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Synthesis, anti-HIV and antitubercular activities of isatin derivatives

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HIV is the most significant risk factor for many opportunistic infections like tuberculosis. In this study, we designed an isatinimino lead compound as a novel non-nucleoside reverse transcriptase inhibitor with antimycobacterial properties for the effective treatment of AIDS and AIDS-related tuberculosis. Among the compounds sythesized, 1-cyclopropyl-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-7[[N^4-[3′-[(4,6-dimethylpyrimidin-2-yl)benzenesulfonamido-4-yl]imino-1′-(5-fluoroisatinyl)]methyl]-3-methyl N^1-piperazinyl]-3-quinoline carboxylic acid (**9**) emerged as the most potent broad-spectrum chemotherapeutic agent active against HIV (EC_{50}: 12.1 μg/ml), and *Mycobacterium tuberculosis* (MIC: 1.22 μg/ml).

1. Introduction

Tuberculosis (TB) is the most common oportunistic infection (OI) in people with AIDS and is the leading killer of people living with HIV. Up to 50% of people with HIV in sub-Saharan Africa develop TB and 1 out of 3 dies of TB. The development of active TB accelerates the progression of HIV disease towards full-blown AIDS, accompanied by enhanced HIV replication. The majority of people infected with the HIV virus develop TB as the first manifestation of AIDS. Thus, it appears that an ideal drug for HIV/AIDS patients should suppress HIV replication treat OIs like TB. Earlier studies in our laboratory have identified various isatinimino derivatives exhibiting broad-spectrum chemotherapeutic properties (Sriram and Yogeeswari 2003). As a continuation to our efforts in developing broad-spectrum chemotherapeutics, we undertook the present study to design, synthesize and evaluate isatin analogues, which could suppress HIV replication and also inhibit the opportunistic microorganism *M. tuberculosis*.

2. Investigations, results and discussion

2.1. Synthesis

The synthesis of various isatin derivatives was achieved in two steps (Scheme) (Pandeya et al. 1998). Isatin and its 5-fluoro derivative were condensed with 4-amino-*N*-(4,6-dimethylpyrimidin-2-yl) benzenesulfonamide in the presence of glacial acetic acid to form a Schiff base. The N-Mannich bases of the above Schiff base were synthesized by condensing the acidic imino group of isatin with formaldehyde and various aryl piperazines. All compounds (Table 1) gave satisfactory elemental analysis. IR and ^{1}H NMR spectra were consistent with the assigned structures.

2.2. Biological investigation and discussion

The synthesized compounds were evaluated for their inhibitory effect on the replication of HIV-1 in MT-4 and CEM cell lines (Table 2) (Pandeya et al. 1999). In the MT-4 cell lines, compound **9** was found to be the most active against replication of HIV-1 with an EC_{50} value of 12.1 μg/ml and the selectivity index ($SI = CC_{50}/EC_{50}$) was found to be more than 13 with maximum protection of 106%. Other compounds (**1**–**4**, **7**, **8** and **10**) showed a maximum protection of 69–102%, and a SI of 3–10. In the T4 lymphocytes (CEM cell lines), the compounds showed marked anti-HIV activity (10–44%) 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.

Scheme

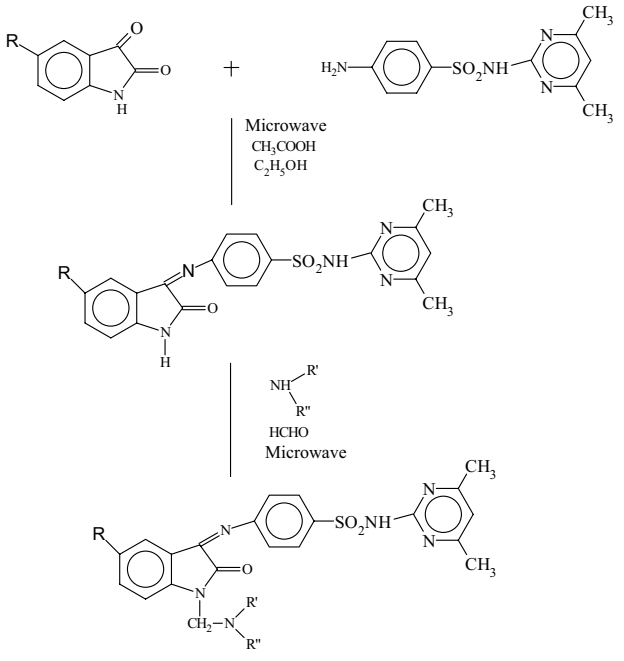
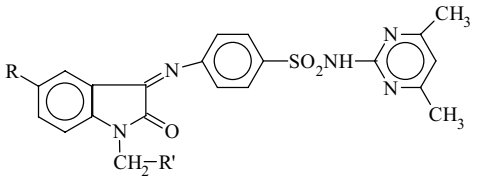


Table 1: Physical constants of the synthesized compounds 1–7



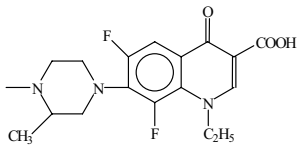
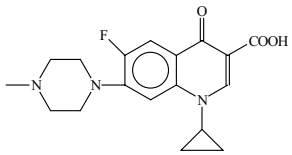
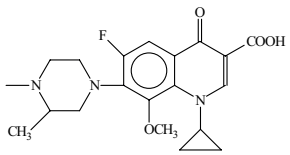
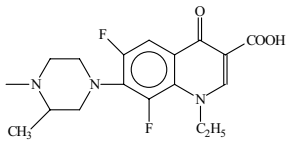
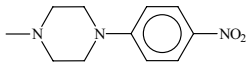
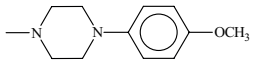
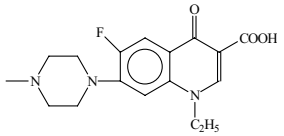
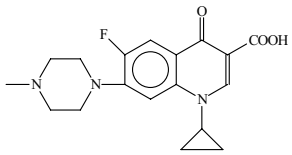
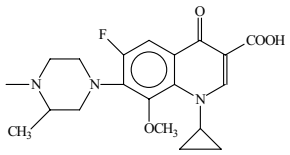
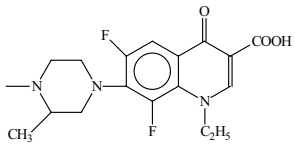
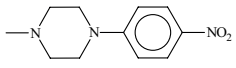
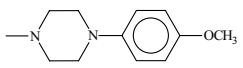
Compd.	R	R′	Molecular formula	Molecular weight	Yield (%)	M.P. (°C)	Log P
1	H		$C_{37}H_{35}N_8O_6SF$	738	59.58	214	1.73
2	H		$C_{38}H_{35}N_8O_6SF$	750	78.71	220	1.68
3	H		$C_{38}H_{35}N_8O_6SF$	794	54.57	186	2.30
4	H		$C_{38}H_{36}N_8O_6SF_2$	770	43.9	175	2.02
5	H		$C_{31}H_{30}N_8O_5S$	626	72.65	144	4.42
6	H		$C_{32}H_{33}N7O4S$	611	73.55	165	5.23
7	F		$C_{37}H_{34}N_8O_6SF_2$	756	67.11	226	2.29
8	F		$C_{38}H_{34}N_8O_6SF_2$	768	78.22	235	2.25
9	F		$C_{38}H_{34}N_8O_6SF_2$	812	70.10	215	2.78
10	F		$C_{38}H_{35}N_8O_6SF_3$	788	68.00	225	2.37
11	F		$C_{31}H_{29}N_8O_5SF$	644	67.94	185	4.76
12	F		$C_{32}H_{32}N7O4SF$	629	62.73	171	5.31

Table 2: Anti-HIV and antimycobacterial activity

Compd.	CEM cell line			MT-4 cell line			Antimycobact erial activity at 6.25 μg/ml % inhibition
	EC_{50}[a]	CC_{50}[b]	% Inhibition	EC_{50}[a]	CC_{50}[b]	% Protection	
1	>116.0	116.0	12.82	22.60	128.60	86.12	100
2	NT	NT	NT	31.06	114.20	69.66	100
3	>133.0	133	11.64	15.60	151.60	98.12	100
4	>117.0	117.0	44.10	15.90	122.0	96.36	100
5	NT	NT	NT	>42.62	42.62	20.10	64
6	>34.80	34.80	12.32	>44.40	44.40	13.60	62
7	>117.0	117.0	10.37	19.12	132.10	88.12	100
8	NT	NT	NT	30.12	119.0	76.42	100
9	>135.0	135.0	41.07	12.10	166.80	106.20	100
10	>115.0	115.0	12.47	14.80	126.16	102.10	100
11	NT	NT	NT	>44.12	44.12	20.68	43
12	> 35.20	35.20	13.17	>43.32	43.32	24.12	59

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

Two compounds (**9** and **10**) were evaluated for their inhibitory effects on the HIV-1 RT enzyme (Balzarini et al. 1992) and their IC_{50} values were found to be 18.4 ± 2.4 and 20.2 ± 4.6 μM respectively. The *in vitro* IC_{50} values for HIV-1 RT with Poly (vC) oligo (dG) as the template/primer were significantly higher than the corresponding EC_{50} values for inhibition 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 (Garcia et al. 1999).

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 (Table 2) (Sriram et al. 2004). Eight compounds (**1**–**4** and **7**–**10**) showed complete inhibition (100%) of *M. tuberculosis* in the primary screening. In the secondary level screening the actual minimum inhibitory concentration (MIC) and cytotoxicity in VERO cells of these three compounds were determined. The MIC's of compounds **1**–**4**, **7**, **8** and **10** were found to be 3.13 μg/ml and for compound **9** it was 1.22 μg/ml, and all these compounds were not cytotoxic to VERO cells up to 62.5 μg/ml.

To conclude, eight out of the twelve new derivatives showed inhibition against replication of HIV-1 in MT-4 cells with EC_{50} values ranging from 12.1–31.6 μg/ml. Eight compounds inhibited *M. tuberculosis* $H_{37}Rv$ with MIC values of 1.22–3.13 μg/ml. Among the synthesized compounds compound **9** emerged as the most promising broad-spectrum chemotherapeutic agent.

3. Experimental

3.1. Chemistry

Melting points were determined in one end open capillary tubes on a Büchi 530 melting point apparatus and are uncorrected. IR and ^{1}H NMR spectra were recorded 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 with a Perkin-Elmer model 240C analyzer. The homogeneity of the compounds was monitored by ascending TLC on silicagel-G (Merck) coated aluminium plates, visualized by iodine vapour. Developing solvents were chloroform-methanol (9 : 1). A domestic microwave oven with the following specifications had been used: Make LG; Input 220 V ~ 50 Hz, 980 W, 4.7 A; Frequency 2450 MHz.

3.2. Synthesis of 3-{[(4,6-dimethylpyrimidin-2-yl) benzenesulfonamido-4′-yl] imino}-5-fluoro-1,3-dihydro-2H-indol-2-one

Equimolar quantities (0.01 mol) of 5-fluoroisatin and 4-amino-*N*-(4,6-dimethylpyrimidin-2-yl)benzenesulfonamide 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/cycle for 3 min and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol-chloroform mixture. Yield 84.2%; m.p.: 213 °C.

3.3. General procedure for the preparation of Mannich bases

To a suspension of 3-{[(4,6-dimethylpyrimidin-2-yl) benzenesulfonamido-4′-yl] imino}-5-fluoro-1,3-dihydro-2H-indol-2-one (0.02 mol) in ethanol the appropriate aryl piperazine (0.02 mol) and 37% formaldehyde (0.5 ml) were added and irradiated in a microwave oven at an intensity of 80% with 30 s/cycle. The number of cycles in turn depended on the completion of the reaction, which was checked by TLC. The reaction time varied from 1.5–3 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.

3.4. 1-Ethyl-6-fluoro-1,4-dihydro-4-oxo-7[[N^4-[3′-[(4,6-dimethylpyrimidin-2-yl) benzenesulfonamido-4-yl]imino-1′-isatinyl] methyl]-3-methyl N^1-piperazinyl]-3-quinoline carboxylic acid (**1**)

Yield: 59.58%; m.p.: 214 °C; IR (KBr): 3320, 1730, 1654, 1153, 1129 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ (ppm): 1.28 (t, 3 H, CH_3 of C_2H_5), 2.25 (s, 6 H, CH_3), 3.7–4.1 (m, 8 H, -piperazine-H), 4.25 (q, 2 H, CH_2 of C_2H_5), 5.1 (s, 2 H, $-NCH_2N$), 6.58–8.60 (m, 11 H, Ar–H), 8.66 (s, 1 H, C_2–H), 11.06 (s, 1 H, SO_2NH) 14.20 (bs, 1 H, COOH).

3.5. 1-Cyclopropyl-6-fluoro-1,4-dihydro-4-oxo-7-[[N4-[(4,6-dimethylpyrimidin-2-yl) benzenesulfonamido-4-yl]imino-1′-isatinyl] methyl] N′-piperazinyl]-3-quinoline carboxylic acid (**2**)

Yield: 78.71%; m.p.: 220 °C; IR (KBr): 3326, 1730, 1652, 1153, 1129, cm^1 ; ^{1}H NMR ($CDCl_3$) δ (ppm): 0.88–1.1 (m, 4 H, cyclopropyl-H), 2.28 (s, 6 H, CH_3), 3.5 (m, 1 H, cyclopropyl-H), 3.7–4.1 (m, 8 H, -piperazine-H), 5.1 (s, 2 H, $-NCH_2N$), 6.68–8.42 (m, 11 H, Ar–H), 8.6 (s, 1 H, C_2–H) 11.12 (s, 1 H, SO_2NH), 14.22 (bs, 1 H, COOH).

3.6. 1-Cyclopropyl-6-fluoro-8-methoxy-1,4-dihydro-4-oxo-7[[N^4-[3′-[(4,6-dimethylpyrimidin-2-yl) benzenesulfonamido-4-yl]imino-1′-isatinyl] methyl]-3-methyl N^1-piperazinyl]-3-quinoline carboxylic acid (**3**)

Yield: 76%; m.p.: 222 °C; IR (KBr): 3326, 1730, 1652, 1153, 1129 cm^1; ^{1}H NMR ($CDCl_3$) δ (ppm): 0.92–1.10 (m, 4 H, cyclopropyl-H), 1.14 (d, 3 H, CH_3 of pip), 2.25 (s, 6 H, CH_3), 3.46 (s, 3 H, $-OCH_3$), 3.6–3.8 (m, 1 H, cyclopropyl-H), 3.62–4.18 (m, 7 H, -piperazine-H), 5.2 (s, 2 H, $-NCH_2N$), 6.68–8.42 (m, 10 H, Ar–H), 8.6 (s, 1 H, C_2–H) 11.12 (s, 1 H, SO_2NH), 14.22 (bs, 1 H, COOH).

3.7. 3-{[(4,6-Dimethylpyrimidin-2-yl) benzenesulfonamido-4′-yl] imino}-5-chloro-1-[(4-nitrophenyl piperazinyl) methyl]-1,3-dihydro-2H-indol-2-one (**11**)

Yield: 67.94%; m.p.: 185 °C; IR (KBr): Yield: 69.60%; m.p.: 162 °C; IR (KBr): 3332, 1730, 1648, 1153, 1129 cm^1; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.25 (s, 6 H, CH_3), 3.9–4.26 (m, 8 H, piperazine-H), 5.26 (s, 2 H, $-NCH_2N-$), 6.67–7.80 (m, 12 H, Ar–H), 11.12 (s, 1 H, SO_2NH).

3.8. Anti-HIV screening

3.8.1. 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 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.

3.8.2. 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.

3.8.3. 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 albumin, an appropriate concentration of the radiolabelled substrate [^{3}H] dGTP, 0.1 mM poly(dC) 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), 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 analysed for radioactivity. For the experiments in which 50% inhibitory concentration (IC_{50}) of the test compounds was determined, a fixed concentration of 2.5 μM [^{3}H] dGTP was used.

3.9. Antimycobacterial screening

Primary screening was conducted at 6.25 μg/ml 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).

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