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 N^4-hydrogen of piperazine in fluoroquinolones with mannich reactions. The structures of the synthetic compounds were characterized by means of their IR, ^{1}H-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 cells[1]. 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-α) and interleukin 6 (IL-6) production in blood mononuclear cells, suggesting a mechanism of action mediated by inhibition of Tat functions[2]. Recently, newer synthesized arylpiperazinyl fluoroquinolones were studied for anti-HIV activity[3,4,5].

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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.

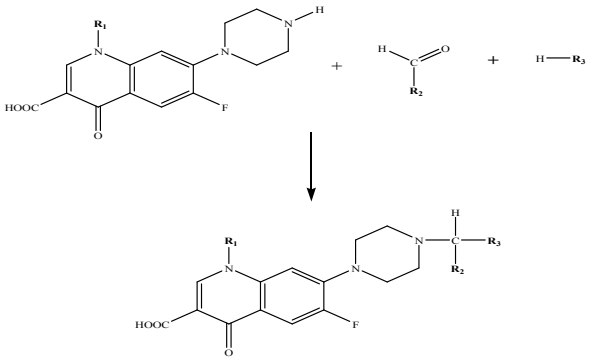
Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a Jasco-410 infrared spectrophotometer, ^{1}H-NMR spectra were determined BRUKER AMX 400 M_{HZ} with tetramethyl silane as an internal standard. The sample is dissolved in DMSO-d_6 and the value is measured in δ 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-d_6)-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-d_6)-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) ,1.4(s,3H,methyl)PD-FAP:IR(KBr)- 436(OH),1626(C=O), 1475(C=N), 751(C-F); PMR (DMSO-d_6)- 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-d_6)-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)-3444(OH), 1628(C=O), 1487(C=N), 1292(NH). PMR (DMSO-d_6)-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-d_6)-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-d_6)-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-d_6)-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-d_6)-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 (III_B) in MT-4 cells[6,7,8]. The MT-4 cells were grown and maintained in RPMI 1640 DM Medium supplemented with 10% (v/v) heat-inactivated Fetal Calf Serum (FCS), 2 mM-glutamine, 0.1% Sodium bicarbonate and 20μg/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 μl of HIV-1 (100-300 $CCID_{50}$) were added to a flat-bottomed microtiter tray with 50 μl of medium containing various concentrations of compounds.



Scheme 1: Synthetic protocol of Studied compounds

Table 01: List of Studied Compounds

Compound code	R_1	R_2	R_3
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 $6x10^5$cells/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 (°)	Rf value@
PD-FBI	$C_{24}H_{24}FN_5O_3$	449.47	145-148	0.35
PD-FBT	$C_{23}H_{23}FN_6O_3$	450.46	145-150	0.44
PD-FAP	$C_{22}H_{24}FN_5O_3$	425.45	170	0.65
PD-FMZ	$C_{27}H_{29}FN_6O_6S$	584.62	125	0.83
PD-FSD	$C_{27}H_{28}FN_7O_5S$	581.62	150-153	0.71
PD-FSX	$C_{28}H_{31}FN_6O_6S$	598.2	135-140	0.61
PD-FSA	$C_{23}H_{25}FN_4O_6S$	504.53	190-195	0.56
PD-FSM	$C_{29}H_{32}FN_7O_5S$	609.67	190-195	0.54
PD-FSN	$C_{23}H_{26}FN_5O_5S$	503.54	170-175	0.57
PD-NFIN	$C_{25}H_{25}FN_4O_3$	448.48	195	0.30
PD-DBI	$C_{32}H_{33}FN_6O_3$	568.64	96-104	0.42
PD-DBM	$C_{32}H_{34}FN_5O_4$	571.64	90	0.72
PD-DPH	$C_{33}H_{32}FN_5O_5$	597.63	98-102	0.82
PD-DPZ	$C_{29}H_{37}FN_6O_3$	536.29	185	0.74
PD-NDIN	$C_{33}H_{34}FN_5O_3$	556.65	198	0.87
PD-BAP	$C_{28}H_{28}FN_5O_3$	501.55	116-120	0.41
PD-CFI	$C_{25}H_{24}FN_5O_3$	461.48	110-115	028
PD-CFT	$C_{24}H_{23}FN_6O_3$	462.47	110	0.44
PD-CFA	$C_{23}H_{24}FN_5O_3$	437.46	130	0.57
PD-CFM	$C_{25}H_{24}FN_5O_3S$	493.55	72-75	0.86
PD-CFIN	$C_{26}H_{25}FN_4O_3$	460.5	95	0.26
PD-CDI	$C_{33}H_{33}FN_6O_3$	580.65	110	0.31
PD-CDT	$C_{32}H_{33}FN_7O_3$	581.64	90	0.51
PD-CDA	$C_{31}H_{33}FN_6O_3$	556.63	60-65	0.46
PD-CDM	$C_{33}H_{33}FN_6O_3S$	612.71	82-87	0.83
PD-CDIN	$C_{34}H_{34}FN_5O_3$	579.66	110	0.50
PD-CBI	$C_{33}H_{27}FN_5O_3$	566.65	88-92	0.37
PD-CBT	$C_{30}H_{27}FN_6O_3$	538.57	140	0.39
PD-CBA	$C_{29}H_{28}FN_5O_3$	513.22	140-145	0.44

@ $CHCl_3$:CH_3OH

Table 03:
Anti HIV activity of fluoroquinolones in MT-4 cells

Code	EC_{50}[a](µM)	CC_{50} [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

[a]50% Effective concentration of compound, achieving 50% protection of MT-4cells 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%.

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.

Results and Discussion

Synthesized compounds were screened for antiviral activity against HIV-1 in MT-4 cells using AZT-as standard. Cytotoxic activity (CC_{50}) 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 10 percent maximum protection against replication of HIV-1 in MT-4 cells at sub toxic concentration.

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