***Original Article***

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SYNTHESIS, ANTI-HIV AND CYTOTOXICITY STUDIES OF SOME NOVEL

N-HETEROARYL METHYL PIPERAZINYL FLUOROQUINOLONE DERIVATIVES

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**Abstract**

A series of novel N- substituted piperzinyl fluoroquinolones were synthesized and screened antiviral activity. 29 compounds were synthesized through modifying the N4-hydrogen of piperazine in fluoroquinolones with mannich reactions. The structures of the synthetic compounds were characterized by means of their IR, 1H-NMR data. The anti-HIV activities of the new compounds were screened antiviral activity against replication of HIV-1(III B) in MT-4 cells among the compounds tested two compounds, PD-NDIN and PD-CFA have shown more toxic in these series. Compounds PD-CDIN and PDNDIN exhibited 27 and 10 percent maximum protection against replication of HIV-1 in MT-4 cells at subtoxic concentration.

**Key words:** Mannich base, Fluoroquinolones, HIV-1, MT-4 cells.

# Introduction

Quinolone derivatives have been shown to inhibit HIV-1 replication in do novo- and chronically infected cells1. Limited work is available in the literature for Fluoroquinolone derivatives with different substitutions. A new fluoroquinolone, K12, bearing o-methoxypheny

-piperazinyl group and a difluoromethoxyl group at positions 7 & 8, respectively, was reported to have strong and selective antiHIV-1 activity. The antiviral activity seemed to be related to an inhibitory effect at the transcriptional level. Two K12 analogues bearing a phenyl dehydropiperidinyl moiety at position 7 were effective at inhibiting HIV-1 long terminal repeat (LTR)- driven gene expression, as well as suppressing tumor necrosis factor alpha (TNF-) and interleukin 6 (IL-6) production in blood mononuclear cells, suggesting a mechanism of action mediated by inhibition of Tat functions2. Recently, newer synthesized arylpiperazinyl fluoroquinolones were studied for anti-HIV activity3,4,5.

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In view of this we have synthesized some novel Mannich bases fluoroquinolone derivatives and tested for their antiviral activity against the replication of HIV-1 (IIIB) in MT-4 cells and cytotoxicity of the compounds were also tested in uninfected MT-4 cells by MTT assay.

Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a Jasco-410 infrared spectrophotometer, 1H-NMR spectra were determined BRUKER AMX 400 MHZ with tetramethyl silane as an internal standard. The sample is dissolved in DMSO-d6 and the value is measured in  PPM.

**General procedure for synthesis** Equimolar(0.01mol)mixture of aldehyde(formaldehyde, benzaldehyde and p-dimethylaminobenzaldehyde) active hydrogen compounds(benzamide,benzimidazole, 2-mercaptobenzimidazole,benztriazole, phthalimide, 2-aminopyridine, sulfamethoxazole, sulfadiazine, sulfamaxozole, sulfanilic acid, indole, piperazine, sulfadimidine, sulfanilamide) and fluoroquinolone (norfloxacin and cipropfloxacin) was stirred in magnetic stirrer with ethanol for 3 hrs. The mixture allows cooling over night in refrigerator. The solid thus

obtained was recrystallized from DMF with ethanol (Scheme 1, Table 01). The physical data of the synthesized compounds are presented in Tables 02.

PD-FBI: IR (KBr)-3488(OH), 1719(C=O), 1482(C=N),

742(C-F); PMR (DMSO-d6)-9.0 (s, 1H, COOH), 8.3-

7.1(m, 7H, Ar-H), 2.5-3.3(m, 8H, piperanzyl), 3.1 and

2.5 (s, 4H, methylene). PD-FBT: IR(KBr)-3433(OH), 1624(C=O), 1475(C=N), 751(C-F), PMR (DMSO-d6)- 8.9(s, 1H, COOH), 8.2-7.1(m,6H,Ar-H), 3.4 and 2.5 (m,

8H,piperanzyl),3.1(s,2H,methylene),4.1(s,2H,methylene)

Calf Serum (FCS), 2 mM-glutamine, 0.1% Sodium bicarbonate and 20g/ml gentamicin (culture medium). Inhibitory effect of test compounds on HIV-1 replications was monitored by inhibition of virus- induced cytopathic effect in MT-4 cells and was estimated by MTT assay. Briefly, 50 l of HIV-1 (100- 300 CCID50) were added to a flat-bottomed microtiter tray with 50 l of medium containing various concentrations of compounds.

H

**R1**

N

N

N

F

O

H O

C

,1.4(s,3H,methyl)PD-FAP:IR(KBr)- 436(OH),1626(C=O),

1475(C=N), 751(C-F); PMR (DMSO-d6)- 9.5(s, 1H,

COOH), 8.3-6.5(m, 6H, Ar-H), 3.3 and 2.5(s, 8H,

piperanzyl), 1.5(s, 3H, methyl), 4.5(d, 2H, methylene). PD-FSX: IR (KBr)-3358(OH), 1624(C=O), 1476(C=N),

664(C-F). PMR(DMSO-d6)-9.0(s, 1H, COOH), 8.1-

6.5(m, 6H, Ar-H), 2.7(m, 8H, piperanzyl), 2.41-1.9(s,

6H, methyl), 2.7(s, 2H, methylene). PD-BAP: IR (KBr)-

HOOC

HOOC

+ **+** H **R3**

**R2**

H

**R1** N C **R3**

N N **R2**

F

3444(OH), 1628(C=O), 1487(C=N), 1292(NH). PMR (DMSO-d6)-9.0 (s,1H, COOH), 8.2-6.7(m, 11H, Ar-H),

2.4-2.6(m, 8H, piperanzyl), 4.5 (s, 1H, aromatic C-NH),

3.3 (s, 2H, methylene), 1.4 (s, 3H, methyl). PD-DBI: IR (KBr)-3415(OH), 1625(C=O), 1486 (C=N), 749(C-F); PMR (DMSO-d6)-9.8(s, 1H, COOH), 8.3-6.7(m, 11H, Ar-H), 4.5 (q, 8H, piperanzyl), 2.8(s, 6H, methyl), 3.1(s, 2H, methylene). PD-CFT: IR (KBr)-3365(OH), 1627(C=O), 1495(C=N), 725(C-F); PMR (DMSO-d6)- 11(s,1H,COOH), 8.2-7.3(m, 7H, Ar-H), 3.5-.5(m, 8H, piperanzyl), 2.5 (s, 2H, methylene), 3.5 (s, 2H, methylene). PD-CBI: IR (KBr)-3369(OH), 1624(C=O), 1450(C=N), 755(C-F), PMR (DMSO-d6)-9.9(s, 1H, COOH), 7.7-6(m, 11H, Ar-H), 3.9-2.5(m, 8H, piperanzyl), 1.2 (s, 2H, cyclopropane), 1.1(s, 2H, cyclopropane).PD-CDA:IR-(KBr)-3356(OH), 627(C=O), 1473(C=N),775(C-F), 1332(NH); PMR (DMSO-d6)-9.7 (s, 1H, COOH), 8.3-6.7(m, 9H, Ar-H), 3.5 and 2.5(s, 8H, piperanzyl), 1.3(s, 1H, N-CH), 3.0(s, 6H, N-methyl), 3.9(s, 1H, Ar-NH). PD-CDIN:IR (KBr)-3249(OH), 1627(C=O), 1457(C=N), 1335(NH), 744(C-F). PMR (DMSO-d6)-9.6(s, 1H, COOH), 8.2-6.5.(m, 6H, Ar-H), 3.5 and 2.5(s, 8H, piperanzyl), 1.4-1.2 (d, 2H, cyclopropane) 3.1(s, 6H, methyl). Compounds were tested for their inhibitory effects against replication of HIV-1 (IIIB) in MT-4 cells6,7,8. The MT-4 cells were grown and maintained in RPMI 1640 DM Medium supplemented with 10% (v/v) heat-inactivated Fetal

O

**Scheme 1: Synthetic protocol of Studied compounds**

 **Table 01: List of Studied Compounds**

**Compound R1 R2 R3 code**

|  |  |  |  |
| --- | --- | --- | --- |
| PD-FBI | Ethyl | H | Benzimidazole |
| PD-FBT | Ethyl | H | Benztriazole |
| PD-FAP | Ethyl | H | 2-aminopyridine |
| PD-FMZ | Ethyl | H | Sulphamethoxazole |
| PD-FSD | Ethyl | H | Sulphdiazine |
| PD-FSX | Ethyl | H | Sulphamethoxazole |
| PD-FSA | Ethyl | H | sulphanilic acid |
| PD-FSM | Ethyl | H | Sulphadimidine |
| PD-FSN | Ethyl | H | Sulphanilamide |
| PD-NFIN | Ethyl | H | Indole |
| PD-BAP | Ethyl | benzene | 2-aminopyridine |
| PD-DBI | Ethyl | p-dimethylamino | Benzimidazole |
| PD-DBM | Ethyl | p-dimethylamino | Benzamide |
| PD-DPH | Ethyl | p-dimethylamino | Phthalimide |
| PD-DPZ | Ethyl | p-dimethylamino | Piperazine |
| PD-NDIN | Ethyl | p-dimethylamino | Indole |
| PD-CFI | Cyclopropyl | H | Benzimidazole |
| PD-CFT | Cyclopropyl | H | Benztriazole |
| PD-CFA | Cyclopropyl | H | 2-aminopyridine |
| PD-CFM | Cyclopropyl | H | 2-mercaptobenzimidazole |
| PD-CFIN | Cyclopropyl | H | Indole |
| PD-CBI | Cyclopropyl | benzene | Benzimidazole |
| PD-CBT | Cyclopropyl | benzene | Benztriazole |
| PD-CBA | Cyclopropyl | benzene | 2-aminopyridine |
| PD-CDI | Cyclopropyl | p-dimethylamino | Benzimidazole |
| PD-CDT | Cyclopropyl | p-dimethylamino | Benztriazole |
| PD-CDA | Cyclopropyl | p-dimethylamino | 2-aminopyridine |
| PD-CDM | Cyclopropyl | p-dimethylamino | 2-mercaptobenzimidazole |
| PD-CDIN | Cyclopropyl | p-dimethylamino | Indole |

MT-4 cells were added at a final concentration of 6x105cells/ml. After 5 days of incubation at 37C, the number of viable cells were determined by the 3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) method.

**Table 02: Physical constant of synthesized compounds**

**Compound code**

**Molecular formula**

**Molecular weight**

**Melting Point (º)**

**Rf value@**

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-1 in human MT-4 cells. The anti-HIV and cytotoxicity data are presented in Table3.

@ CHCl3:CH3OH

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| PD-FBI | C24H24FN5O3 | 449.47 | 145-148 | 0.35 |
| PD-FBT | C23H23FN6O3 | 450.46 | 145-150 | 0.44 |
| PD-FAP | C22H24FN5O3 | 425.45 | 170 | 0.65 |
| PD-FMZ | C27H29FN6O6S | 584.62 | 125 | 0.83 |
| PD-FSD | C27H28FN7O5S | 581.62 | 150-153 | 0.71 |
| PD-FSX | C28H31FN6O6S | 598.2 | 135-140 | 0.61 |
| PD-FSA | C23H25FN4O6S | 504.53 | 190-195 | 0.56 |
| PD-FSM | C29H32FN7O5S | 609.67 | 190-195 | 0.54 |
| PD-FSN | C23H26FN5O5S | 503.54 | 170-175 | 0.57 |
| PD-NFIN | C25H25FN4O3 | 448.48 | 195 | 0.30 |
| PD-DBI | C32H33FN6O3 | 568.64 | 96-104 | 0.42 |
| PD-DBM | C32H34FN5O4 | 571.64 | 90 | 0.72 |
| PD-DPH | C33H32FN5O5 | 597.63 | 98-102 | 0.82 |
| PD-DPZ | C29H37FN6O3 | 536.29 | 185 | 0.74 |
| PD-NDIN | C33H34FN5O3 | 556.65 | 198 | 0.87 |
| PD-BAP | C28H28FN5O3 | 501.55 | 116-120 | 0.41 |
| PD-CFI | C25H24FN5O3 | 461.48 | 110-115 | 028 |
| PD-CFT | C24H23FN6O3 | 462.47 | 110 | 0.44 |
| PD-CFA | C23H24FN5O3 | 437.46 | 130 | 0.57 |
| PD-CFM | C25H24FN5O3S | 493.55 | 72-75 | 0.86 |
| PD-CFIN | C26H25FN4O3 | 460.5 | 95 | 0.26 |
| PD-CDI | C33H33FN6O3 | 580.65 | 110 | 0.31 |
| PD-CDT | C32H33FN7O3 | 581.64 | 90 | 0.51 |
| PD-CDA | C31H33FN6O3 | 556.63 | 60-65 | 0.46 |
| PD-CDM | C33H33FN6O3S | 612.71 | 82-87 | 0.83 |
| PD-CDIN | C34H34FN5O3 | 579.66 | 110 | 0.50 |
| PD-CBI | C33H27FN5O3 | 566.65 | 88-92 | 0.37 |
| PD-CBT | C30H27FN6O3 | 538.57 | 140 | 0.39 |
| PD-CBA | C29H28FN5O3 | 513.22 | 140-145 | 0.44 |

**Table 03:**

# Results and Discussion

Synthesized compounds were screened for antiviral activity against HIV-1 in MT-4 cells using AZT-as standard. Cytotoxic activity (CC50) of the compounds was also tested in mock-infected MT-4 cells (C-type Adults Leukemia-T cell). All the compounds displayed cytotoxic properties in MT-4 cells. Among the compounds tested two compounds, PD-NDIN (7-[4-{(4- (dimethylamino) phenyl) (1H-indole-1-yl) methyl} piperazine-1-yl]-1-ethyl-6-fluoro-4-oxo-1,4-dihydro quinoline -3-carboxlic acid) and PD-CFA(1cyclo propyl-6-fluoro-4-oxo-7[4-{(pyridine-2-yalmino) methyl} piperazine-1yl}-1,4-dihydroquinolines-3-carb

-oxylic acid) have shown more toxic in these series. Compounds PD-CDIN and PD-NDIN exhibited 27 and

**Anti HIV activity of fluoroquinolones in MT-4 cells**

|  |  |  |  |
| --- | --- | --- | --- |
| **Code** | **EC50a(µM)** | **CC50 b (µM)** | **Max Prot (%)** |
| PD-BAP | > 104.82 | 104.82 | 3 |
| PD-DBI | > 91.43 | 91.43 | 2 |
| PD-DBM | > 94.01 | 94.01 | 3 |
| PD-DPH | > 109.28 | 109.28 | 5 |
| PD-DPZ | > 124.61 | 124.61 | 2 |
| PD-FAP | > 142.55 | 142.55 | 2 |
| PD-FBI | > 92.13 | 92.13 | 2 |
| PD-FBT | > 72.22 | 72.22 | 0 |
| PD-FSA | > 90.24 | 90.24 | 1 |
| PD-FSM | > 37.41 | 37.41 | 3 |
| PD-NDIN | > 35.73 | 35.73 | 10 |
| PD-SMZ | > 120.12 | 120.12 | 1 |
| PD-NFFIN | > 23.56 | 23.56 | 0 |
| PD-CBA | > 119.46 | 119.46 | 0 |
| PD-CBI | > 130.96 | 130.96 | 0 |
| PD-CBT | > 97.92 | 97.92 | 0 |
| PD-CDI | > 121.16 | 121.16 | 3 |
| PD-CDIN | > 61.60 | 61.60 | 27 |
| PD-CDT | > 72.65 | 72.65 | 1 |
| PD-CFA | > 20.22 | 20.22 | 0 |
| PD-CFI | > 115.68 | 115.68 | 1 |
| PD-CFIN | > 70.69 | 70.69 | 1 |
| PD-CFT | > 73.16 | 73.16 | 1 |
| AZT | 0.0062 | 65.45 | 106 |

a50% Effective concentration of compound, achieving 50% protection of MT-4cells against the cytopathic effect of HIV. b50% Cytotoxic concentration of compound, required to reduce the viability of mock- infected MT-4 cells by 50%.

10 percent maximum protection against replication of HIV-1 in MT-4 cells at sub toxic concentration.

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# References

1. Okamoto H, Cujee TP, Okamoto M, Peterlin BM, Baba M, Okamoto T. Inhibition of the RNA- dependent transactivation and replication of human immuno- deficiency virus type-1 by a fluoroquinoline derivative K-37, Virology, 272, 2000; 402-408.
2. Hagihara M, Kashiwase H, Katsube T, Kimura T, Komai, Tomoaki M, *et al.* Synthesis and anti-HIV activity of arylpiperazinyl fluoroquinolones: a new class of anti-HIV agents. Bioorg Med Chem Lett, 9: 1999; 3063-3068.
3. Pandeya SN, Sriram D, Nath G, De Clercq E, synthesis, antibacterial, antifungal and anti-HIV activites of norfloxacin mannich bases Eur J Med Chem, 35: 2000; 249-255.
4. P. Selvam, Rajapandi, C. Pannecouque And E. De Clercq , Synthesis anti HIV and cytotoxicity studies of some novel N-substitutedpiperazinyl flouro - quinolone derivative, Indian Drugs, 448, 2007; 102-105.
5. P. Selvam, P. Rathore, S.Karthikumar, K. Velkumar, P.Palanichamy, Vijalakhsmi, M.Witvrouw. Synthesis and antiviral studies of novel N-sulphonamidomethyl piperazinyl flouroquinolones. Indian Journal of Pharmaceutical Sciences, 71, 2009, 432-436.
6. Pauwels R, DeClercq E, Desmyter J, Balzarini J, Goubau P, Herdewijn P, *et al.* Sensitive and rapid

assay on MT-4 Cells for the detection of antiviral compounds against the AIDS virus**.** J Virol Methods, 16:1987; 171-185.

1. Pauwels R, Balzarini J, Baba M, Snoeck R, Schols DJ, De Clercq E. *et al.* Rapid and automated tetrazolium based colourimetric assay for the detection of anti-HIV compounds. J Virol Methods, 20:1988; 309-321.
2. Miyoshi I, Taguchi H, Kubonishi I, Yoshimoto S, Ohtsuki Y, Shiraishi Y, Akagi T. Type C virus- producing cell lines derived from adult T cell leukemia. Can Res, 28:1982; 219-228.