treated with the test compounds. The anti viral activity of the compounds were expressed as the concentration required to inhibit viral cytopathogenicity by 50% (EC_{50}). Cytotoxicity of the compounds was determined as the minimum concentration required to cause microscopically detectable alteration of normal cell morphology. The anti viral and cytotoxicity data are presented in tables 05 to 08.

In vitro cytotoxicity studies

In–vitro cytotoxicity studies of the synthesised compounds were determined by MTT assay in Human liver cancer cells[23] (Hep G2 cells). This assay is based on the assumption that dead cells or their products do not reduce tetrazolium salt into a blue coloured product-formazan. The monolayer cell culture was trypsinized and the cell count was adjusted to $1.0x10^5$ cells/ml using medium containing 10% new born calf serum. To each well of the 96 well microtitre plate, 0.1ml of the diluted cell suspension (approximately 10,000 cells) was added. After 24 hours, when a partial monolayer was formed, the supernatant was flicked off, washed the monolayer once and 100µl of different drug concentrations was added to the cells in microtitre plates. The plates were then incubated at 37°C for 3 days in 5% CO_2 atmosphere, and microscopic examination was carried out and observations recorded every 24 hours. After 72 hours, the drug solutions in the wells were discarded and 50µl of MTT in MEM was added to each well. The plates were gently shaken and incubated for 3 hours at 37°C in 5% CO_2 atmosphere. The supernatant was removed and 50µl of propanol was added and the plates were gently shaken to solubilize the formed formazan. The absorbance was measured using a microplate reader at a wavelength of 540nm. The percentage growth inhibition was calculated using the formula below:

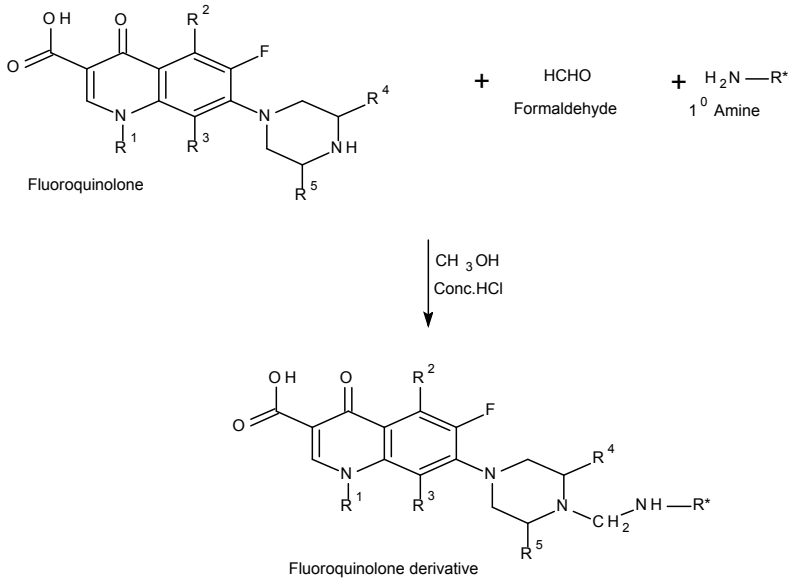
$$\%\ Growth\ Inhibition = 100 - \frac{Mean\ OD\ of\ Individual\ Test\ Group}{Mean\ OD\ of\ control\ group}\ X\ 100$$

50% cytotoxic concentration (CTC_{50}) of the compounds was then calculated from the plot of concentration v/s % growth inhibition. The cytotoxicity data are presented in table 09.

Results and Discussion

This research work described the synthesis of N-Mannich bases of fluoroquinolones and *in-vitro* evaluation of their cytotoxicity and anti viral activities. Four series of N-Mannich bases of fluoroquinolones have been prepared through Mannich reaction. The formation of mannich base can be confirmed from the appearance of new ^1H NMR signal in the range of δ 3.8-5.5 due to 2H; CH_2 of methylene linkage formed during mannich base formation. The anti-HIV activities of the derivatives were screened against replication of HIV-1(III B) and HIV-2(ROD) in MT-4 cells through MTT- assay. Cytotoxicity of test compounds against mock-infected MT-4 cells was also assessed by the MTT method. All the compounds displayed cytostatic properties in T-lymphocyte MT-4 cells. Among the derivatives tested, compounds CF-APH (CC_{50}:2.39±0.43 µg/ml) and CF-PME-APH (CC_{50}:3.47±2.01 µg/ml) was found to be more toxic in this series. The

synthesised compounds were tested for anti-viral activity against HeLa cells (VSV and RSV) HEL cells (HSV-1 and HSV-2), CRFK cells (Feline Corona and Feline Herpes Virus) and Vero cells (Para influenza-3, Reovirus-1, Sindbis virus, Coxsackie virus B4 and Punta Toro virus). Among the derivatives tested, compound SF-2A4PT and GF-2A4PT exhibited anti-viral activity against Vesicular Stomatitis virus in HeLa cells at the concentration of 7µg/ml and 12µg/ml, respectively, and their cytotoxicity was found to be more than 100µg/ml. Compound SF-2A4PT also inhibit the replication of Respiratory Syncytial Virus (RSV) at the concentration of 45µg/ml. Hence these broad-spectrum antiviral properties should be further explored in order to assess the potential of fluoroquinolone derivatives in the treatment of viral infection. *In–vitro* cytotoxicity studies of the synthesised compounds were determined by using MTT assay in Human liver cancer cells (Hep G2 cells). All the tested compounds exhibited significant cytotoxicity. Among the compounds, SF-SG (CTC $_{50}$ = 164.93±4.11µg/ml) was found to be more toxic, where as the CTC $_{50}$ of standard cisplatin was found to be 11.09 ± 0.59 µg/ml. Hence SF-SG merits further investigation to screen its anti cancer activity using *in-vitro* and *in-vivo* models. From the results it has been found that introduction of heterocyclic primary amine at N-4 hydrogen of piperazine in fluoroquinolone through mannich reaction will substantially affect the antiviral activity and cytotoxicity of fluoroquinolones and many future drugs may be produced by the structural diversity of Mannich bases of fluoroquinolones.

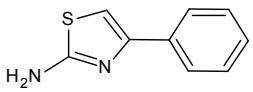
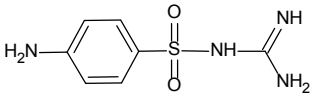
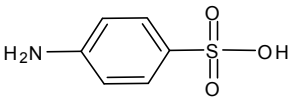
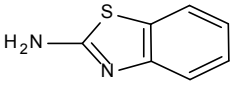
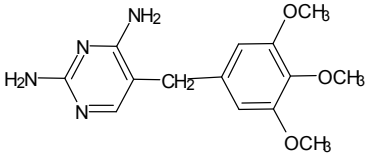
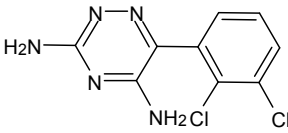
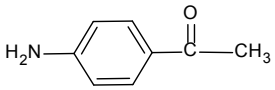
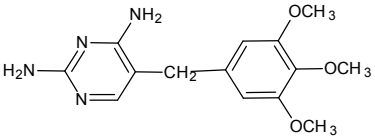
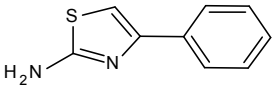
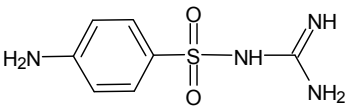
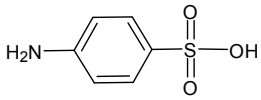
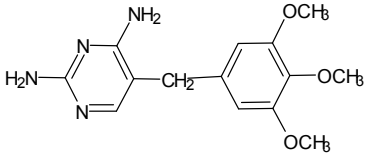
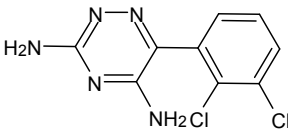
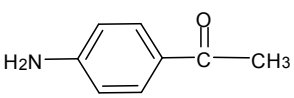


Scheme 1: Synthetic Protocol of studied Compounds

Table No. 01: List of synthesized fluoroquinolone derivatives

Compound	R^1	R^2	R^3	R^4	R^5
Ciprofloxacin		H	H	H	H
Norfloxacin	C_2H_5	H	H	H	H
Sparfloxacin		NH_2	F	CH_3	CH_3
Gatifloxacin		H	OCH_3	CH_3	H

Table No. 02: List of studied compounds

Compound Code	H_2N - R*	IUPAC Name
CF-2A4PT		1-Cyclopropyl-6-fluoro-4-oxo-7-{4-[(4-phenyl-thiazol-2-ylamino)-methyl]-piperazin-1-yl}-1,4-dihydro-quinoline-3-carboxylic acid.
CF-SG		1-Cyclopropyl-7-(4-{[4-(diaminomethylene-sulfamoyl)-phenylamino]-methyl}-piperazin-1-yl)-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
CF-SA		1-Cyclopropyl-6-fluoro-4-oxo-7-{4-[(4-sulfo-phenylamino)-methyl]-piperazin-1-yl}-1,4-dihydro-quinoline-3-carboxylic acid.
CF-2ABT		7-[4-(Benzothiazol-2-ylaminomethyl)-piperazin-1-yl]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
CF-TRM		7-(4-{[4-Amino-5-(3,4,5-trimethoxy-benzyl)-pyrimidin-2-ylamino]-methyl}-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
CF-LTG		7-(4-{[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-ylamino]-methyl}-piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
CF-PAA		7-{4-[(4-Acetyl-phenylamino)-methyl]-piperazin-1-yl}-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
CF-PNA		1-Cyclopropyl-6-fluoro-7-{4-[(4-nitro-phenylamino)-methyl]-piperazin-1-yl}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
NF-2A4PT		1-Ethyl-6-fluoro-4-oxo-7-{4-[(4-phenyl-thiazol-2-ylamino)-methyl]-piperazin-1-yl}-1,4-dihydro-quinoline-3-carboxylic acid.
NF-SG		7-(4-{[4-(Diaminomethylene-sulfamoyl)-phenylamino]-methyl}-piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
NF-SA		1-Ethyl-6-fluoro-4-oxo-7-{4-[(4-sulfo-phenylamino)-methyl]-piperazin-1-yl}-1,4-dihydro-quinoline-3-carboxylic acid.
NF-TRM		7-(4-{[4-Amino-5-(3,4,5-trimethoxy-benzyl)-pyrimidin-2-ylamino]-methyl}-piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
NF-LTG		7-(4-{[5-Amino-6-(2,3-dichloro-phenyl)-[1,2,4]triazin-3-ylamino]-methyl}-piperazin-1-yl)-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
NF-PAA		7-{4-[(4-Acetyl-phenylamino)-methyl]-piperazin-1-yl}-1-ethyl-6-fluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.

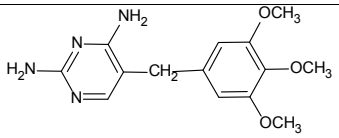
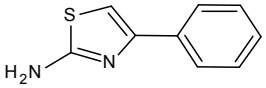
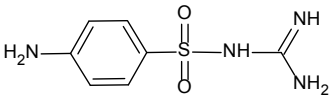
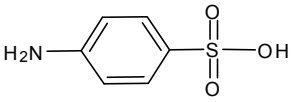
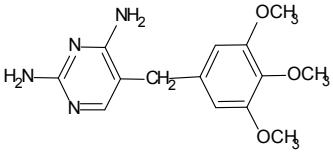
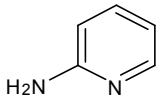
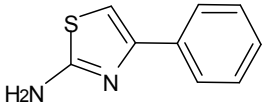
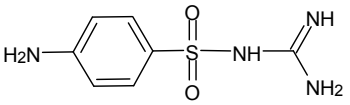
Compound Code	H_2N - R*	IUPAC Name
NF-PNA		1-Ethyl-6-fluoro-7-{4-[(4-nitro-phenylamino)-methyl]-piperazin-1-yl}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
SF-2A4PT		5-Amino-1-cyclopropyl-7-{3,5-dimethyl-4-[(4-phenyl-thiazol-2-ylamino)-methyl]-piperazin-1-yl}-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
SF-SG		5-Amino-1-cyclopropyl-7-(4-{[4-(diaminomethylene-sulfamoyl)-phenylamino]-methyl}-3,5-dimethyl-piperazin-1-yl)-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
SF-SA		5-Amino-1-cyclopropyl-7-{3,5-dimethyl-4-[(4-sulfo-phenylamino)-methyl]-piperazin-1-yl}-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
SF-TRM		5-Amino-7-(4-{[4-amino-5-(3,4,5-trimethoxy-benzyl)-pyrimidin-2-ylamino]-methyl}-3,5-dimethyl-piperazin-1-yl)-1-cyclopropyl-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
SF-2AP		5-Amino-1-cyclopropyl-7-[3,5-dimethyl-4-(pyridin-2-ylaminomethyl)-piperazin-1-yl]-6,8-difluoro-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
GF-2A4PT		1-Cyclopropyl-6-fluoro-8-methoxy-7-{3-methyl-4-[(4-phenyl-thiazol-2-ylamino)-methyl]-piperazin-1-yl}-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.
GF-SG		1-Cyclopropyl-7-(4-{[4-(diaminomethylene-sulfamoyl)-phenylamino]-methyl}-3-methyl-piperazin-1-yl)-6-fluoro-8-methoxy-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid.

Table No. 03: Physical Constant of Synthesized Compounds

Compound code	Molecular formula	M W	M p (°C)	R_f value	Percentage yield
CF-2A4PT	$C_{27}H_{26}FN_5O_3S$	519.59	220- 224	0.64	51.96
CF-SG	$C_{25}H_{28}FN_7O_5S$	557.60	244-248	0.66	55.6
CF-SA	$C_{24}H_{25}FN_4O_6S$	516.54	262-265	0.68	81.08
CF-2ABT	$C_{25}H_{24}FN_5O_3S$	493.55	258-264	0.72	68.96
CF-TRM	$C_{32}H_{36}FN_7O_6$	633.67	250-254	0.67	34.72
CF-LTG	$C_{27}H_{25}Cl_2FN_8O_3$	599.44	252-258	0.69	70.07
CF-PAA	$C_{26}H_{27}FN_4O_4$	478.52	226-230	0.54	62.7
CF-PNA	$C_{24}H_{24}FN_5O_5$	481.48	220-225	0.66	72.76
NF-2A4PT	$C_{26}H_{26}FN_5O_3S$	507.58	248-252	0.60	19.7
NF-SG	$C_{24}H_{28}FN_7O_5S$	545.59	258-260	0.62	75.09
NF-SA	$C_{23}H_{25}FN_4O_6S$	504.53	260-262	0.67	29.73
NF-TRM	$C_{31}H_{36}FNO_6$	621.66	260-265	0.70	64.44
NF-LTG	$C_{26}H_{25}Cl_{12}FN_8O_3$	587.43	255-260	0.74	37.48
NF-PAA	$C_{25}H_{27}FN_4O_4$	466.51	260-264	0.63	42.87
NF-PNA	$C_{23}H_{24}FN_5O_5$	469.47	220-224	0.78	72.42
SF-2A4PT	$C_{29}H_{30}F_2N_6O_3S$	580.65	274-278	0.65	34.44
SF-SG	$C_{27}H_{32}F_2N_8O5S$	618.66	262-265	0.64	50.11
SF-SA	$C_{26}H_{29}F_2N_5O_6S$	577.60	265-270	0.70	72.71
SF-TRM	$C_{34}H_{40}F_2N_8O_6$	694.73	258-262	0.66	66.22
SF-2AP	$C_{25}H_{28}F_2N_6O_3$	498.53	268-272	0.70	56.17
GF-2A4PT	$C_{29}H_{30}F_2N_6O_4S$	563.64	235-240	0.62	67.42
GF-SG	$C_{27}H_{32}FN_7O_6S$	601.65	240-242	0.67	60.87

Table No. 04: Anti-HIV activity and cytotoxicity of synthesised compounds in MT-4 cells.

Compound code	Strain	IC_{50}[a] (µg/ml)	CC_{50}[b] (µg/ml)	Max. Protection
2A4PT	IIIB	>70.30	70.30±3.25	3
	ROD	>70.30	70.30±3.25	3
2ABT	IIIB	>11.50	11.50±0.71	1
	ROD	>11.50	11.50±0.71	2
CF-2A4PT	IIIB	>34.75	34.75±16.93	1
	ROD	>34.75	34.75±16.93	4
CF-SG	IIIB	>61.93	61.93±3.01	3
	ROD	>61.93	61.93±3.01	5
NF-2A4PT	IIIB	>47.25	47.25±5.02	1
	ROD	>47.25	47.25±5.02	4
NF-SA	IIIB	>46.10	46.10±3.25	0
	ROD	>46.10	46.10±3.25	7
SF-2A4PT	IIIB	>66.33	66.33±5.18	4
	ROD	>66.33	66.33±5.18	7
SF-SG	IIIB	>56.05	56.05±12.23	2
	ROD	>56.05	56.05±12.23	9
GF-2A4PT	IIIB	>64.10	64.10±2.36	1
	ROD	>64.10	64.10±2.36	3
CF-2A4PT-OMe	IIIB	>39.85	39.85±9.33	2
	ROD	>39.85	39.85±9.33	4
CF-APH	IIIB	>2.39	2.39±0.43	0
	ROD	>2.39	2.39±0.43	3
CF-PAA	IIIB	>56.73	56.73±14.45	1
	ROD	>56.73	56.73±14.45	4
CF-PME-APH	IIIB	>3.47	3.47±2.01	1
	ROD	>3.47	3.47±2.01	3
CF-SA	IIIB	>59.88	59.88±14.08	7
	ROD	>59.88	59.88±14.08	4
NF-PME-APH	IIIB	>11.20	11.20±5.80	1
	ROD	>11.20	11.20±5.80	3
NF-PAA	IIIB	>46.0	46.00±5.07	0
	ROD	>46.0	46.00±5.07	14
SF-SA	IIIB	>53.85	53.85±6.61	0
	ROD	>53.85	53.85±6.61	5
DDN/AZT	IIIB	0.0022±0.0022	>25.00	68
Retrovir	ROD	0.0022±0.0004	>25.00	63

[a] Effective concentration of compound , achieving 50% protection of MT-4 cells against the cytopathic effect of HIV.
[b] 50% Cytotoxic concentration of compound, required to reduce the viability of mock infected MT-4 cells by 50%.
IIIB = HIV-1, ROD = HIV-2. All the values are SD of two independent experiment.

Table No. 05: Cytotoxicity and antiviral activity of compounds in HEL cell.

Compound	Minimum cytotoxic concentration[a] (µg/ml)	EC_{50}[b] (µg/ml)			
		Hepes simplex virus-1 (KOS)	Herpes simplex virus-2 (G)	Vaccinia virus	Herpes simplex virus-1 TK-KOS ACV^r
CF-2A4PT	>100	>20	>20	>20	>20
CF-SG	>100	>100	>100	>100	>100
NF-2A4PT	>100	>100	>100	>100	>100
SF-2A4PT	>100	>100	>100	>100	>100
GF-2A4PT	>100	>100	>100	>100	>100
Brivudin	>250	0.02	183	10	50
Cidofovir	>250	2	2	17	0.9
Acyclovir	>250	0.4	0.4	>250	50
Ganciclovir	>100	0.03	0.03	>100	10

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table No. 06: Anti-feline corona virus (FIPV) and anti-feline herpes virus activity and cytotoxicity in CRFK cell cultures.

Compound	CC_{50}[a] (µg/ml)	EC_{50}[b] (µg/ml)	
		Feline Corona Virus (FIPV)	Feline Herpes Virus
CF-2A4PT	>100	>100	>100
CF-SG	>100	>100	>100
NF-2A4PT	95.4	>20	>20
SF-2A4PT	>100	>100	>100
GF-2A4PT	>100	>100	>100
HHA	>100	35.5	11.1
UDA	>100	9.8	4.5
Ganciclovir (µM)	>100	>100	5.7

[a] 50% Cytotoxic concentration, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay.
[b] 50% Effective concentration , or concentration producing 50% inhibition of virus-induced cytopathic effect, as determined by measuring the cell viability with the colorimetric formazan-based MTS assay
CRFK cells: Crandell-Rees Feline Kidney cells.

Table No. 07: Cytotoxicity and antiviral activity of compounds in VERO cells.

Compound	Minimum cytotoxic concentration[a] (µg/ml)	EC_{50}[b] (µg/ml)			
		Para-inflluenza-3 virus	Reovirus-1	Sindbis virus	Punta Toro virus
CF-2A4PT	≥20	>20	>20	>20	>20
CF-SG	>100	>100	>100	>100	>100
NF-2A4PT	100	>20	>20	>20	>20
SF-2A4PT	100	>20	>20	>20	>20
GF-2A4PT	≥100	>100	>100	>100	>100
DS-5000	>100	>100	>100	>100	100
(S)-DHPA (µM)	>250	>250	>250	>250	>250
Ribavirin (µM)	>250	50	146	>250	112

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table No. 08a: Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compound	Minimum cytotoxic concentration[a] (µg/ml)	EC_{50}[b] (µg/ml)		
		Vecicular stomatitis virus	Coxsackie virus	Respiratory syncytial virus
CF-2A4PT	>100	>20	>20	>20
CF-SG	100	>100	>100	>100
NF-2A4PT	100	>20	>20	>20
SF-2A4PT	**>100**	**7**	**>100**	**45**
GF-2A4PT	**≥100**	**12**	**>20**	**>100**
DS-5000	>100	2	45	0.6
(S)-DHPA(µM)	>250	112	>250	>250
Ribavirin (µM)	>250	4	>250	2

Table No. 08b: Cytotoxicity and antiviral activity of compounds in HeLa cell cultures.

Compound	Minimum cytotoxic concentration[a] (µg/ml)	EC_{50}[b] (µg/ml)		
		Vesicular stomatitis virus	Coxsackie virus	Respiratory syncytial virus
SF-2A4PT	**>100**	**10**	**>100**	**45**
GF-2A4PT	**100**	**12**	**>20**	**>20**
DS-5000	>100	2	45	2
(S)-DHPA(µM)	>250	112	>250	>250
Ribavirin (µM)	>250	4	85	5

[a] Required to cause a microscopically detectable alteration of normal cell morphology.
[b] Required to reduce virus-induced cytopathogenicity by 50%.

Table No. 09: Determination of CTC_{50} by using MTT assay in HepG2 cells (human liver cancer) cell cultures.

Sl.No	Compound	CTC_{50}* in (µg/ml)
1.	SF-2A4PT	174.95 ± 3.28
2.	SF-SG	164.93 ± 4.11
3.	SF-2AP	177.93 ± 4.19
4.	CF-SG	205.25 ± 4.66
5.	CF-TRM	217.76 ± 3.53
6.	CF-2ABT	216.31 ± 5.61
7.	CF-LTG	203.20 ± 4.12
8.	NF-SA	259.03 ± 5.15
9.	Cis-platin (STD)	11.09 ± 0.59

*CTC_{50} = 50% cytotoxic concentration

* Average of six independent determinations, values are mean ± S.E.M.

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