Available online at www.sciencedirect.com
SCIENCE DIRECT®
Il Farmaco 60 (2005) 377–384
IL FARMACO
http://france.elsevier.com/direct/FARMAC/

Newer aminopyrimidinimino isatin analogues as non-nucleoside HIV-1 reverse transcriptase inhibitors for HIV and other opportunistic infections of AIDS: design, synthesis and biological evaluation

D. Sriram *, T.R. Bal, P. Yogeeswari

Medicinal Chemistry Research Laboratory, Pharmacy Group, Birla Institute of Technology and Science, Pilani 333031, India

Received 24 September 2004; received in revised form 8 February 2005; accepted 5 March 2005

Available online 03 May 2005

Abstract

Human immuno deficiency virus (HIV) weakens the immune system so that many opportunistic infections (OIs) like tuberculosis, hepatitis, bacterial infections etc can develop. In this paper, we designed aminopyrimidinimino isatin lead compound as a novel non-nucleoside reverse transcriptase inhibitor (NNRTI) with broad-spectrum chemotherapeutic properties for the effective treatment of AIDS and AIDS-related OIs. Compound 1-cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[[N^4-[3'-(4'-amino-5'-trimethoxybenzyl pyrimidin-2'-yl)imino-1'-(5-methylisatinyl)]methyl]-N^1-piperazinyl]-3-quinoline carboxylic acid (**10**) emerged as the most potent broad-spectrum chemotherapeutic agent active against HIV, HCV, *Mycobacterium tuberculosis* and various pathogenic bacteria.

Keywords: Anti-HIV; Anti-HCV; Antituberculosis; Antibacterial; Isatin

1. Introduction

Human immuno deficiency virus (HIV) has been identified as the probable causative agent for AIDS. The HIV infection, which targets the monocytes expressing surface CD4 receptors, eventually produces profound defects in cell-mediated immunity [1]. Overtime infection leads to severe depletion of CD4 T-lymphocytes (T-cells) resulting in opportunistic infection (OIs) like tuberculosis (TB), fungal, viral, protozoal and neoplastic diseases and ultimately death. TB is the most common OI in people with AIDS and it is the leading killer of people with AIDS. The co-infection by hepatitis C virus (HCV) and HIV is quite common, mainly because these infections share the same parenteral, sexual and vertical routes of transmission [2]. Although classical OIs are now rarely seen, the toxicity of antiretroviral drugs as well as liver diseases caused by HCV represent an increasing cause of morbidity and mortality among HIV-positive persons. Predisposing liver damage favors a higher rate of hepatotoxicity of antiretroviral drugs, which can limit the benefit of HIV treatment in some individuals [3]. An ideal drug for HIV/AIDS patients should suppress HIV replication thereby acting as anti-HIV drug and also should treat OIs like TB, hepatitis and other bacterial infections. Earlier works in our laboratory have identified various isatinimino derivatives exhibiting broad-spectrum chemotherapeutic properties [4]. As a continuation to our effort in developing broad-spectrum chemotherapeutics, we undertook the present study to design, synthesize and evaluate aminopyrimidinimino isatin analogs, which could suppress HIV replication and also inhibit the opportunistic microorganisms.

2. Experimental

Melting points (m.p.) were determined in one end open capillary tubes on a Büchi 530 m.p. apparatus and are uncorrected. Infrared (IR) and proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded for the compounds on Jasco IR Report 100 (KBr) and Brucker Avance (300 MHz) instruments, respectively. Chemical shifts are reported in parts per million (ppm) using tetramethyl silane (TMS) as an internal standard. Elemental analyses (C, H, and N) were undertaken

* Corresponding author. Tel.: +91 1596 24 4684; fax: +91 1596 24 4183.
E-mail address: dsriram@bits-pilani.ac.in (D. Sriram).

0014-827X/$ - see front matter © 2005 Elsevier SAS. All rights reserved.
doi:10.1016/j.farmac.2005.03.005

with Perkin–Elmer model 240C analyzer. The homogeneity of the compounds was monitored by ascending thin layer chromatography (TLC) on silica gel-G (Merck) coated aluminium plates, visualized by iodine vapor. Developing solvents were chloroform/methanol (9:1). The pharmacophoric distance map and log *P* values were determined using Alchemy-2000 and Scilog P softwares (Tripos Co.).

2.1. Synthesis of (3-{[4'-amino-5(3″,4″,5″-trimethoxybenzyl) pyrimidin-2'-yl]} imino}-5-methyl-1,3-dihydro-2H-indol-2-one)

Equimolar quantities (0.01 mol) of 5-methylisatin and 5-(3',4',5'-trimethoxybenzyl)-2,4-diaminopyrimidine were dissolved in warm ethanol containing 1 ml of glacial acetic acid. The reaction mixture was irradiated in an unmodified domestic microwave oven at 80% intensity with 30 s per cycle for 3 min and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol–chloroform mixture. Yield: 81.4%; m.p.: 232 °C; IR (KBr): 3300, 2045, 1660, 1620, 1580, cm^{-1}; ^{1}H-NMR ($CDCl3$) δ (ppm): 1.82 (s, 3H, CH_3), 3.22 (s, 2H, CH_2), 3.7 (s, 9H, $–OCH_3$), 5.46 (s, 2H, NH_2), 6.8–7.6 (m, 6H, Ar–H), 10.68 (s, 1H, –NH).

2.2. General procedure for the preparation of Mannich bases

To a suspension of 3-{[4'-amino-5-(3″,4″,5″-trimethoxybenzyl) pyrimidin-2'-yl]} imino}-5-methyl-1,3-dihydro-2H-indol-2-one (0.02 mol) in ethanol was added appropriate secondary amines (0.02 mol) and 37% formaldehyde (0.5 ml) and irradiated in a microwave oven at an intensity of 80% with 30 s per cycle. The number of cycle in turn depended on the completion of the reaction, which was checked by TLC. The reaction timing varied from 1.5 to 3 min. The solution obtained after the completion of the reaction was kept at 0 °C for 30 min and the resulting precipitate was recrystallized from a mixture of DMF and water.

*2.2.1. (3-{[4'-Amino-5'-(3″,4″,5″-trimethoxybenzyl) pyrimidin-2'-yl]} imino}-5-methyl-1-[(4-chlorophenyl piperazinyl) methyl]-1,3-dihydro-2H-indol-2-one) (**2**)*

Yield: 65.6%; m.p.: 79 °C; IR (KBr): 3010, 2850, 2830, 1730, 1620, 1500, 1240, cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.8 (s, 3H, CH_3), 3.18 (s, 2H, CH_2 of trimethoxybenzyl), 3.6 (s, 9H, $–OCH_3$), 3.9–4.2 (m, 8H, piperazine-H), 5.2 (s, 2H, $–NCH_2N–$), 5.65 (s, 2H, NH_2), 6.7–7.82 (m, 10H, Ar–H); Calculated for $C_{34}H_{36}N_7O_4Cl$: C, 63.59; H, 5.65; N, 15.27; found: C, 63.60; H, 5.60; N, 15.12.

*2.2.2. (3-{[4'-Amino-5'-(3″,4″,5″-trimethoxybenzyl) pyrimidin-2'-yl]} imino}-5-methyl-1-[(4-phenyl piperazinyl) methyl]-1,3-dihydro-2H-indol-2-one) (**5**)*

Yield: 64.5%; m.p.: 97 °C; IR (KBr): 3010, 2850, 2840, 1730, 1616, 1500, 1240, cm^{-1}; ^{1}H-NMR ($CDCl_3$) δ (ppm): 1.8 (s, 3H, CH_3), 3.17 (s, 2H, CH_2 of trimethoxybenzyl), 3.65 (s, 9H, $–OCH_3$), 3.9–4.1 (m, 8H, piperazine-H), 5.2 (s, 2H, $–NCH_2N–$), 5.65 (s, 2H, NH_2), 6.67–7.82 (m, 11H, Ar–H); Calculated for $C_{34}H_{37}N_7O_4$: C, 67.2; H, 6.14; N, 16.13; found: C, 67.02; H, 6.20; N, 16.32.

*2.2.3. 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[[N^4-[3'-(4'-amino-5'-trimethoxybenzyl pyrimidin-2'-yl)imino-1'-(5-methylisatinyl)] methyl] N'-piperazinyl]-3-quinoline carboxylic acid (**10**)*

Yield: 70.5%; m.p.: 164 °C; IR (KBr): 3010, 2850, 2840, 1736, 1620, 1506, 1236, 1125, cm^{-1}; 1H-NMR ($CDCl_3$) δ (ppm): 0.88–1.12 (m, 4H, cyclopropyl-H), 1.84 (s, 3H, CH_3) 3.3 (s, 2H, CH_2 of benzyl), 3.5 (m, 1H, cyclopropyl-H), 3.62 (s, 9H, $–OCH_3$), 3.7–4.1 (m, 8H, –piperazine-H), 5.16 (s, 2H, $–NCH_2N$), 5.8 (s, 2H, NH_2), 6.58–8.40 (m, 9H, Ar–H), 8.6 (s, 1H, C_2-H); Calcd. for $C_{41}H_{41}N_8O_7F$: C, 63.39; H, 5.32; N, 14.42; found: C, 63.28; H, 5.36; N, 14.40.

2.3. Anti-HIV screening

2.3.1. In MT-4 cells

The compounds were tested for anti-HIV activity against replication of HIV-1 (III B) in MT-4 cells. The MT-4 cells were grown in RPMI-1640 DM (Dutch modification) medium (Flow lab, Irvine Scotland), supplemented with 10% (v/v) heat-inactivated calf serum and 20-μg ml^{-1} gentamicin (E. Merck, Darmstadt, Germany). HIV-1 (III B) was obtained from the culture supernatant of HIV-1 infected MT-4 cell lines and the virus stocks were stored at –70 °C until used. Anti-HIV assays were carried out in microtitre plates filled with 100 μl of medium and 25 μl volumes of compounds in triplicate so as to allow simultaneous evaluation of their effects on HIV and mock-infected cells. Fifty microlitres of HIV at 100 $CCID_{50}$ medium were added to either the HIV-infected or mock-infected part of the microtitre tray. The cell cultures were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in air. Five days after infection the viability of mock and HIV-infected cells were examined spectrophotometrically by the MTT method.

2.3.2. In CEM cells

Candidate agents were dissolved in dimethylsulfoxide, and then diluted 1:100 in cell culture medium before preparing serial half-log_{10} dilutions. T4 lymphocytes (CEM cell-line) were added and after a brief interval HIV-1 was added, resulting in a 1:200 final dilution of the compound. Uninfected cells with the compound served as a toxicity control, and infected and uninfected cells without the compound served as basic controls. Cultures were incubated at 37 °C in a 5% carbon dioxide atmosphere for 6 days. The tetrazolium salt, XTT was added to all the wells, and cultures were incubated to allow formazan color development by viable cells. Individual wells were analyzed spectrophotometrically to quantitative formazan production, and in addition were viewed microscopically for detection of viable cells and confirmation of protective activity.

2.3.3. HIV-1RT assay

The reaction mixture (50 μl) contained 50 mM Tris–HCl (pH 7.8), 5 mM dithiothreitol, 30 mM glutathione, 50 μM EDTA, 150 mM KCl, 5 mM $MgCl_2$, 1.25 μg of bovine serum albumine, an appropriate concentration of the radiolabeled substrate [3H] dGTP, 0.1 mM poly(νC)·oligo(dG) as the template/primer, 0.06% Triton X-100, 10 μl of inhibitor solution (containing various concentrations of compounds), and 1 μl of RT preparation. The reaction mixtures were incubated at 37 °C for 15 min, at which time 100 μl of calf thymus DNA (150 μg ml^{-1}), 2 ml of $Na_4P_2O_7$ (0.1 M in 1 M HCl), and 2 ml of trichloroacetic acid (10% v/v) were added. The solutions were kept on ice for 30 min, after which the acid-insoluble material was washed and analyzed for radioactivity. For the experiments in which 50% inhibitory concentration (IC_{50}) of the test compounds was determined, fixed concentration of 2.5 μM [^{3}H] dGTP was used.

2.4. Antiviral and cytotoxicity assays for HCV

2.4.1. Cell culture

Huh-7 cells the subgenomic HCV replicon BM4-5 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Life Technologies) supplemented with 10% fetal bovine serum, 1% L-glutamine, 1% L-pyruvate, 1% penicillin and 1% streptomycin supplemented with 500 mg ml^{-1} G418 (Geneticin, Invitrogen). Cells were passaged every 4 days.

2.4.2. Cytotoxicity

Huh-7 cells were, respectively, seeded at a density of 3×10^{-4} cells per well in 96-well plates for the cell viability assay, or at a density of 6×10^{-5} cells per well in six-well plates for the antiviral assay. Sixteen hours post seeding, cells were treated with the compounds at 50 μg ml^{-1} for 3 days. The administration of each drug was renewed each day. Other drugs, including ribavirin (ICN Pharmaceuticals, USA), mycophenolic acid (Sigma, USA), and interferon alpha-2b (IntronA) were used in the same conditions as positive controls. At the end of treatment, cell viability assays were performed with the 96-well plates using Neutral Red assay (Sigma).

2.4.3. Antiviral assay

Total RNA (tRNA) was extracted from six-well plates with the 'Extract All' reagent (Eurobio), which is a mixture of guanidinium thiocyanate–phenol–chloroform. Northern Blot analysis was then performed using the NorthernMaxTM-Gly (Ambion) kit, following manufacturer's instruction. Ten micrograms of tRNA was denatured in glyoxal buffer at 50 °C for 30 min and separated by agarose gel electrophoresis, then transferred for 12 h onto a charged nylon membrane (Biodyne B, Merck Eurolab). Hybridization was carried out with three different [^{32}P] CTP-labeled riboprobes obtained by in vitro transcription (Promega). These probes were complementary to the NS5 A region of the HCV genome, and to the cellular gene GAPDH, respectively. First, the blot was hybridized with two riboprobes directed against the negative strand of HCV RNA and the GAPDH mRNA, respectively. After one night of hybridization at 68 °C, the membrane was washed then exposed to X-ray film and a phosphor screen for quantitative analysis. The amount of GAPDH mRNA was used as an internal loading control to standardize the amount of HCV RNA detected. The same membrane was subsequently hybridized with a negative-sense riboprobe to determine the level of HCV-positive strand RNA using the same approach.

2.5. Antimycobacterial screening

Primary screening was conducted at 6.25 μg ml^{-1} against *Mycobacterium tuberculosis* strain $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium using a broth micro dilution assay, the Microplate Alamar Blue Assay (MABA) [14].

2.6. In vitro antibacterial activity

Compounds were evaluated for their in vitro antibacterial activity against 28 pathogenic bacteria procured from the Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, India. The agar dilution method was performed using Mueller-Hinton agar (Hi-Media) medium. Suspensions of each microorganism were prepared to contain approximately 10^6 colony forming units (cfu ml^{-1}) and applied to plates with serially diluted compounds in DMF to be tested and incubated at 37 °C overnight (approximately 18–20 h). The minimum inhibitory concentration (MIC) was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculums.

2.7. In vivo antibacterial activity (mouse protection test)

The in vivo antibacterial activity of the test compounds was determined in CF-strain male mice (20–25 g body weight, six per group). The mice were infected intraperitoneally with a suspension containing an amount of the indicated organism slightly greater than its lethal dose 100 (LD_{100}). The mice were treated orally (p.o.) with a specific amount of the test compound administered at 1 and 4 h after infection. ED_{50} values were calculated by interpolation among survival rates in each group after a week. They express the total dose of compound (mg kg^{-1}) required to protect 50% of the mice from an experimentally induced lethal systemic infection of the indicated organism.

3. Results and discussion

3.1. Design

To qualify as a non-nucleoside reverse transcriptase inhibitors (NNRTI), the compound should interact specifically with

a non-substrate binding site of the reverse transcriptase (RT) of HIV-1, and inhibit the replication of HIV-1 at a concentration that is significantly lower than the concentration required to affect normal cell viability [5]. Based on these premises, more than thirty different classes of NNRTI's could be considered [6]. Although the NNRTI's seemingly belong to widely diverging classes of compounds, closer inspection reveals that most have some features in common, that is a carboxamide, or (thio) urea entity ('body'), surrounded by two hydrophobic, mostly aryl moieties ('wings'), one of which is quite often substituted by a halogen group (Fig. 1). Thus, the overall structure may be considered reminiscent of a butterfly with hydrophilic center ('body') and two hydrophobic outskirts ('wing'). In the present study, the aminopyrimidinimino isatin analogs are designed in accord to this hypothesis. The iminocarbamoyl moiety (–N=C–CO–N–) constitutes the 'body' and the aryl ring of isatin and the pyrimidine derivative constitute the 'wings' as depicted in Fig. 1. The distance between the hydrophilic center (A) and hydrophobic outskirts (B and C) and the angle between the two aryl rings (B and C) were measured using Tripos Alchemy-2000 software [7] from the energy minimized structures using MM3 program. The lead compound was found to comply within the specification of the pharmacophoric distance map (Fig. 2 and Table 1).

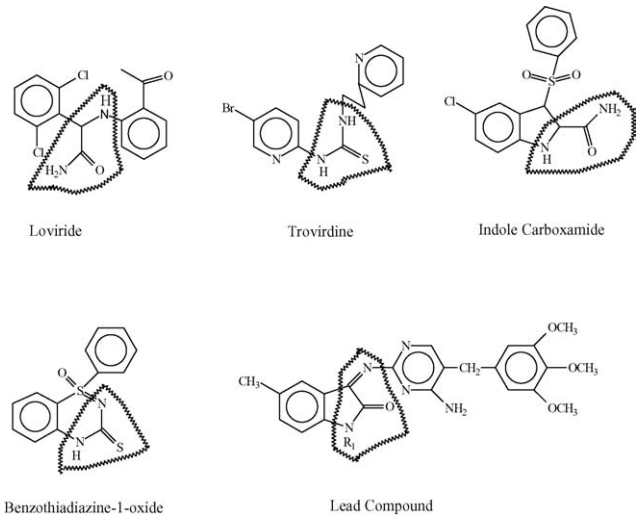


Fig. 1. Existing NNRTI's and lead compound.

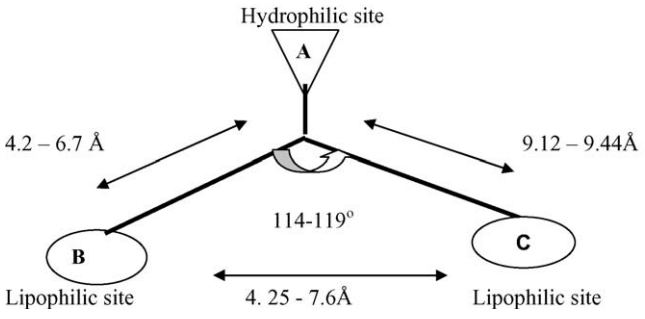


Fig. 2. Schematic representation of a butterfly-like configuration of NNRTI'S and the pharmacophoric distance map.

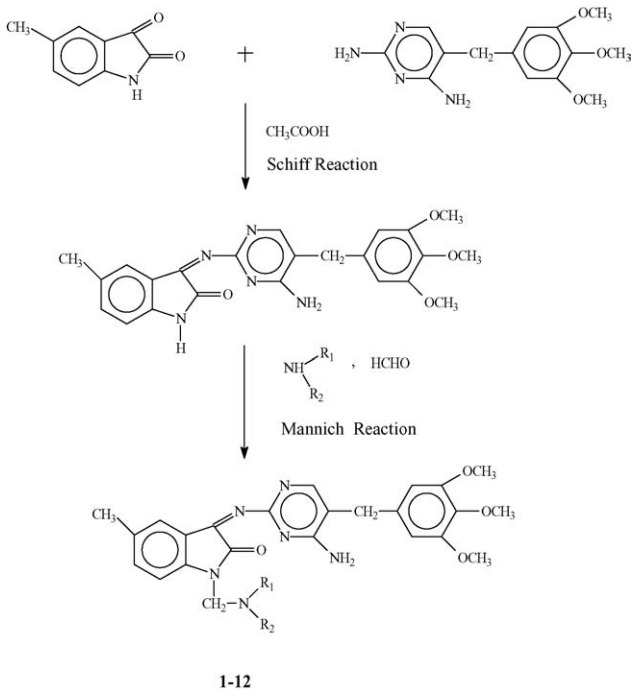


Fig. 3. Protocol for the synthetic compounds.

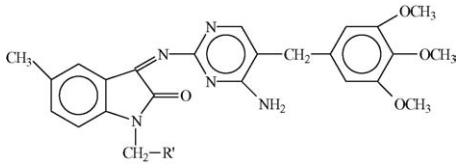
3.2. Synthesis

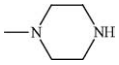
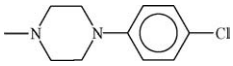
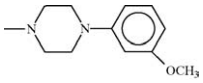
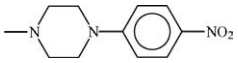
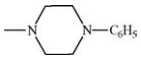
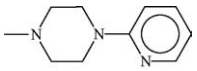
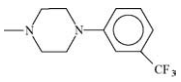
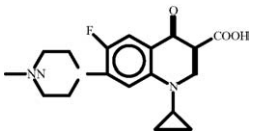
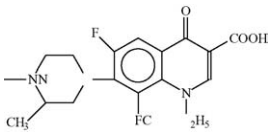
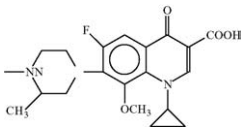
The synthesis of various aminopyrimidinimino isatin derivatives was achieved in two steps (Fig. 3) [8]. 5-Methylisatin was condensed with 5-trimethoxybenzyl-2,4-diamino pyrimidine in the presence of glacial acetic acid to form Schiff's base. The *N*-Mannich bases of the above Schiff's base were synthesized by condensing acidic imino group of isatin with formaldehyde and various secondary amines. All compounds (Tables 2,3) gave satisfactory elemental analysis. IR and ^{1}H-NMR spectra were consistent with the assigned structures.

Table 1
The distance between the pharmacophoric functional groups of anti-HIV drugs and the lead compound

Drugs	AB (in Å)	BC (in Å)	CA (in Å)	Angle Bac
Loviride	4.36–5.19	4.25–6.25	9.4	119°
Trovirdine	4.22–6.67	6.53–7.6	9.44	117.5°
Indole carboxamide	4.20–6.72	4.44–7.08	9.44	118°
Benzothiadiazine-1-oxide	4.25–6.60	4.86–7.02	9.12	114.9°
Range	4.20–6.72	4.25–7.6	9.12–9.44	114.9–119°
Lead compound	4.23–5.34	4.07–6.63	9.113	116.7°

Table 2
Physical constants of the synthesized compounds **1–12**



Compound	R'	Molecular formula	Molecular weight	Yield (%)	M.p. (°C)	log *P*
1		$C_{28}H_{33}N_7O_4$	531.60	65.60	162	6.12
2		$C_{34}H_{36}N_7O_4Cl$	642.14	60.25	79	8.04
3		$C_{35}H_{39}N_7O_5$	637.72	57.85	132	7.99
4		$C_{34}H_{36}N_8O_6$	652.7	66.70	80	7.01
5		$C_{34}H_{37}N_7O_4$	607.70	64.50	97	7.84
6		$C_{33}H_{36}N_8O_4$	608.69	68.00	87	7.54
7		$C_{35}H_{36}N_7O_4F_3$	675.70	65.30	64	9.29
8		$C_{28}H_{32}N_6O_4$	516.59	60.50	117	4.01
9		$C_{29}H_{34}N_6O_4$	530.61	61.40	60	4.52
10		$C_{41}H_{41}N_8O_7F$	776.81	70.50	164	5.69
11		$C_{41}H_{42}N_8O_7F_2$	796.819	69.00	147	6.29
12		$C_{43}H_{45}N_8O_8F$	820.86	72.50	141	6.20

3.3. Biological activities

The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in MT-4 and CEM cell lines (Table 4) [9]. In the MT-4 cell lines, compound **10** was found to be the most active against replication of HIV-1 with EC_{50} of 11.6 μM and their selectivity index ($SI = CC_{50}/EC_{50}$) was found to be more than 7 with maximum protection of 84.9%. Other compounds (**3**, **11** and **12**) showed maximum protection of 64–72% with SI of approximately 3. In the T4 lymphocytes (CEM cell lines), tested compounds showed marked anti-HIV activity (10–20%) at a concentration below their toxicity threshold. The loss of activity might be due to degeneration/rapid metabolism in the culture conditions used in the screening procedure.

Compound **10** was evaluated for the inhibitory effects on HIV-1 RT enzyme [10] and their IC_{50} value was found to be 28.4 ± 3.4 μM. The in vitro IC_{50} values for HIV-1 RT with Poly (νC) oligo (dG) as the template/primer were significantly higher than the corresponding EC_{50} values for inhibi-

Table 3
Anti-HIV, anti-HCV and antimycobacterial activity

Compound	Anti-HIV activity (μM)						Anti-HCV activity at 50 μg ml^{-1}		Antimycobacterial activity at 6.25 μg ml^{-1}
	MT-4 cell line			CEM cell line			Cell growth (%)	Viral RNA replication (%)	% Inhibition
	EC_{50}[a]	CC_{50}[b]	% Protection	EC_{50}[a]	CC_{50}[b]	% Protection			
1	> 94.2	94.2	19.2	> 89.2	89.2	13.02	57	96	24
2	17.2	49.2	72.6	> 36.1	36.1	28.21	67	90	28
3	> 32.1	32.1	26.94	> 11.9	11.9	13.23	15	100	15
4	> 47.96	47.96	16.4	> 37.4	37.4	17.64	74	93	30
5	> 116.1	116.1	26.2	> 121.0	121.0	11.24	78	91	18
6	> 119.6	119.6	14.20	> 121.0	121.0	11.20	78	72	25
7	> 106.2	106.2	26.4	> 118.0	118.0	10.01	81	87	21
8	> 124	124	34.6	> 122.0	122.0	11.86	93	90	19
9	> 124.6	124.6	24.6	> 105.0	105.0	10.8	74	99	22
10	11.6	86.1	84.9	NT	NT	NT	81	97	100
11	28.4	92.6	64.0	NT	NT	NT	68	92	100
12	21.2	81.4	66.8	NT	NT	NT	76	65	100

NT indicates not tested.
[a] 50% effective concentration, or concentration required to inhibit HIV-1 induced cytopathicity in cell lines by 50%.
[b] 50% cytotoxic concentration, or concentration required to reduce the viability of mock-infected cell lines by 50%.

tion of the cytopathic effect of HIV-1 in MT-4 cell culture. This discrepancy is not unusual for NNRTI's as it may reflect the differences between the in vitro HIV-1 RT assay, in which a synthetic template/primer is used, and the cellular systems [11].

All the synthesized compounds were also evaluated for their inhibition of HCV viral RNA replication in HUH-7 cells at 50-μg ml^{-1} [12], and the results are presented in Table 4. Among these, eight compounds inhibited HCV viral RNA replication at about 90–100%. Compound **8** was found to be most active with inhibition on viral replication of 90% and non-toxic to Huh-7 cells (93% cell growth). This paper is first of its kind in which isatin derivatives are reported to possess anti--HCV activity.

The synthesized compounds were also screened against *M. tuberculosis* strain $H_{37}Rv$ (ATCC 27294) in BACTEC 12B medium initially at 6.25 μg ml^{-1} (Table 4) [13]. Three compounds (**10**–**12**) showed complete inhibition (100%) of

Table 4
In vitro antibacterial activity (MIC's in μM)

Microorganism	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
K. ozaenae	0.0459	0.0095	0.0096	0.0094	0.0100	0.0090	0.0118	0.0236	0.1840
K. pneumoniae	11.7562	38.95	19.6	9.575	20.5675	20.535	18.5	0.0118	1.4723
S. sonnei	0.0115	0.0095	0.0096	0.0748	0.0803	0.1604	0.0301	0.0945	0.0115
Plesiomonas	0.0115	0.1521	0.0766	0.0374	0.0201	0.1604	0.0301	0.0945	0.0115
S. boydii	11.7562	38.95	19.6	19.15	20.5675	20.535	18.5	48.39	1.4723
M. morganii	11.7562	38.95	19.6	19.15	20.5675	20.535	18.5	48.39	23.5575
S. aureus	0.0229	0.0380	0.0096	0.0094	0.0100	0.0090	0.0118	0.1890	0.0920
P. aeruginosa	47.025	77.9	78.4	38.3	41.135	41.07	37.00	48.39	23.5575
V. mimicus	0.0115	0.0190	0.0096	0.0374	0.0402	0.0802	0.0722	0.0473	0.0460
V. fluvialis	23.5125	77.9	19.6	19.15	20.5675	41.07	37	48.39	1.4723
V. cholerae 0139	23.5125	38.95	19.6	38.3	10.2838	41.07	37	0.0118	1.4723
V. cholerae 01	0.0115	0.0095	0.1531	0.1496	0.1607	0.1604	0.0722	0.0236	0.0460
V. parahaemolyticus	0.1837	0.0380	0.1531	0.0374	0.0803	0.0401	0.0181	0.0945	0.0460
E. Coli NCTC10418	11.7562	38.95	19.6	19.15	20.5675	41.07	18.5	48.39	23.5575
E. tarda	11.7562	1.2172	19.6	19.15	20.5675	20.535	18.5	24.195	23.5575
P. vulgaris	11.7562	19.475	19.6	19.15	20.5675	20.535	18.5	48.39	0.0115
P. mirabilis	0.0459	0.0761	0.0383	0.0094	0.0100	0.0090	0.1445	0.1890	0.0115
S. typhimurium	11.7562	38.95	19.6	19.15	20.5675	20.535	37.00	48.39	0.0115
S. paratyphi A	2.9390	0.0095	9.8	4.7875	20.5675	10.2675	9.25	1.5122	5.8894
S. typhi	11.7562	38.95	19.6	19.15	20.5675	20.535	18.5	48.39	0.0115
S. enteritidis	2.9390	19.475	9.8	19.15	20.5675	20.535	18.5	24.195	23.5575
C. ferundii	2.9390	2.4343	4.9	4.7875	5.1418	20.535	18.5	24.195	23.5575
Enterobacter	2.9390	2.4343	1.225	19.15	20.5675	20.535	18.5	1.5122	11.7788
B. megatherius	11.7562	38.95	0.6125	4.7875	10.2838	10.2675	9.25	24.195	11.7788

Table 5
In vitro antibacterial activity (MIC's in μM)

Microorganism	**10**	**11**	**12**	**Cipro**	**Lome**	**Gati**
K. ozaenae	0.0002	0.0613	0.0149	0.0092	0.0629	0.0037
K. pneumoniae	0.0002	0.0002	0.0074	0.0023	0.1259	0.0037
S. sonnei	0.0079	0.0002	0.0149	0.0023	2.0156	0.0037
Plesiomonas	0.0079	0.0002	0.0019	0.0023	0.0629	0.1182
S. boydii	0.0079	0.0005	0.0019	0.0023	0.5039	0.0590
M. morganii	0.0079	0.0002	0.0019	0.0023	0.0629	0.0009
S. aureus	0.0002	0.0002	0.0297	0.0023	0.0314	0.0009
P. aeruginosa	0.0002	0.0613	0.0074	0.0092	0.2519	0.0074
V. mimicus	0.0157	0.0306	0.0074	0.0023	0.0629	0.0074
V. fluvialis	0.0157	0.0613	0.0037	0.0023	0.0629	0.0009
V. cholerae 0139	0.0157	0.0153	0.0074	0.0023	0.1259	0.0009
V. cholerae 01	0.0005	0.0153	0.0297	0.0023	0.0009	0.0009
V. parahaemolyticus	0.0002	0.0002	0.0005	0.0023	2.0156	0.4727
E. Coli NCTC10418	0.0002	0.0010	0.0005	0.0011	0.0314	0.0009
E. tarda	0.0002	0.0005	0.0009	0.0023	0.2519	0.0009
P. vulgaris	0.0002	0.0002	0.0009	0.0023	0.0314	0.0009
P. mirabilis	0.01571	0.0002	0.0019	0.0023	0.1259	0.0009
S. typhimurium	0.0002	0.0077	0.0037	0.0023	0.2519	0.0009
S. paratyphi A	0.0002	0.0002	0.0074	0.0023	0.0314	0.0009
S. typhi	0.0002	0.0002	0.0149	0.0023	0.5039	0.0009
S. enteritidis	0.0002	0.0002	0.0074	0.0023	1.0078	0.0009
C. ferundii	0.0002	0.0002	0.0037	0.0023	1.0078	0.0037
Enterobacter	0.0005	0.0002	0.0018	0.0023	1.0078	0.0009
B. megatherius	0.0157	0.0002	0.0009	0.0023	1.0078	0.0037

M. tuberculosis in the primary screening. In the secondary level screening the actual MIC and cytotoxicity in VERO cells of these three compounds were determined. The MIC's of these compounds were found to be 3.13 μg ml^{-1} and they were not cytotoxic upto 62.5 μg ml^{-1} to VERO cells.

All the compounds were evaluated for their in vitro antibacterial activity against 24 pathogenic bacteria by conventional agar dilution procedures [14] and the results of these assays are summarized in Tables 5,6. The data for ciprofloxacin, lomefloxacin and gatifloxacin were included for comparison. The antibacterial activity data revealed that all the test compounds showed mild to moderate activity against tested bacteria. The most sensitive organisms for the tested compounds were *Kleb. Ozaenae, Shig. Sonnei, Plesiomonas, Staph. aureus, Vibrio cholerae* 01, *V. mimicus, V. parahaemolyticus*, and *Prot. mirabilis*, as these compounds inhibited them at a concentration less than 1 μM. Compound **10**, which containing ciprofloxacin moiety at N-1 position was found to be more active than ciprofloxacin against 15 tested bacteria. When compared to lomefloxacin, compound **11** (lomefloxacin derivative) was found to be more active against 23 tested bacteria. Compound **12** bearing gatifloxacin at N-1 position was found to be equipotent against 4 and more active against 5 tested bacteria, when compared to gatifloxacin. These data are in consistent with our earlier results [15].

Table 6
In vivo antibacterial study on *E.coli* NCTC 10419 strain

Compound	In vitro MIC (in μM)	In vivo EC_{50} (in mg per kg body wt.)
10	0.0002	0.62
11	0.0010	1.87
Ciprofloxacin	0.0011	1.25
Lomefloxacin	0.0314	1.87

In vivo antibacterial activity of some selected compounds against an experimentally induced infection of mice after oral administration [15] are presented in Table 6, along with the in vitro activity against the infecting organism *E. coli* NCTC 10418. Ciprofloxacin and lomefloxacin were used as reference compounds. Compound **10** was twice more active than ciprofloxacin with ED_{50} of 0.62 mg kg^{-1} and compound **11** was equally active as lomefloxacin (ED_{50}: 1.87 mg kg^{-1}) against the tested bacteria.

The enhanced in vitro and in vivo antibacterial activity of the fluoroquinolone derivatives **10**–**12** might be due to the presence of bulky lipophilic isatinyl moiety at the C-7 piperazine.

To conclude, four compounds of the 12 new derivatives developed in this work showed inhibition against replication of HIV-1 in MT-4 cells with EC_{50} ranging from 11.6 to 28.4 μM. All compounds were active against HCV RNA replication showing > 65% inhibition at 50 μg ml^{-1}. Three compounds inhibited *M. tuberculosis* H37Rv with MIC of 3.13 μg ml^{-1}. Three compounds showed very good activity against various pathogenic bacteria. Among the synthesized compounds, compound **10** emerged as more promising broad-spectrum chemotherapeutic agents.

Acknowledgements

One of the authors Ms. Tanushree Bal deeply acknowledges the Council of Scientific and Industrial Research, India,

for providing Senior Research Fellowship. The authors also thank Dr. Robert H. Shoemaker, National Cancer Institute, USA and Dr. Sam Ananthan, TAACF, Southern Research Institute, USA for their support in biological testing.

References

[1] D.L. Bowen, H.C. Hane, A.C. Fauci, Immunopathogenesis of the acquired immunodeficiency syndrome, Ann. Intern. Med. 103 (1985) 704–709.

[2] A. Di Bisceglie, C. Hepatitis, Lancet 351 (1998) 351–355.

[3] R.R. Rosado, P.M. Olmeda, G.J. Samaniego, V. Soriano, Management of hepatitis C in HIV-infected persons, Antiviral Res. 52 (2001) 189–198.

[4] D. Sriram, P. Yogeeswari, Towards the design and development of agents with broad spectrum chemotherapeutic properties for the effective treatment of HIV/AIDS, Curr. Med. Chem. 10 (2003) 1909–1915.

[5] E. De Clercq, Perspectives of non-nucleoside reverse transcriptase inhibitors in the therapy of HIV-1 infection, Farmaco 54 (1999) 26–45.

[6] E. De Clercq, What can be expected from non-nucleoside reverse transcriptase inhibitors in the treatment of human immuno deficiency virus type-1 infections? Med. Virol. 6 (1996) 97–117.

[7] Tripos Alchemy, Alchemy 32 Version 2.0, Copyright 1997, Tripos Inc., St. Louis, MO, USA, 2000.

[8] S.N. Pandeya, D. Sriram, E. De Clercq, C. Pannecouque, M. Witrouw, Anti-HIV activity of some Mannich bases of isatin derivatives, Ind. J. Pharm. Sci. 60 (1998) 207–212.

[9] S.N. Pandeya, P. Yogeeswari, D. Sriram, E. De Clercq, C. Pannecouque, M. Witrouw, Synthesis and screening for anti-HIV activity of some *N*-Mannich bases of isatin derivatives, Chemotherapy 45 (1999) 192–196.

[10] J. Balzarini, P.M.J. Perez, S.A. Felix, M.J. Camarasa, I.C. Batharst, P.J. Barr, Kinetics of inhibition of human immunodeficiency virus type 1 (HIV-1) reverse transcriptase by the novel HIV-1-specific nucleoside analogue [2',5'-bis-*O*-(tert-butyldimethylsilyl)-beta-D-ribofuranosyl]-3'-spiro-5″-(4″-amino-1″,2″-oxathiole-2″,2″-dioxide) thymine (TSAO-T), J. Biol. Chem. 267 (1992) 11831–11838.

[11] C.A. Garcia, M. Micklatcher, J.A. Turpin, T.L. Stup, K. Watson, R.W. Buckheit, Novel modifications in the alkenyldiarylmethane (ADAM) series of non-nucleoside reverse transcriptase inhibitors, J. Med. Chem. 42 (1999) 4861–4874.

[12] J.T. Guo, V.V. Bichkow, C. Seeger, Effect of alpha interferon on the hepatitis C virus replicon, J. Virol. 75 (2001) 8516–8523.

[13] D. Sriram, K. Jyothimallika, P. Yogeeswari, Synthesis of tetrahydro-2H-[1,3,5] thiadiazine-5-(4-pyridylcarboxamido)-2-thione with antitubercular activity, Sci. Pharm. 72 (2004) 35–41.

[14] S.N. Pandeya, D. Sriram, Synthesis and screening for antibacterial activity of Schiff's and Mannich bases of Isatin and its derivatives, Acta Pharm. Turc. 40 (1998) 33–38.

[15] S.N. Pandeya, D. Sriram, G. Nath, E. De Clercq, Synthesis, antibacterial, antifungal and anti-HIV activities of norfloxacin Mannich bases, Eur. J. Med. Chem. 35 (2000) 249–265.