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Pharmacological Screening of Some Novel Isatin Derivatives

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4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene Sulphonamide and its derivatives were evaluated for antibacterial activity, antifungal activity, antiviral activity against ten pathogenic viruses in E_6SM, Vero and HeLa cells and anticancer activity against CNS, breast and lung cancer. The N-acetyl and 5-methyl derivatives showed a minimum inhibitory concentration (MIC) of 48 μg/ml against *herpes simplex virus* type-2 and *respiratory syncytial virus*, without toxicity at a concentration upto 400 μg/ml. The test compounds showed comparable antibacterial activity to that of the parent sulphadimidine, except for the 5-bromo and N-acetyl derivatives.

Isatin (2,3-dioxoindole), a versatile lead molecule for potential bioactive agents, and its derivatives were reported to possess anticancer[1], antibacterial, antifungal and antiHIV activities[2-11]. Methisazone (N-methylisatin-β-thiosemicarbazone) was one of the first clinically used synthetic antiviral agents[12]. Isatin derivatives were reported for antiviral activities against a variety of pathogenic viruses[13] and N,N-disubstituted thiosemicarbazone derivatives of isatin were tested for inhibition of HIV-1 replication[14]. Previously we synthesized some novel isatin derivatives and evaluated for their activities against HIV-1 and HIV-2 in MT-4 cells[15], significant activity was noted with these compounds against HIV-1 replication[16].

In view of the broad spectrum biological activities of isatin derivatives, we aimed at evaluating the antiviral activity of some novel 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene) amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamide and its derivatives (fig. 1) against pathogenic viruses in E_6SM, Vero and HeLa cells cultures and compared their activity with that of the standard antiviral agents brivudin (BVDU) and ribavirin. Anticancer activity test were also performed against breast, lung and CNS cancer cell in culture technique (NCI, NIH, USA). The compounds were also tested for antibacterial and antifungal activities in compari-

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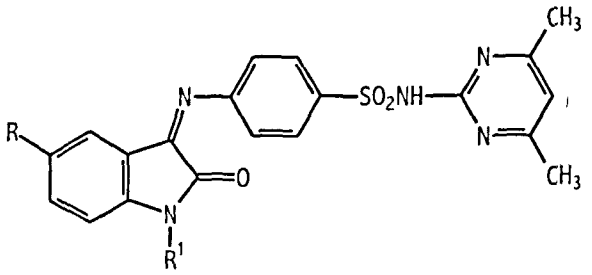

Fig. 1: Structure of the 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamide and its derivatives

son with the parent sulphadimidine. These compounds were also tested for their activities against HIV-1 and HIV-2 in MT-4 cells.

4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamide and its derivatives were prepared by combining the isatin and its derivatives (5- chloro, 5-bromo, 5-methyl and N-acetyl) with sulphadimidine in the presence of glacial acetic acid[16]. Log P is a hydrophobic parameter (derived from Oct/water partition co-efficient), used to study the correlation between lipophilicity of the molecules with their pharmacodynamic/ kinetic properties. The log P values of the test compounds were calculated by the methodology developed by molinspiration software programme as a sum of fragment based contribution and correction factor[17]. The Log P values of test compounds are given in Table 1.

E_6SM cells were used for evaluating activity against *herpes simplex virus* (HSV) 1, 2 and *vaccinia virus*. Vero

TABLE 1: LOG P VALUES OF THE COMPOUNDS

COMP.	R	R^1	Molecular Weight	Log P value
SPIII-5H	-H	-H	407.446	2.59
SPIII-5Cl	-Cl	-H	441.891	3.217
SPIII-5Br	-Br	-H	486.342	3.346
SPIII-5Me	$-CH_3$	-H	421.473	2.915
SPIII-NA	-H	$-COCH_3$	449.483	3.087

cells were used for *parainfluenza virus*-3, *reovirus*-1, *sindbis virus*, *coxsackie virus* B4, *punta toro virus* and HeLa cells were used for *vesicular stomatitis virus* (VSV) and *respiratory syncytial virus* (RSV).Antiviral activity and cytotoxicity of the test compounds were determined by an *in vitro* cell culture technique[18]. Parameter of activity was the minimum inhibitory concentration (MIC). Parameter of toxicity was the minimum cytotoxic concentration (MCC). Antiviral activity (MIC) and cytotoxicity (MCC) of the test compounds were compared with the standard compounds brivudin (BVDU) and ribavirin when tested under similar conditions. The antiviral activity and cytotoxicity of the test compounds are presented in Table 2 and 3.

The test compounds were evaluated for anticancer activity against breast, non small cell lung and CNS cancer by an *in vitro* cell culture technique[19]. In the current protocol, each cell line was inoculated and preincubated on microtiter plates. Test agents are then added at a single (dose) concentration and the culture incubated for 48 h. End point

TABLE 2: ANTIVIRAL ACTIVITY OF COMPOUNDS IN E_6SM CELLS

Compound	Minimum Cytotoxic Concentration[a] (μg/ml)	Minimum Inhibitory Concentration[b] (μg/ml)		
		Herpes Simplex Virus-1	*Herpes Simplex Virus*-2	*Vaccinia Virus*
SPIII – 5H	≥400	240	240	>80
SPIII – 5Cl	≥80	>80	>80	>80
SPIII – 5Br	≥80	>80	>80	>80
SPIII – 5Me	≥80	>80	>80	>80
SPIII – NA	≥400	>80	48	>80
BVDU(STD)	400	0.0256	>80	0.384

[a]Required to cause a microscopically detectable alteration of normal cell morphology. [b]Required to reduce a virus-induced cytopathogenicity by 50%.

TABLE 3: ANTIVIRAL ACTIVITY OF COMPOUNDS IN VERO AND HELA CELLS

Comp.	MCC[a] (μg/ml) Vero cells	Minimum Inhibitory Concentration[b] (μg/ml) in Vero cells					MCC[a] (μg/ml) HeLa cells	Minimum Inhibitory Concentration[b] (μg/ml) HeLa Cells	
		Para influenza-3 Virus	*Reo virus-1*	*Sindbis Virus*	*Coxsackie Virus B4*	*Punta Toro Virus*		*VSV*	*RSV*
SPIII – 5H	≥400	>80	>80	>80	>80	>80	400	>80	>80
SPIII – 5Cl	≥400	>80	>80	>80	>80	>80	400	>80	>80
SPIII – 5Br	≥400	>80	>80	>80	>80	>80	≥400	>80	>80
SPIII– 5Me	≥400	>80	>80	>80	>80	>80	>400	>400	48
SPIII – NA	≥400	>80	>80	>80	>80	>80	>400	>400	>400
Ribavirin	≥400	48	80	240	>400	48	>400	48	1.92

[a]Required to cause a microscopically detectable alteration of normal cell morphology. [b]Required to reduce a virus-induced cytopathogenicity by 50%. MCC-Minimum cytotoxic concentration, VSV- *Vesicular Stomatitis Virus* and RSV - *Respiratory Syncytial Virus.*

determinations are made with alamar blue. Results for each test agent are reported as the percent of growth of the treated tumour cells as compared to the untreated control cells. Compounds which reduce the growth of the any one of the cell lines to approximately 32% (or less) indicated a meaningful cytotoxic effect of the compounds. The results are presented in Table 4.

The compounds were screened for antibacterial activity against *E. coli, P. aeruginosa, S. typhi and S. aureus*, and antifungal activity was tested against *Candida albicans* by the cup plate method[20] at 100 μg/ml in dimethylformamide using nutrient agar medium. The results were evaluated by measuring average zone of inhibition in mm and compared with parent sulphadimidine at the same concentration. The results are presented in Table 5.

TABLE 4:*IN VITRO* ANTICANCER ACTIVITY DATA

Comp.	Growth Percentage[a]		
	Breast (MCF7)	Non-Small cell Lung (NCI-H460)	CNS (SF-268)
SPIII-5H	104	94	115
SPIII-5Cl	99	101	97
SPIII-5Me	102	100	107
SPIII-NA	108	98	120

[a]The percent of growth of the treated tumour cells as compared to the untreated control cells.

The compounds were tested for antiHIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells[15]. The cells were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum (FCS), 2 mM glutamine, 0.1% sodium bicarbonate and 20 μg/ml gentamicin (culture medium). HIV-1(HTLV-IIIB/LAI) and HIV-2 ($LAV\text{-}2_{ROD}$) were used in all experiments. The virus strains were propagated in MT-4 cells. Titer of virus stock was determined in MT-4 cells and the virus stock was stored at -70° until used.

The inhibitory effects of the compounds on HIV-1 and HIV-2 replication were monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and were estimated by the MTT method. Briefly, 50 μl of HIV-1 and HIV-2 (100-300 $CCID_{50}$) were added to a flat-bottomed microtiter tray with 50 μl of medium containing various concentrations of the test compounds. MT-4 cells were added at a final concentration of $6x10^5$ cells/ml. After 5 days of incubation, at 37°

TABLE 5: ANTIMICROBIAL ACTIVITY OF THE SYNTHESIZED COMPOUNDS

Compounds	Zone of Inhibition[a]				
	E. coli	*S. aureus*	*P. aeruginosa*	*S. typhi*	*C. albicans*
SP III – 5H	17	16	24	24	16
SP III – 5Cl	17	20	21	19	15
SP III – 5Br	NA	NA	NA	NA	NA
SP III – 5Me	20	18	21	23	NA
SP III – NA	NA	NA	NA	NA	NA
SULPHADIMIDINE	17	17	NA	19	NA

[a]Average zone of inhibition measured in mm, NA-not active.

the number of viable cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Cytotoxicity of the compounds for mock-infected MT-4 cells was also assessed by the MTT method. AntiHIV activity and cytotoxicity of the standard compound AZT were also performed by a similar method in MT-4 cells. The results on antiHIV activity and cytotoxicity are shown in Table 6.

From the antiviral and cytotoxicity assays we learned that the compound 4-[(1,2-dihydro-2-oxo-3H-indol-3-ylidene)amino]-N(4,6-dimethyl-2-pyrimidiny)-benzene sulphonamide had a minimum inhibitory concentration (MIC) of 240 μg/ml against *herpes simplex virus* (HSV) 1 and 2. N-acetyl derivative showed on MIC of 48 μg/ml against HSV-2 in E_6SM cells, where the minimum cytotoxic concentration (MCC) was found to be 400 μg/ml in mock-infected E_6SM cells. The 5-methyl derivative exhibited on MIC of 48 μg/ml against respiratory syncytial virus and the MCC was found to be greater than 400 μg/ml in HeLa cells. The test compounds showed comparable cytotoxicity with that of the stan-

TABLE 6: ANTIHIV ACTIVITY AND CYTOTOXICITY IN MT-4 CELLS

Compounds	Strain	EC^a_{50} (μg/ml)	CC^b_{50} (μg/ml)	Max. Protection
SP III – 5H	HIV-1 (III B)	8.03	> 125	128
	HIV-2 (ROD)	41.45	> 125	99
SP III – 5Cl	HIV-1 (III B)	10.28	> 125	88
	HIV-2 (ROD)	118	> 125	40
SP III – 5Br	HIV-1 (III B)	15.25	> 125	76
	HIV-2 (ROD)	78.15	> 125	65
SP III – 5Me	HIV-1 (III B)	8.07	> 125	138
	HIV-2 (ROD)	> 125	> 125	30
SP III – 5NA	HIV-1 (III B)	7.99	> 125	120
	HIV-2 (ROD)	90.90	> 125	61
AZT (STD)	HIV-1 (III B)	0.0012	65.9	126
	HIV-2 (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%.

dard compound ribavirin in mock-infected Vero cells. Compounds which reduced the growth of any one of the tumour cell lines to approximately 32% (or less) could be considered as cytotoxic agents. However, none of the compounds was found to inhibit tumour cell growth by 10% and, therefore, they were considered inactive in the *in vitro* anticancer screening.

The results of the antibacterial screening indicated that compounds SPIII-5H, SPIII-5Cl, SPIII-5Me had comparable activity against *E. coli, S. aureus* and *S. typhi,* with that of the parent sulphadimidine (zone of inhibition:17-24 mm), the 5-bromo and N-acetyl derivatives were inactive in the antibacterial and antifungal screening tests. From the antibacterial studies it appeared that sulphadimidine was ineffective against *P. aeruginosa,* on the other hand, the compounds SPIII-5H, SPIII-5Cl, SPIII-5Me had antibacterial activity (zone of inhibition: 21-24 mm). The compounds SPIII-5H, SPIII-5Cl (zone of inhibition: 15-16 mm) had antifungal activity against *Candida albicans.*

Effective concentration (EC_{50}) of synthesized compounds on HIV-1 and HIV-2 replication were measured by inhibition of virus induced cytopathic effect in MT-4 cells. Cytotoxicity of test compounds in mock infected MT-4 cells was also measured by MTT- Method. AntiHIV activity and cytotoxicity of compounds were compared with standard AZT against the replication of HIV-1 and HIV-2 in acutely infected MT-4 cells. Effective concentration (EC_{50}) for inhibition of replication of HIV-1 ranges from 8.0 to 15.3 mg/ml and 41.5 to>125 mg/ml for HIV-2. Where as the standard AZT had an effective concentration (EC_{50}) of 0.0012 and 0.00062 µg/ml against the HIV-1 and HIV-2 respectively in MT-4 cells. Cytotoxic concentration of test compounds was found to be more than 125 µg/ml, where as the standard AZT showed 65.9 mg/ml in mock infected MT-4 cells.

The present study was aimed at investigating some novel isatin derivatives for antibacterial, antifungal, antiviral and anticancer activities to identify potential bioactive agent in the series. Form the results of biological activities it appeared that some of the derivatives showed comparable antibacterial activity with that of the standard sulphadimidine, N-acetyl and 5-methyl derivatives inhibited the replication of HSV-2 and RSV but only at a relatively high concentration (48 µg/ml). The test compounds were inactive in the *in vitro* anticancer activity. In the present study, the test compounds also inhibited the replication of HIV-1 and -2 in MT-4 cells (Table 6). Further molecular modification in this series may help in optimizing antiHIV activity.

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