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[Research article]

Synthesis, antiviral and cytotoxicity activities of N-Sulphonamidomethyl benztriazole derivatives

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ABSTRACT

A series of novel N-sulphonamido methyl benztriazole derivatives had been synthesized by combining benztriazole, formaldehyde and sulphonamides. Structure of synthesized compounds was elucidated by spectral analysis. Synthesized compounds were evaluated for *in-vitro* antiviral activity against HIV, HSV and Vaccinia viruses in cell culture. N-Sulphonamido methyl benzotriazole (BT-SN) inhibits Herpes Simplex Virus (HSV) -2 and Vaccinia virus at 34 µg/ml, respectively. HSV-1 at the concentration of 45 µg/ml. The minimum cytotoxic concentration was found to be more than 100µg/ml. So these compounds are suitable for designing newer derivatives and molecular modifications in them may help in optimizing antiviral activity.

Key words: N-sulphonamidomethyl benztriazole, HIV-1 and HIV-2, Antiviral, Cytotoxicity.

INTRODUCTION

Benztriazole is a versatile lead molecule for designing potential bioactive agents and its derivatives were reported to possess broad spectrum activities. Benztriazole was screened for their wide spectrum antiviral activity[1,2] and they have rich potential for further studies. Novel sulphoanamide derivatives with variety of heterocyclic compounds were reported for wide spectrum of antiviral activity[3-15]. Based on this fact present work is to design series of novel N-sulphonamido methyl benztriazole derivatives have been synthesized through Mannich reaction by combining benztriazole, formaldehyde and sulphonamides(sulphamethoxazole, sulphadimidine and sulphanilamide). Synthesized compounds were screened for *invitro* antiviral activity and cytotoxicity.

MATERIALS AND METHODS

Melting points were determined using open ended capillary tube method and are uncorrected. FT-IR recorded on Perkin Elmer–1605 series FT-IR in KBr disc. ^{1}H NMR Spectra were recorded at 400 MHz on Bruker FT-NMR spectrophotometer using TMS as internal standard. Mass spectra were recorded on a Varian Atlas CH-7 Mass Spectrophotometer at 70 eV.

Synthesis of N-Sulphonamido methyl benzotriazole derivatives

An equimolar mixture (0.001mol) of benztriazole, formaldehyde and sulphonamides with primary

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aromatic amino functional group (sulphamethoxazole, sulphadimidine and sulphanilamide) were mixed and the mixture was stirred for 3 hours in 10 ml methanol. The contents were kept overnight. The precipitated solid was collected and recrystallized from methanol to give the desired title compounds.

4-[(Benzotriazol-1-ylmethyl)-amino]-benzene sulfonamide (BT-SN) Yeild 68 %, Mp 234^0C, FT-IR (KBr): 3432 (NH), 1654 (C=C), 1554 (C=N), 1154 (SO_2), 675 (Ar-H). PMR (DMSO-d_6): 2.0 (s,1H, -SO_2NH-), 4.0 (s, 1H, NH), 5.50 (s, 2H,-N-CH_2-) 6.71-7.98 (m, 8H, Ar-H).EI-MS (m/e) 303.34

4-[(Benzotriazol-1-ylmethyl)-amino]-N-(4,6-dimethyl - pyrimidin-2-yl) - benzene sulfonamide (BT-SDM) Yield 78 %, Mp 273^0C, FT-IR (KBr): 3466 (NH), 1667 (C=C), 1563 (C=N), 1158 (SO_2), 675 (Ar-H). PMR (DMSO-d_6): 2.0 (s,1H, -SO_2NH-), 2.4 (s,6H, 2 X CH_3), 4.2 (s, 1H, NH), 5.50 (s, 2H,-N-CH_2-), 8.2(s, 1H, pyrimidinyl) 6.71-7.98 (m, 8H, Ar-H).EI-MS (m/e) 409.46

4-[(Benzotriazol-1-ylmethyl)-amino]-N-(4-methyl-oxazol-2-yl)-benzenesulfonamide (BT-SMZ) Yield 64 %, Mp 279^0C, FT-IR (KBr): 3476 (NH), 1644 (C=C), 1548 (C=N), 1152 (SO_2), 670 (Ar-H).PMR (DMSO-d_6): 2.0 (s,1H, -SO_2NH-), 2.2 (s, 1H, CH_3) 4.0 (s, 1H, NH), 5.50 (s, 2H,-N-CH_2-) 6.70-7.98 (m, 8H, Ar-H).EI-MS (m/e) 384.10

Anti-HIV activity and cytotoxicity assay

The synthesized compounds were tested for anti-HIV activity against the replication of HIV-1(III_B) and HIV-2(ROD) in MT-4 cells[16]. 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) strain and HIV-2 ($LAV\text{-}2_{ROD}$) strain were used in the experiment. 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°C until used. 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 MTT assay. Briefly, 50 µl of HIV-1 and HIV-2 (100-300 $CCID_{50}$) was added to a flat-bottomed MT–4 cells ($6x10^5$ cells/ml). After 5 days of incubation, at 37°C the number of viable cells were determined by the 3 - (4, 5 – dimethyl thiazol-2-yl) - 2, 5-diphenyl tetrazolium bromide (MTT) method. Cytotoxicity 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 activity and cytotoxicity data are presented in Table 2

ANTIVIRAL ACTIVITY

Anti viral assay

Antiviral activity and cytotoxicity of the synthesized compounds were determined by in vitro cell culture techniques[16]. The antiviral assays were based on inhibition of virus-induced cytopathicity in HEL (HSV-1 and HSV-2, VV, VSV) cultures. Briefly, confluent cell culture in 96-well microtiter plates were inoculated with 100 CCID50 of virus, 1 CCID 50 being the virus dose required to infect 50% of the cell cultures. After a 1 h virus adsorption period, residual virus was removed and the cell cultures were incubated in the presence of varying concentrations of the test compounds. Viral cytopathicity was recorded as soon as it reached completion in the control virus-infected cell cultures that were treated with the test compounds. The antiviral activity and cytotoxicity data are presented in Table 3.

RESULTS AND DISCUSSIONS

All these compounds calculated for Lipinski's rule of 5 by molinspiration software[17,18] and exhibited the drug like properties (Table 1). N-Sulphonamido methyl benzotriazole (BT-SN) inhibits Herpes Simplex Virus (HSV) -2 and Vaccinia virus at 34 µg/ml, respectively. HSV-1 at the concentration of 45 µg/ml, The minimum cytotoxic concentration was found to be more than 100µg/ml. So these compounds are suitable for designing newer derivatives and molecular modifications in them may help in optimizing antiviral activity. N-Sulphonamidomethyl benztriazole (BT-SN) exhibited antiviral activity against Herpes Simplex Virus -1 & -2 and Vaccinia viruses in HEL cell cultures. Free sulphonamyl group in BT-SN lead molecule is essential for antiviral activity and any other substitution will abolish antiviral activity. All the compounds displayed cytostatic properties in T lymphocytes cells (Adult T cell Leukemia cells) and devoid of anti-HIV activity. Compound **BT-SN** (CC_{50}= 3.3 µg/ml) was found to be more toxic in this series.

Table 1: Physical Data of Synthesized compounds

Compounds	Molecular Weight	Log P	Number of Rotatable bond or molecular flexibility
BT-SN	303.34	1.52	4
BT-SDM	409.64	2.65	6
BT-SMZ	384.10	2.23	6

Table 2: Anti-HIV activity and Cytotoxicity Data

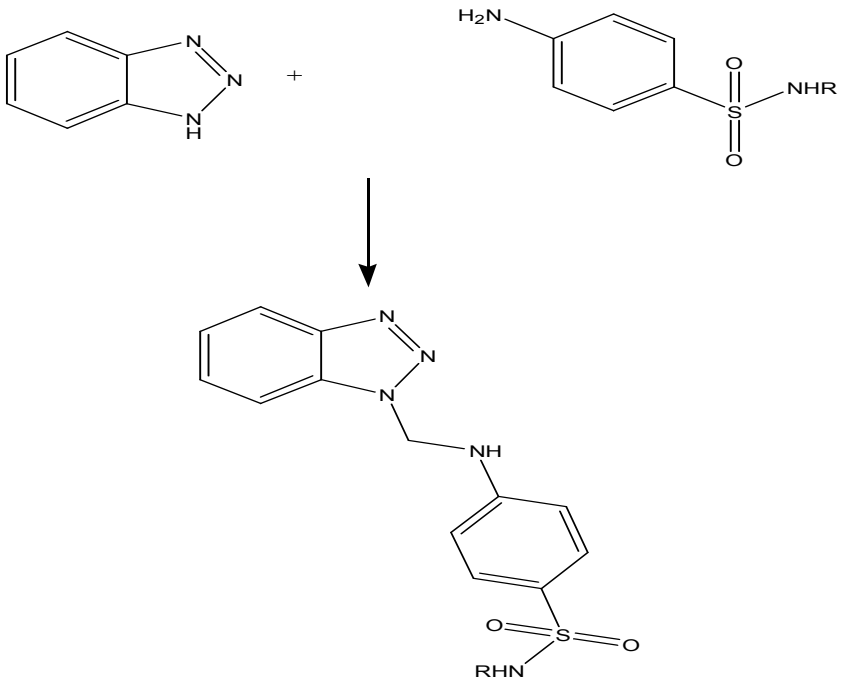
Compounds	Strain	IC_{50} [a] (µg/ml)	CC_{50} [b] (µg/ml)
BT-SDM	HIV-1	>11.27	11.27 ±0.61
	HIV-2	>11.27	11.27±0.61
BT-SMZ	HIV-1	>51.98	51.98 ±3.53
	HIV-2	>51.98	51.98 ±3.53
BT-SN	HIV-1	>3.30	3.30 ±2.07
	HIV-2	>3.30	3.30 ±2.07
AZT	HIV-1	0.0015	>25.00
(STD)	HIV-2	0.0016	>25.00

[a] Effective concentration of compound, achieving 50% protection of MT-4 cells against cytopathic effect of HIV.
[b]Cytotoxic concentration of compounds, required to reduce the viability of mock infected MT-4 cells by 50%.

Table 3: Antiviral activity and Cytotoxicity Data in HEL cells

COMPOUND	MINIMUM CYTOTOXIC CONCENTRATION (µg/ml)	MINIMUM INHIBITORY CONCENTRATION (µg/ml)			
		Herpes simplex virus-1	Herpes simplex Virus-2	Vaccinia virus	Herpes simplex virus-1 TK- ACV
BT-SDM	**100**	**>20**	**>20**	**>20**	**>20**
BT-SMZ	**100**	**>20**	**>20**	**>20**	**>20**
BT-SN	**>100**	**45**	**34**	**34**	**34**
Cidofovir	**>250**	**0.9**	**0.9**	**10**	**2**

Scheme 1.Synthesis of N-Sulphanamidomethyl benzotriazole derivatives



Where	R
BT-SN	-H
BT-SDM	-2,6-dimethylpyrimidine
BT-SMZ	-4-Methyl-2-isoxazole

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