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Synthesis and Antiviral Activity of Some Novel Isatin Derivatives

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Some novel Schiff's and mannich base isatin derivatives were synthesized. Their chemical structure was elucidated by means of spectral (FT-IR, ^{1}H-NMR, MS) analysis. The synthesized compounds were screened for antiviral activity against influenza virus A and B in MDCK cells, HIV-1 (IIIB) and HIV-2 (ROD) in acutely infected MT-4 cells. All the compounds displayed cytotoxic properties in the lymphocyte cell line (MT-4) cells. Compound SPIII-5H-AC exhibits weak activity against influenza A (H_3N_2) virus in MDCK cells.

Key Words: Isatin, Anti-HIV activity, Influenza virus.

INTRODUCTION

Isatin (2,3-dioxoindole), a versatile lead molecule for potential bioactive agents. Schiff's and mannich base isatin derivatives were reported to possess anticancer[1], antibacterial[2–4] and anti-HIV[5–12] activities. Methisazone (N-methylisatin-β-thiosemicarbazone) was one of the first clinically used synthetic antiviral agents[13]. Isatin derivatives were reported for antiviral activities against a variety of pathogenic viruses[14]. Previously, some novel isatin derivatives and evaluated for activity against HIV-1 and HIV-2 in MT-4 cells have also been synthesized[15], significant activity was noted with these compounds against HIV-1 replication[16]; In continuation of this work herein, the synthesis **(Scheme-1)** and antiviral activity against influenza and HIV viruses of newer isatin derivatives have been reported.

EXPERIMENTAL

Melting points were determined by open ended capillary tube and are

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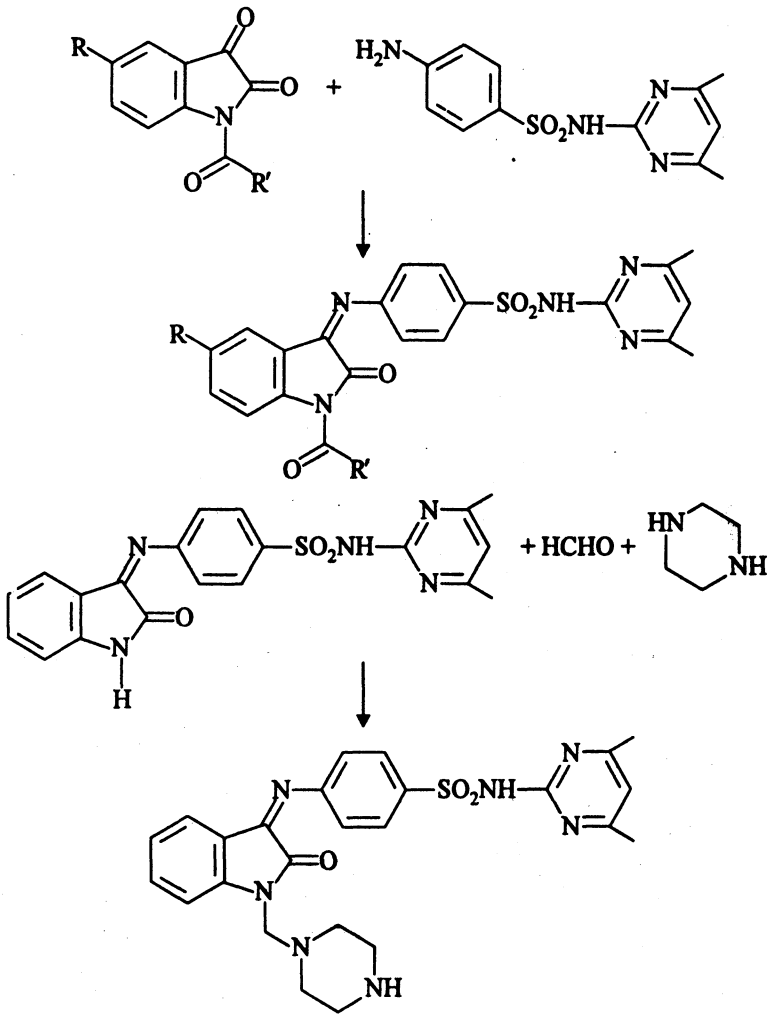

Scheme-1. Synthetic protocol of the compounds

uncorrected. Purity of the compounds were checked by TLC using silica gel G as stationary phase. The structures of the compounds were elucidated by FT-IR (Shimadzu) in KBr disc and FT-PMR (Brucker 400 Amx) DMSO-d_6, mass spectra on a Varian Atlas CH-7 mass spectrometer at 70 eV.

4-(1-Acetyl-5-chloro-2-oxo-1,2-dihydro-indol-3-ylideneamino)-N-(4,6-dimethyl-pyrimidin-2-yl) -benzenesulfonamide (SPIII-5Cl-Ac)

Equimolar quantities (0.01 mol) of 1-acetyl-5-chloroisatin and sulphadimidine were dissolved in 10 mL of glacial acetic acid and refluxed for 4 h. The resultant solid was filtered, washed with ethanol and recrystallized from ethanol.

IR (KBr, cm^{-1}): 3300 ν(NH), 1595 ν(C═O), 1541 ν(C═N), 1473 ν(C═C), 1153.4 ν(SO_2), 573.6 ν(C—Cl); PMR (DMSO-d_6, δ ppm): 2.0 (s, 3H, 1 × CH_3), 2.4 (s, 6H, 2 × CH_3), 6.4–7.4 (m, 4H, Ar—H), 7.5–8.2 (m, 3H, -phenyl indole ring), 10.4 (b, 1H, —SO_2NH); EI-MS (m/z): 483.928.

4-(1-Benzoyl-2-oxo-1,2-dihydro-indol-3-ylidene amino)-N-(4,6-dimethyl-pyrimidin-2-yl)-benzene sulfonamide (SPIII-5H-BZ)

Equimolar quantities (0.01) of 1-benzoyl isatin and sulphadimidine were dissolved in 10 mL of glacial acetic acid and refluxed for 4 h. The resultant solid was filtered, washed with ethanol and recrystallized from ethanol.

IR (KBr, cm^{-1}): 3350 ν(NH), 1790 ν(C═O), 1595 ν(C═N), 1541 ν(C═C), 1153.4 ν(SO_2), PMR (DMSO-d_6, δ ppm): 2.4 (s, 6H, 2 × CH_3), 6.4–7.4 (m, 9H, Ar—H), 7.5–8.2 (m, 4H, -phenyl indole ring), 10.4 (b, 1H, —SO_2NH); EI-MS (m/z): 511.554.

N-(4,6-Dimethyl-pyrimidin-2-yl)-4-(2-oxo-1-piperazin-1-ylmethyl-1,2-dihydro-indol-3-ylidene amino)benzene sulfonamide (SPIII-5H-PP)

To a slurry consisting of 3-(4-sulphadimidinyl)isatin[16] (0.002 mol), ethanol (5 mL) and 37% formalin (1 mL) was added. To this piperazine (0.002 mol) was added slowly with constant shaking. The reaction mixture was allowed to stand at room temperature for 1 h with occasional shaking after which it was warmed on a steam bath for 15 min, cooled and the product was recovered with absolute ethanol.

IR (KBr, cm^{-1}): 3375 ν(NH), 1733 ν(C═O), 1716 ν(C═N), 1697 ν(C═C), 1153 ν(SO_2); PMR (DMSO-d_6, δ ppm): 2.35 (s, 6H, 2 × CH_3), 2.5 (s, 4H, —CH_2, piperazinyl), 2.7 (s, 4H, —CH_2 piperazinyl), 4.6 (s, 2H, —CH_2), 5.9 (s, 1H, NH piperazinyl), 6.5–7.4 (m, 4H, Ar—H), 7.5 (m, 4H, phenyl indole ring), 10.3 (b, 1H, —SO_2NH) EI-MS (m/z): 505.593.

RESULTS AND DISCUSSION

All the synthesized compounds were identified by spectral studies.

Anti-HIV Activity

The compounds were tested for anti-HIV activity against replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells[15]. 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 20 μg/mL gentamycin (culture medium). HIV-1 (HTLV-IIIB/LAI) and HIV-2 (LAV-2_{ROD}) were used in the experiment. Inhibitory effects of the compounds on HIV-1 and HIV-2 replications were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by MTT assay. Briefly, 50 μL of HIV-1 and HIV-2 (100–300 $CCID_{50}$) was added to a flat-bottomed microtitre tray with 50 μL of medium containing various concentrations of test compounds. MT-4 cells were added at a final concentration of 6×10^5 cells/mL. After 5th day 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. Cytotox-

icity of the compounds for mock-infected MT-4 cells was assessed by the MTT method. Anti-HIV activity and cytotoxicity of standard AZT were also performed by a similar method in MT-4 cells. The anti-HIV data are present in Table-2. The 50% effective concentration (EC_{50}) values of the synthesized compounds against the replication of HIV-1 and HIV-2 in acutely infected MT-4 cells was found to have higher than the cytototoxic concentration (CC_{50}). Compound SPIII-5H-BZ exhibited 47% maximum protection against HIV-1 (IIIB) and SPIII-5CL-AC exhibited 31% maximum protection against HIV-2 (ROD) at subtoxic concentration.

TABLE-1
PHYSICAL DATA OF THE COMPOUNDS

Compound	Yield (%)	m.p. (°C)	R_f value	mi log P[a]	m.f.
SPIII-5H-BZ	52	210	0.74	4.203	$C_{27}H_{21}O_4N_5S$
SPIII-5Cl-AC	34	238	0.88	3.715	$C_{22}H_{18}O_4N_5S$
SPIII-5H-PP	89	225	0.72	2.432	$C_{25}H_{27}O_3N_7S$

mi log P was calculated by mol inspiration software (edited by Ertl P. Novartis).

TABLE-2
ANTI-HIV ACTIVITY AND CYTOTOXICITY IN MT-4 CELLS

Compound code	Strain	EC_{50}^{a} (μg/mL)	EC_{50}^{b} (μg/mL)	Max protection (%)
SIII-5Cl-AC	IIIB	>54.5	54.5	21
	IIIB	>57.6	57.6	3
	ROD	>65.1	65.1	3
	ROD	>66.9	66.9	31
SPIII-5H-PP	IIIB	>64.4	64.4	15
	IIIB	>47.8	47.8	4
	ROD	>65.8	65.8	3
	ROD	>64.8	64.8	15
SPIII-5H-BZ	IIIB	>108.0	108.0	47
	IIIB	>89.4	89.4	18
	ROD	>90.1	90.1	4
	ROD	>109.0	109.0	24
AZT (STD)	IIIB	0.0012	65.9	126
	ROD	0.00062	65.9	148

[a]50% effective concentration required to reduce virus induced cytopathicity by 50%.

[b]50% cytotoxic concentration required to reduce host cell viability by 50%.

Antiviral screening

Antiviral activity of the synthesized compounds was tested against influenza A(H_1N_1 and H_3N_2) and B virus in MDCK cell by visual cytopathic effect and neutral red method[17, 18]. Parameters such as effective concentration (EC_{50}), a concentration required to reduce virus-induced cytopathogenicity to 50% and cytotoxic concentration (IC_{50}), a concentration of substance required to cause microscopically detectable alteration of normal cell morphology in mock infected MDCK. Inhibitory effect and cytotoxic effect of test compounds were compared with standard ribavirin under similar conditions. The compounds SPIII-5Cl-AC, SPIII-5H-BZ inhibited the replication of influenza A and B viruses in MDCK cell. All the compounds exhibited cytotoxity > 100 μg/mL (Table-3).

TABLE-3
ANTIVIRAL AND CYTOTOXICITY OF COMPOUNDS IN MDCK CELLS

Compounds	Methods	Virus	EC_{50}^a	IC_{50}^b	SI[c]
	CPE inhibition	IVA (H_1N_1)	32	>100	>3.1
SPIII-5Cl-AC	Neutral Red	IVA (H_1N_1)	32	>100	>3.1
	CPE inhibition	IVA (H_3N_2)	32	>100	>3.1
	Neutral Red	IVA (H_3N_2)	28	>100	>3.6
	CPE inhibition	IVB	32	>100	>3.1
	Neutral Red	IVB	35	>100	>2.9
SPIII-5H-BZ	CPE inhibition	IVA (H_1N_1)	35	>100	>2.9
	Neutral Red	IVA (H_1N_1)	48	>100	>2.1
	CPE inhibition	IVA (H_3N_2)	18	>100	>5.6
	Neutral Red	IVA (H_3N_2)	22	>100	>4.5
	CPE inhibition	IVB	45	>100	>2.2
	Neutral Red	IVB	70	>100	>1.4
SPIII-5H-PP	CPE inhibition	IVA (H_1N_1)	>100	>100	0
	Neutral Red	IVA (H_1N_1)	>100	>100	0
	CPE inhibition	IVA (H_3N_2)	>100	>100	0
	Neutral Red	IVA (H_3N_2)	>100	>100	0
	CPE inhibition	IVB	>100	>100	0
	Neutral Red	IVB	>100	>100	0
RIBAVIRIN	CPE inhibition	IVA (H_1N_1)	7.0	>100	>14
(STD)	Neutral Red	IVA (H_1N_1)	6.5	>100	>15
	CPE inhibition	IVA (H_3N_2)	5.5	>100	>18
	Neutral Red	IVA (H_3N_2)	5.5	>100	>18
	CPE inhibition	IVB	5.5	>100	>18
	Neutral Red	IVB	5.5	>100	>18

[a]50% effective concentration required to reduce virus induced cytopathicity by 50%.
[b]50% cytotoxic concentration required to reduce host cell viability by 50%.
[c]Selectivity index-ratio of IC_{50} to EC_{50}.

Conclusion

All the compounds displayed cytotoxic properties in the lymphocyte cell line (MT-4 cells). Substitution in NH group of isatin (N-acylation and piperazinylmethylene) abolishes the anti-HIV activity of lead compound 3-(4-sulphadimidinyl)isatin[16] (SPIII-5H). SPIII-5H-AC was found to possess weak activity against influenza A virus (SI > 5).

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