Research Article

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International Journal of Pharmacy and Industrial Research

ISSN	Print	2231 – 3648
	Online	2231 – 3656

STUDIES ON ANTIVIRAL ACTIVITIES AND CYTOTOXICITY OF ISATINE-SULPHONAMIDES AGAINST ORTHOPOX VIRUSES

[*1]Periyasamy Selvam

[*1]Raghavendra Institute of Pharmaceutical Education and Research, Anantapur 515 721, AP

Abstract

Series of Isatine-Sulphonamide derivatives were synthesized and tested for antiviral activity against orthopox virus in human foreskin fibroblast (HFF) cells. Cytotoxicity was investigated in uninfected HFF cells. Compound SPIII-5Br-AC (EC_{50} 12 uM and CC_{50} >300 uM) exhibits equipotent activity with that of standard Cidofovir (CDV) (EC_{50} 16.2 uM and CC_{50} >317 uM) against cowpox virus in CPE reduction assays.

Key words: Isatin, Sulphadimidine, Antiviral activity, Vaccinia virus, Cow pox virus

Introduction

Isatin (2,3-dioxoindole), a versatile lead molecule for potential bioactive agents and Methisazone (N-methylisatin-ß-thiosemicarbazone) was one of the first clinically used synthetic antiviral agents[1]. N-Methyl isatin-ß-4':4'-diethyl thiosemicarbazone was found to inhibit Moloney leukemia virus replication[2]. N,N-disubstituted thiosemicarbazone derivatives of isatin were tested for inhibition of HIV-1 replication[3]. Schiff and Mannich bases of isatin derivatives were synthesized and evaluated for antiviral activity. Some of their derivatives showed significant inhibitory activity against the replication of HIV-1[4-10]. In earlier studies, some novel isatin derivatives were synthesized and evaluated for antiviral, anticancer and antibacterial activities[11,12].These compounds showed significant inhibitory effects against HIV-1 replication. In this study we describe the orthopox (cowpox and vaccinia virus) inhibitory activity of some novel of isatine-sulphonamide derivatives (Scheme 1) in HFF cells.

Material and methods

Experimental

Melting points were determined using Thomas melting point apparatus and are uncorrected. The

Author for Correspondence:
Periyasamy Selvam,
Raghavendra Institute of Pharmaceutical Education and Research,
Anantapur 515 721, AP, India.
E mail: periyasamy_selvam@yahoo.co.in

purity was checked by TLC using silica gel G as stationary phase. The structure of the synthesized compounds was elucidated using a Perkin Elmer FT-IR in KBr disc and PMR was taken on a Bruker AMX-(400 MHz) FT-NMR. Mass spectra were obtained on a Varian Atlas CH-7 Mass spectrometer at 70 eV.

Synthesis of isatine-sulphonamide derivatives

Equimolar quantities (0.01 mole) of isatin (isatin, 5-chloro and 5-methyl, 5-chloro-1-acetyl-isatin, 5-bromo-1-acetyl isatin, 5-bromo-1-benzoyl-isatin and 5-methyl-1-benzoyl-isatin) and sulphonamide (sulphanilamide and sulphadimidine) 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 sec/cycle for 3 minutes and set aside. The resultant solid was washed with dilute ethanol dried and recrystallized from ethanol-chloroform mixture.

The structure of synthesized compounds were elucidated by spectral analysis 4-(2-oxoindolin ylideneamino)benzenesulfonamide (SPIII-S): yield: 68%, mp: 116^0 , IR (KBr) cm^{-1}: 3300 (NH), 1510 (C=N), 1674 (C=0), 1583 (C=C), PMR (DMSO-d_6) : 2 (b, 2H, NH_2), 7.1-7.9 (m, 8H, Ar-H), 8.1 (s, 1H, NH), EI- MS (m/e): 301.32.4-(5-methyl-2-oxoindolin-3-ylideneamino)benzene sulphonamide (SPIII-SMe) yield: 72 %, mp: 128^0 , IR (KBr) cm^{-1}: 3320 (NH), 1590 (C=N), 1650 (C=0), 1522 (C=C), PMR (DMSO-d_6) : 2.35 (s, 3H, CH_3), 2.1 (b, 2H, NH_2), 7.0-7.9 (m, 7H, Ar-H), 8.2 (s, 1H, NH), EI-MS (m/e): 315.35. 4- (5-chloro-2-oxoindolin-3 ylideneamino) benzenesulfonamide (SPIII-SCl) :yield: 82 %, mp: 181^0, IR (KBr) cm^{-1}: 3370 (NH), 1577 (C=N), 1680 (C=0), 1526 (C=C), PMR (DMSO-d_6) : 2.1 (b, 2H, NH_2), 7.0-7.9 (m, 7H, Ar-H), 8.0 (s,1H, NH), EI-MS (m/e): 355.77.4- (1-acetyl-5-bromo-2-oxoindolin-3-ylideneamino)-N-(4,6-dimethylpyrimidin-2-yl) benzene sulphonamide (SPIII-5Br-AC): yield: 67 %, mp: 221^0, IR (KBr) cm^{-1}: 3310 (NH), 1690 (C=N), 1705 (C=0), 1510 (C=C), PMR (DMSO-d_6): 2.30 (s, 6H, 2xCH_3), 2.42 (s, 3H, CH_3), 4.1 (b, 1H, -SO_2NH), 6.1 (d, 2H, pyrimidinyl), 7.0-7.92 (m, 7H, Ar-H), EI-MS (m/e):528.384-(1-benzoyl-5-bromo-2-oxoindolin-3-ylideneamino)-N-(4,6-dimethylpyrimidin-2-yl)benzene sulfonamide (SPIII-5Br-BZ): yield: 72 %, mp: 285^0 , IR (KBr) cm^{-1}: 3320 (NH), 1695 (C=N), 1707 (C=0), 1520 (C=C), PMR (DMSO-d_6): 2.32 (s, 6H, 2xCH_3), 4.1 (b, 1H, -SO_2NH), 6.1 (d, 2H, pyrimidinyl), 7.1-8.0 (m, 11H, Ar-H), EI-MS (m/e) :590.044-(1-acetyl-5-methyl-2-oxoindolin-3ylideneamino)-N(4,6dimethylpyrimidin-2-yl) benzene sulfonamide (SPIII-5Me-AC): yield: 72 %, mp: 228^0 , IR (KBr) cm^{-1}: 3360 (NH), 1685 (C=N), 1705 (C=0), 1530 (C=C), PMR (DMSO-d_6): 2.10 (s, 6H, CH_3), 2.32 (s, 3H, CH_3), 2.45 (s, 3H, CH_3), 4.1 (b, 1H, -SO_2NH), 6.1 (d, 2H, pyrimidinyl), 7.0-7.92 (m, 7H, Ar-H), 8.0 (s, 1H, NH), EI-MS (m/e): 463.514-(1-benzoyl-5-chloro-2-oxoindolin-3-ylideneamino)-N-(4,6-dimethylpyrimidin-2-yl) benzene sulfonamide (SPIII-5Cl-BZ): yield: 83 %, mp: 179^0 , IR (KBr) cm^{-1}: 3335 (NH), 1662 (C=N), 1710 (C=0), 1530 (C=C), PMR (DMSO-d_6): 2.35 (s, 6H, CH_3), 4.1 (b, 1H, -SO_2NH), 6.1 (d, 2H, pyrimidinyl), 7.1-8.0 (m, 12H, Ar-H), EI-MS (m/e): 446.02

Antiviral assay

The present work concerns the antiviral activities of ISD derivatives against vaccinia virus and cowpox viruses in HFF cells. Cytotoxicity was investigated in uninfected HFF cells[13].

Efficacy

In all the assays used for primary screening, a minimum of six drug concentrations was used covering a range of 100µg/ml to 0.03µg/ml, in 5-fold increments. These data allow us to obtain good dose response curves. From these data, we calculated the dose that inhibited viral replication by 50% (effective concentration 50; EC_{50}) using the computer software program MacSynergy II (by M.N. Prichard, K. R. Asaltine, and C. Shipman, Jr., University of Michigan, Ann Arbor, Michigan).

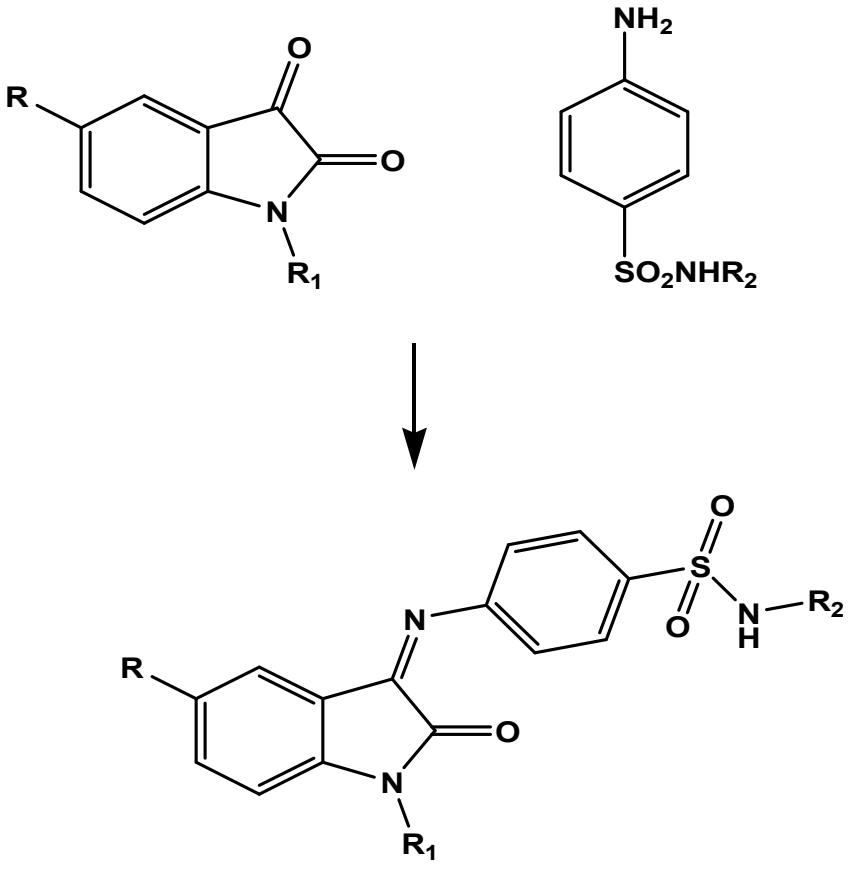
Toxicity

The same drug concentrations used to determine efficacy were also used on uninfected cells in each assay to determine toxicity of each experimental compound. The drug concentration that is cytotoxic to cells as determined by their failure to take up a vital stain, neutral red, (cytotoxic concentration 50; CC_{50}) was determined as above. We have utilized a neutral red uptake assay and found it to be reliable

and reproducible and allows quantitation of toxicity based on the number of viable cells rather than cellular metabolic activity. It is important also to determine the toxicity of new compounds on dividing cells at a very early stage of testing. We have found that a cell proliferation assay using HFF cells is a very sensitive assay for detecting drug toxicity to dividing cells and the drug concentration that inhibits cell growth by 50% (IC_{50}) was calculated as described above. Antiviral activity and cytotoxicity data are presented in Table 1.

Results and discussion

The reaction utilizes the microwave irradiation 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. The yield was found to be 64–89%. Unlike conventional methods[11,12](duration-3 h), microwave-assisted reactions were very facile (2-3 min). The purity of the synthesized compounds was checked by TLC and the compounds of this study were identified by spectral data. Results of antiviral activity activities of ISD derivatives against vaccinia virus and cowpox viruses in HFF cells (Table I). Compound SPIII-5Br-AC (EC_{50} 12 uM and CC_{50} >300 uM) exhibits equipotent activity with that of standard Cidofovir (CDV) (EC_{50} 16.2 uM and CC_{50} >317 uM) against cowpox virus in CPE reduction assays. Compound SPIII-5Br-BZ also inhibits the replication of vaccinia virus and cow pox virus at the concentration of 41.8 and 31.3 uM respectively where as cytotoxicity was found to be >317 uM. All the compounds except SPIII-SCl exhibited cytotoxicity >300 uM.



Scheme1:Synthesis of Isatine-sulphonamide derivatives

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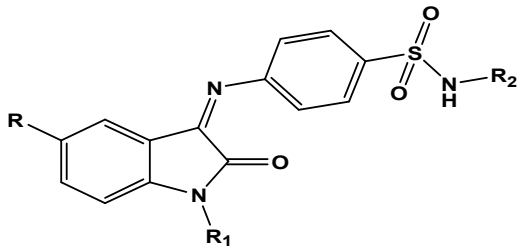

Compound Code	R	R_1	R_2
SPIII-S	H	H	H
SPIII-SMe	CH_3	H	H
SPIII-SCl	Cl	H	H
SPIII-SM	H	H	4,5-dimethyl-2-isoxazolyl
SPIII-5Br-AC	Br	$COCH_3$	4,6-dimethyl-2-pyrimidinyl
SPIII-5Br-BZ	Br	COC_6H_5	4,6-dimethyl-2-pyrimidinyl
SPIII-5Me-AC	CH_3	$COCH_3$	4,6-dimethyl-2-pyrimidinyl
SPIII-5Cl-BZ	Cl	COC_6N_5	4,6-dimethyl-2-pyrimidinyl

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Table No. 01: Anti viral and cytotoxicity of isatine-Sulphonamide derivatives

Drug Name	Virus	EC_{50} [a]	CC_{50} [b]	SI [c]
SPIII-S	Vaccinia	>300	>300	**--**
	Cowpox	>300	>300	**--**
SPIII-SCl	Vaccinia	>60	211	**<3.5**
	Cowpox	>60	211	**<3.5**
SPIII-Sme	Vaccinia	>300	>300	**--**
	Cowpox	>300	>300	**--**
SPIII-5Br-AC	Vaccinia	220	>300	**>1.4**
	Cowpox	12	>300	**>25**
SPIII-5Br-Bz	Vaccinia	41.8	>300	**>7.2**
	Cowpox	31.3	>300	**>7.8**
SPIII-5Cl-Bz	Vaccinia	>300	>300	**-**
	Cowpox	>300	>300	**-**
SPIII-5Me-Ac	Vaccinia	>300	>300	**-**
	Cowpox	>300	>300	**-**
(Cidofovir)CDV	Vaccinia	8.9	>317	**>35.6**
	Cowpox	16.2	>317	**>19.6**

[a] 50% effective (virus-inhibitory) concentration (μM).

[b] 50% cytotoxic concentration (μM) in uninfected cells.

[c] Selectivity index (CC_{50} divided by EC_{50}).

Acknowledgement

The antiviral experiments were performed at Department of Pediatrics, University of Alabama School of Medicine, Alabama, USA under contract NO1-AI from the Virology Branch, NIAID, NIH.

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