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SYNTHESIS, ANTI-VIRAL AND CYTOTOXICITY STUDIES OF SOME NOVEL N-SUBSTITUTED BENZIMIDAZOLE DERIVATIVES

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ABSTRACT

A series of novel N-substituted benzimidazole derivatives were synthesized and screened anti-viral activity against a HIV -1 and -2 in MT-4 cells. New compounds were synthesized through modifying the N-1 hydrogen of benzimidazole moiety with the substitution of sulphanilamide, sulphadimidine, sulphamethoxazole, 2-aminopyridine, pthalamide, benzamide, nicotinamide, anthranilic acid and 2- marcapto- benzimidazole by mannich reaction. The structure of the synthetic compounds was characterized by means of IR and PMR data. The anti-HIV activities of the new compounds were also screened for in vitro anti-viral activity against replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells using AZT- as standard and cytostatic activity were also studied by MT- 4/MTT assay. Benzimidazole derivative BSD inhibited the replication of HIV-1 and 2 (EC_{50}= 35.40µg/ml and CC_{50}>125µg/ml) in MT-4 cells.

INTRODUCTION: Benzimidazoles are an important of heterocyclic compounds possessing a variety of biological activities such as anti-viral, anti-bacterial, anti-fungal and hypoglycemic activities biological activities of these compounds depend upon the functional group attached on the benzimidazole moiety. The benzimidazole derivatives are effective against RNA-virus inhibiting the formation of virus induced RNA-polymerase thereby preventing or retarding the RNA synthesis[1]. Benzimidazoles are remarkably effective compounds both with respect to their degree of virus inhibitory activity and favorable selectivity ratio. Several benzimidazole derivatives with N-1 substitution showed anti-viral activity against human cytomegalovirus and herpes simplex virus type-1. The biological activities of these compounds depend upon the substitution on the benzimidazole at the N-1 or C-2 position. Since benzimidazole heterocyclic ring system mimics the purine bases like adenine and guanine of nucleic acids, the N-1 substituted benzimidazoles may be incorporated into the viral nucleic acids by enzymatic process and subsequently can alter the structure and function of nucleic acids resulting in the inhibition of viral growth. Present work is to synthesis some novel mannich bases of benzimidazole (Scheme 1) and tested for antiviral activity against HIV -1 and -2 in MT-4 cells. Cytotoxicity also tested by MT-4/MTT assay

MATERIAL AND METHODS: Melting points were determined in open capillary tubes on a Thomas-Hoover melting point apparatus and are uncorrected. IR spectra were recorded for KBr pellets on a (SHIMADZU-800) infrared spectrophotometer, PMR spectra were determined BRUKER AMX 400 MHZ with tetramethylsilane as an internal standard. The sample is dissolved in DMSO-d6 and the value is measured in δ ppm.

Synthesis of Benzimidazole Derivatives: An equimolar (0.01 mol) mixture of formaldehyde, active hydrogen compounds (sulphanilamide, sulphadimidine, sulphamethoxazole, 2-aminopyrimidine, phthalimide, anthranilc acid, 2-mercaptobenzimidazole and benzamide) and benzimidazole was stirred in magnetic stirrer or reflux with methanol for 3 hrs. The mixture was allowed to cool over night in a refrigerator. The solid thus obtained was recrystallized from DMF. Physical data of the synthesized compounds are given in **Table 1**.

TABLE 1: PHYSICAL EVALUATION OF SYNTHESIZED COMPOUNDS

Compound Code	Molecular Formula	% Yield	M.P. (^oC)	R_f Value	Log P
BI	$C_7H_6N_2$	69	170 - 175	0.7	2.7
BI-SN	$C_{14}H1_4N_4O_2S$	59.1	270 - 272	0.8	1.35
BI-SD	$C_{20}H_{20}N_6O_2S$	71.3	242 - 245	0.8	3.0
BI-SMZ	$C_{18}H_{17}N_5O_3S$	53.6	173 - 180	0.6	2.24
BI-2AMP	$C_{13}H_{12}N_4$	96.4	260 - 264	0.7	1.99
BI-BA	$C_{15}H_{13}N_3O$	96	125 - 132	0.7	2.25
BI-PTH	$C_{16}H_{11}N_3O_2$	65.9	216 - 220	0.4	1.79
BI-NM	$C_{14}H_{12}N_4O$	73	118 - 120	0.6	1.27
BI-2MBI	$C_{15}H_{12}N_4S$	64	163-178	0.7	3.5
BI-ANTH	$C_{22}H_{16}N_4O_2$	86	145-152	0.6	1.89

Benzimidazole (BI) : IR (KBr) : 3420 (NH), 1640 (C=N), 1458 (C=C), 745 (Ar-H); ^{1}H NMR (DMSO-d_6) : 7.1 – 8.3 (m, 4H, Ar-H), 12.5 (s, 1H, NH), 7.1 (s, 1H, CH).

4 - [(Benzoimidazol – 1 - ylmethyl) - amino] - benzenesulfonamide (BI-SN): IR (KBr): 3502 (NH), 1656 (C=N), 1451 (C=C), 1083 (>N-), 1332 (SO_2), 760,716 (Ar-H); ^{1}H NMR (DMSO-d_6) : 6.8 – 8.2 (m, 8H, Ar-H), 4.2 (s, 1H, NH), 5.1 (s, 2H, -CH_2-).

4 - [(Benzoimidazol - 1 - ylmethyl) - amino] - N- (4, 6-dimethyl- pyrimidin- 2- yl)- benzene sulfonamide (BI-SD) : IR (KBr): 3378 (NH), 1620 (C=N), 1424 (C=C), 1300 (SO_2), 709 (Ar-H); ^{1}H NMR (DMSO-d_6) : 6.5 – 9.0 (m, 9H, Ar-H), 5.4 (s, 2H, -CH_2-), 12.2 (s, 1H, NH), 2-2.2 (s, 6H, 2XCH_3).

4 - [(Benzoimidazol - 1 - ylmethyl) - amino]- N- (5- methyl- isoxazol- 3- yl)- benzenesulfonamide; compound with ethene (BI-SMZ) : IR (KBr) : 3383 (NH), 1663 (C=N), 1458 (C=C), 1386 (CH_3), 1330 (SO_2), 1093 (>N-), 762 (Ar-H); ^{1}H NMR (DMSO-d_6) : 6.5 – 8.5 (m, 8H, Ar-H), 4.3 (s, 1H, NH), 5.6 (s, 2H, -CH_2-).

Benzoimidazol - 1 - ylmethyl - pyridin - 2 - yl- amine (BI 2AMP: IR (KBr) : 3350 (NH), 1663 (C=N), 1490 (C=C), ^{1}H NMR (DMSO-d_6): 4.0 (b, 1H, NH), 5.5 (s, 2H, -CH_2-) 7.26 (t, 2H, Ar-H), 7.7 (d,2H,Ar-H),7.44(t, 3H, pyrimidine- 5H), 6.6-7.0 (m, 2H, Pyrimidine-3 and 4H), 8.02 (s, 1H, -CH-), 8.12 (d, 1H, pyrimidinyl- 6H).

N- Benzoimidazol-1- ylmethyl- benzamide (BI- BA) IR (KBr) : 3370 (NH), 1663 (C=N), 1458 (C=C), 1720 (C=O), 1093 (>N-), 762 (Ar-H); ^{1}H NMR (DMSO-d_6) : 5.5 (s, 2H, -CH_2-) 7.3 -7.95 (m, 9H, Ar-H), 8.0 (b, 1H, NH), 8.05 (s, 1H, -CH-).

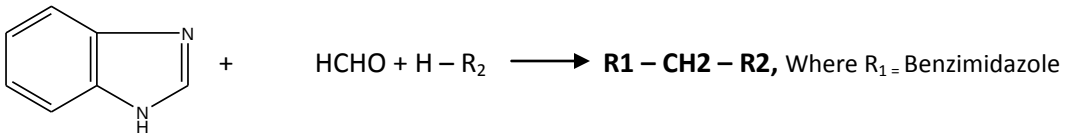
2- Benzoimidazol- 1- ylmethyl- isoindole- 1, 3 - dione (BI-PTH), IR (KBr) : 3370 (NH), 1710 (C=O), 1458 (C=C), 1093 (>N-), 762 (Ar-H); ^{1}H NMR (DMSO-d_6) : 6.07 (s, 2H, -CH_2-) 7.2 -8.0 (m, 8H, Ar-H), 8.05 (s, 1H, -CH-).

1 - Benzoimidazol - 1 - ylmethyl - 1H - benzoimidazole-2- thiol (BI-2MBI), IR (KBr) : 3394 (NH), 1720 (C=O), 1663 (C=N), 1458 (C=C), 1093 (>N-), 762 (Ar-H); ^{1}H NMR (DMSO-d_6) : 4.5 (b, 1H, NH), 5.5 (s, 2H, -CH_2-) 7.2 -7.9 (m, 8H, Ar-H), 8.05 (s,1H,-CH-), 11.0(s, IH, COOH).

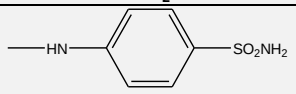
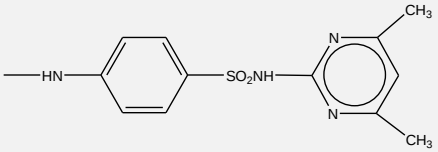
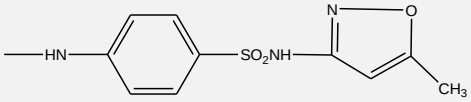
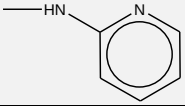
2- [(Benzoimidazol- 1- ylmethyl)- amino]- benzoic acid (BI-ANTH): IR (KBr) : 1663 (C=N), 1458 (C=C), 1093 (>N-), 762 (Ar-H); ^{1}H NMR (DMSO-d_6) : 3.0 (s,1H,SH),6.7 (s,2H,-CH_2-), 6.7 -7.95 (m,8H,Ar-H), 8.05 (s,1H,-CH-).

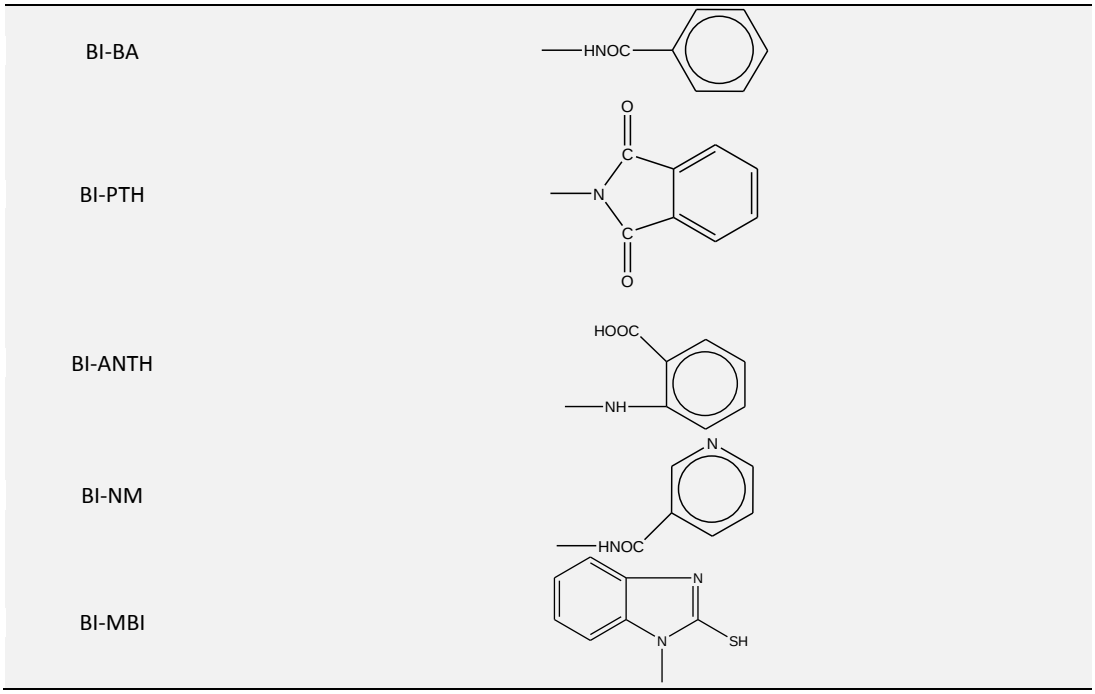
N- Benzoimidazol- 1- ylmethyl- nicotinamide (BI-NM): IR (KBr) : 3350 (NH), 1663 (C=N), 1490 (C=C), ^{1}H NMR (DMSO-d_6): 8.0 (b, 1H, NH),5.4 (s, 2H, -CH_2-) 7.26 (t, 2H, Ar-H), 7.7 (d, 2H, Ar-H), 7.6 (t, 3H, pyrimidine-5H), 8.3-8.8 (m, 2H, Pyrimidine- 4 and 6H), 8.02 (s, 1H, -CH-), 9.12 (d, 1H, pyrimidinyl- 2H).

SCHEME 1: SYNTHETIC PROTOCOL OF HETEROCYCLIC COMPOUNDS



DIFFERENT SUBSTITUTIONS OF SYNTHESIZED COMPOUNDS

Compound Code	R_2
BI-SN	
BI-SD	
BI-SMZ	
BI-2AMP	



Anti-HIV Assay: Anti HIV Assay compounds were tested for their inhibitory effects against replication of HIV-1 (IIIB) and HIV-2 (ROD) in MT-4 cells[2]. 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 mcg/mL gentamicin (culture medium). Inhibitory effect of test compounds on HIV-1 and HIV-2 replications was monitored by inhibition of virus-induced cytopathic effect in MT-4 cells and was estimated by MTT assay. Briefly, 50 mL of HIV-1 and HIV-2 (100-300 CCID50) were added to a flat-bottomed microtiter tray with 50 mcL of medium containing various concentrations of compounds. MT-4 cells were added at a final concentration of 6x105 cells/mL. After 5 days of incubation at 37^{o}, the number of viable cells were determined by the 3- (4, 5- dimethylthiazol- 2- yl)- 2, 5-diphenyl tetrazolium bromide (MTT) method. Cytotoxicity of test compounds against mock-infected MT-4 cells was also assessed by the MTT method. Compounds were evaluated for their inhibitory effect on the replication of HIV-1 & HIV-2 in human MT-4 cells. Anti-HIV activity and cytotoxicity data of synthesized compounds are given in Table 2.

RESULTS: We report our results from a study of replacing the N-1 hydrogen of novel benzimidazole moiety with different types of substitutions like sulphanilamide,sulphadimidine, ulphamethoxazole, 2- aminopyridine, pthalamide, benzamide, anthranilic acid, nicotinamide, 2-marcaptobenzimidazole to form N- methyl substituted Benzimidazole derivatives by Mannich reaction. Synthesized compounds were screened for anti-viral activity against HIV-1 and HIV-2 in MT-4 cells using AZT-as standard. Cytotoxic activities in CC_{50} of the compounds were also tested in mock-infected MT-4 cells. Compound BSD inhibits the replication of HIV-1 and 2 in MT-4 cells at a concentration of 35.40 µg/ml and the cytotoxicy was found to be more than 125µg/ml. All the compounds except BSD displayed cytotoxic properties in MT-4 cells (**Table 2**). The tested compound (BI-2MBI) 1- Benzoimidazol- 1- ylmethyl-1H- benzoimidazole- 2-thiol have shown more toxicity in these series.

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

COMPOUND CODE	STRAIN	EC_{50} [a] (µg/ml)	CC_{50} [b] (µg/ml)
BI	IIIB	>125	>125
	ROD	>125	>125
BI-ANTH	IIIB	>10.90	>10.90
	ROD	>10.90	>10.90
BI-BA	IIIB	>125	>125
	ROD	>125	>125
BI-N	IIIB	>78.50	78.50 ± 10.15
	ROD	>78.50	78.50 ± 10.15
BI-NM	IIIB	>125	>125
	ROD	>125	>125
BI-PTH	IIIB	>69.30	69.30
	ROD	>69.30	69.30
BI-SMZ	IIIB	>125	>125
	ROD	>125	>125
BI-SN	IIIB	>58.18	58.18 ± 4.97
	ROD	>58.18	58.18 ±4.97
BI-2AMP	IIIB	>55.80 4.07	55.80 ± 4.07
	ROD	>55.80 4.07	55.80 ± 4.07
BSD	IIIB	35.40	>125
	ROD	3540	>125
AZT (STD)	IIIB	0.0012 ± 0.003	65.40
	ROD	0.00016 ±0.00027	65.40

[a] Concentrations required to inhibit the cytopathic effect of HIV-1(III_B) in MT-4 cells by 50%;

[b] Concentrations required to cause cytotoxicity to 50% of the MT-4 cells;

Whereas HIV-1 = (IIIB), HIV-2 = (ROD), All the value of SD of two independent experiment

DISCUSSION: We have previously reported the antiviral activity of novel heterocyclic compounds against vaccinia virus, and many of those compounds also exhibited marked cytostatic properties in lymphocytes [3, 4]. Though a variety of heterocyclic compounds had been synthesized and studied for wide range of antiviral activity, the antiviral activities of benzimidazole against HIV-1 and 2 viruses have not been extensively explored. In this study we synthesized 10 derivatives of benzimidazole and evaluated them for antiviral activity against HIV 1 and 2 in MT-4 cells. Newly synthesized benzimidazole derivative (BSD) inhibited the replication of the HIV 1 and 2 in MT4 cells. These lead molecules are suitable for designing newer derivatives against HIV based upon promising antiviral activity seen. Recently we reported the activities of certain quinazolinone derivatives with sulphanamide against biodefence viruses in cell culture [5]. The potencies of some of them exceeded those of the present quinazolinone series. The compounds were found to inhibit virus replication as a result of interfering with virus adsorption [6]. There is a need to discover new compounds that are inhibitory to HIV due to the emergence of potentially pandemic virus strains and viral resistance against approved drugs. The methodology reported here allows for rapid synthesis of a number of Benzimidazole derivatives that can be tested for antiviral activity, as well as for activity against other viruses of concern to the medical community.

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