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Design, synthesis, anti-HIV and antimicrobial study of some 3-(1H-benzo[d][1,2,3]triazol-1-yl)-*N*-phenylalkylamide derivatives

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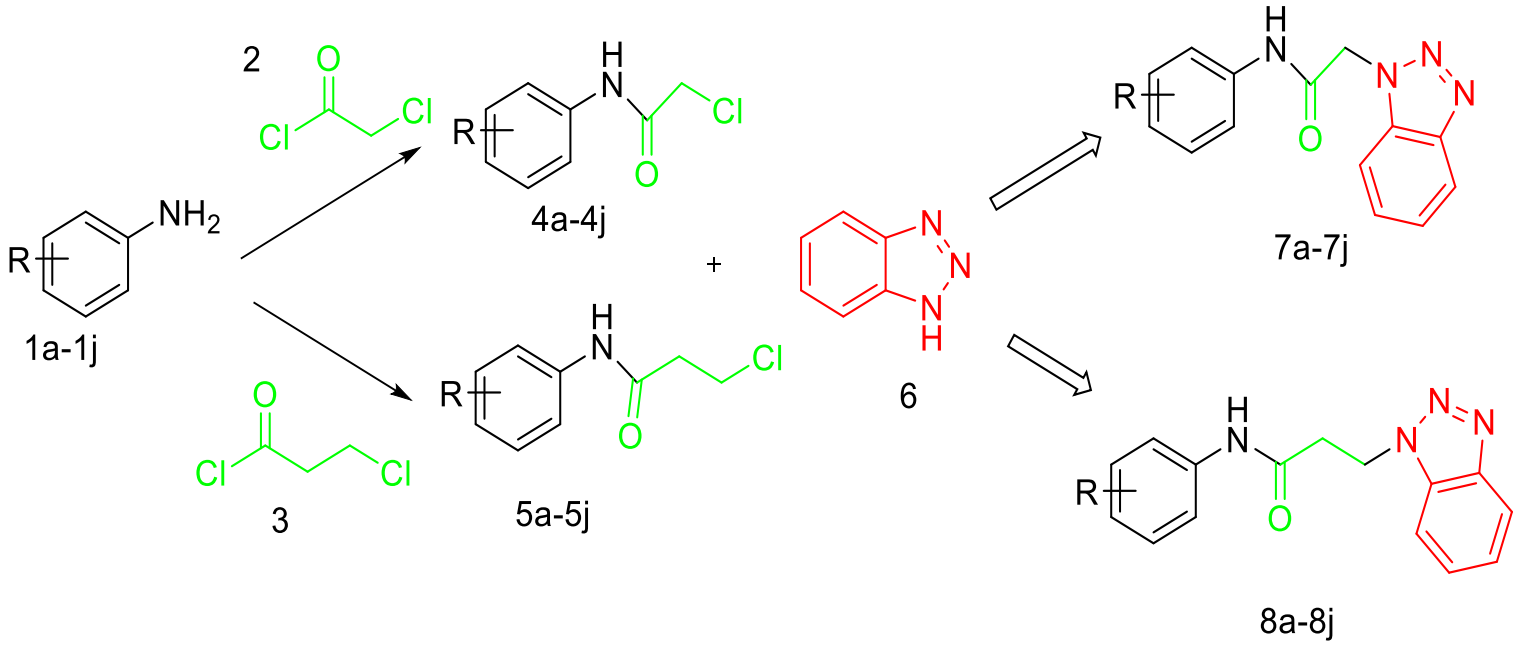
Graphical Abstract



ABSTRACT

Objective-

In the present study, a series of twenty benzotriazolyl-N-phenylalkylamide derivatives (**7a-7j**) and (**8a-8j**) were synthesized, characterized by physicochemical and spectral data (IR, ^{1}H NMR, ^{13}C NMR and mass spectroscopy) and evaluated for their anti-HIV activity, antibacterial, antifungal and anthelmintic activity.

Method-

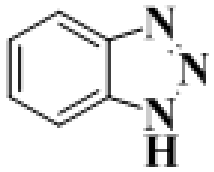
A series of twenty benzotriazolyl-N-phenylalkylamide derivatives were synthesized by reacting substituted anilines (1) with 2-chloro acetylchloride (2) and 3-chloro propionylchloride (3) to form intermediate (4a-4j) and (5a-5j). Intermediates further reacted with benzotraizole (6) to form benzotriazolyl-N-alkylamide derivatives (7a-7j) and (8a-8j). The synthesized test compounds (7a-7j) and (8a-8j) were assessed by MTT colorimetric assay on C8166 cells and screened for antibacterial activity against Gram-positive bacteria: *Staphylococcus aureus* (NCIM 2122), *Bacillus subtilis* (MTCC 121) and Gram-negative bacteria: *Escherichia coli* (MTCC118), *Pseudomonas aeruginosa* (MTCC 647), *Salmonella typhi* (NCIM 2501), *Klebsiella pneumonia* (MTCC 3384) and *in vitro* antifungal activity [19-24] against *Candida albicans* (MTCC 227) and *Aspergillus niger* (NCIM 1056) by two fold broth serial dilution method.

Result- Compounds 7h, 7j, 8i and 8j being the most active showed therapeutic index that were >24.4, 31.1, 30.5 and 51.5 compared to Zidovudine (AZT) having therapeutic index (TI) 514342.6. The test compounds 7h, 7i, 7j, 8h, 8i and 8j exhibited very high activity against all the strains of Gram (+) ve and Gram (-) ve bacteria and antifungal strains.

Keywords: Benzotriazole derivatives; Anti-HIV; Antibacterial; Antifungal agents.

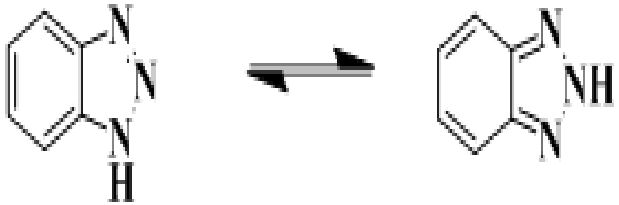
Introduction

➢ Benzotriazole **(1)** is bicyclic heterocyclic system enclosing three nitrogen atoms in the five membered ring which is fused with six membered ring benzene bearing the chemical formula $C_6H_5N_3$.[1]

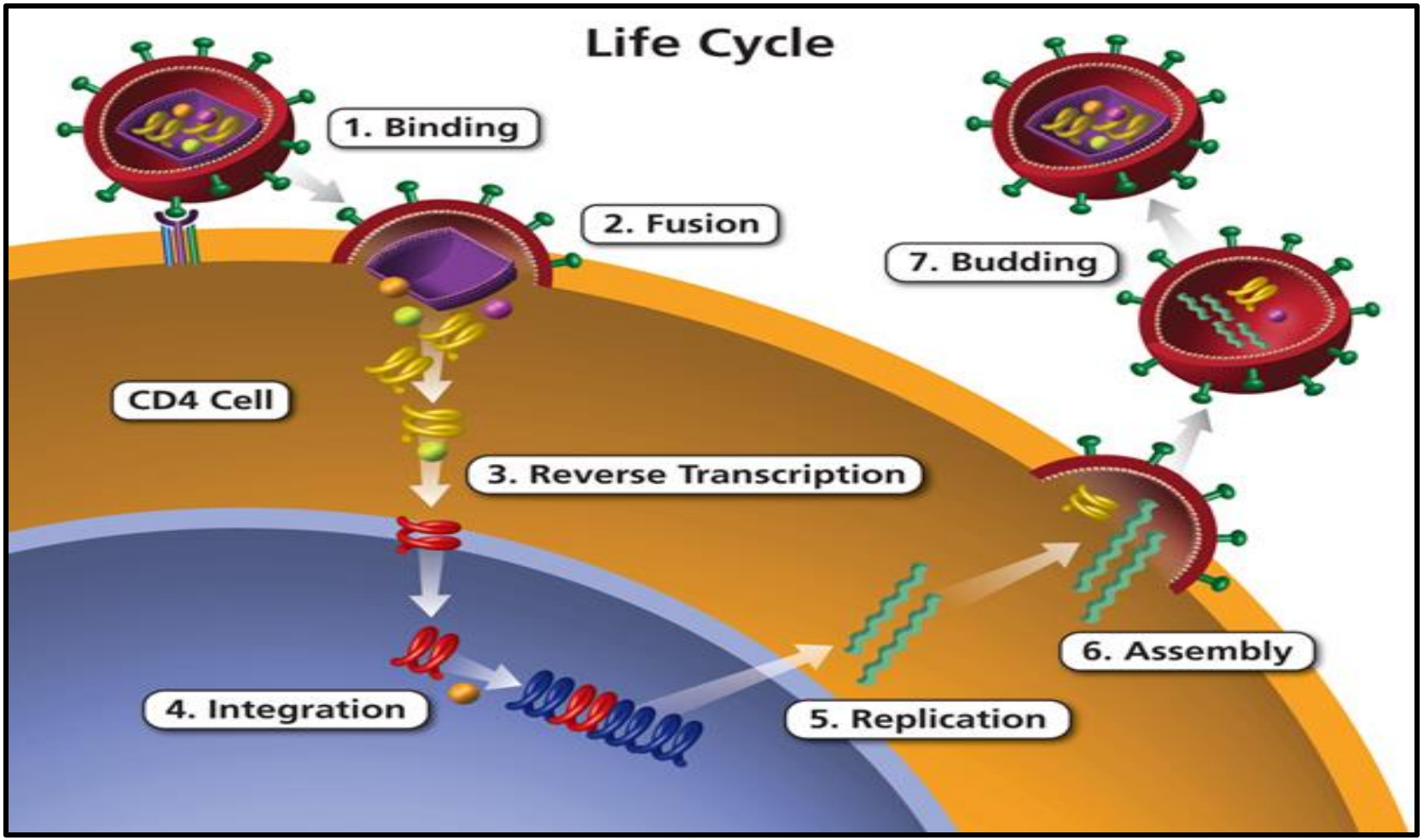


(1)

➢ The triazole nucleus of the benzotriazole moiety exits in two tautomers as the hydrogen substituent rapidly changes its position between fIrst and second nitrogen.



HIV Life Cycle



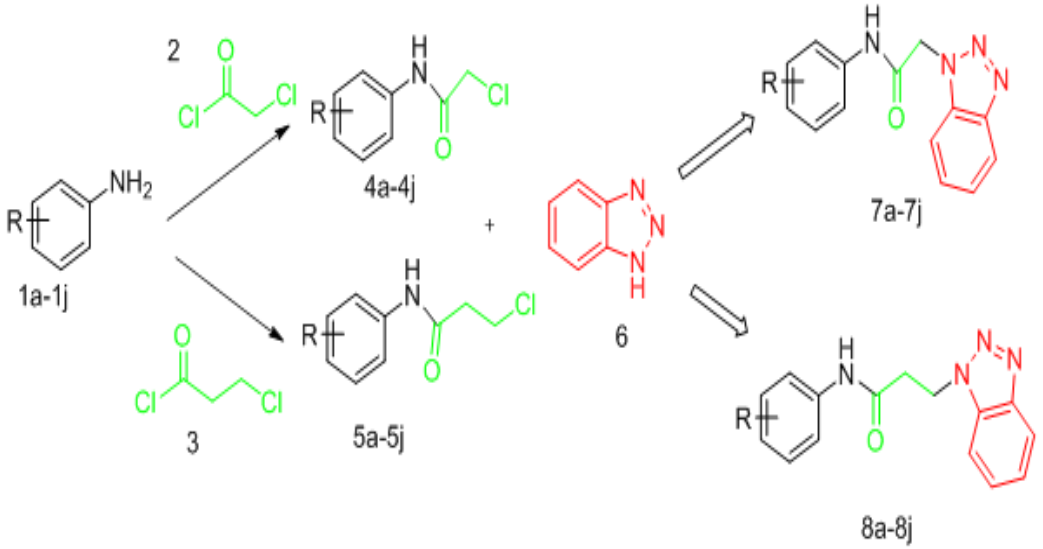
➢ Benzotriazole derivatives have gained attractions round the globe due to its flexibility in its wide pharmacological applications.

➢Benzotriazoles have exhibited high activity as antifungal [2], antibacterial [3], antiviral [4], antiserotonergic, antiadrenergic, antihistaminic [5], anti-inflammatory and antineoplastic [6] agents. Besides benzotriazoles also possess DNA cleavage activities [6], herbicidal [7], anti-tubercular [8], antiemetic [9], protein kinase inhibitory [10] and respiratory syndrome protease inactivation activities [11] and as agonist of peroxisome proliferator activated receptor.

➢In continuation of previous work on imidazole [12-15], a series of 2-(1H-benzo[d][1,2,3]triazol-1-yl)-N-phenylacetamide and 3-(1H-benzo[d][1,2,3]triazol-1-yl)-N-phenylpropanamide were synthesized.

Results and discussion

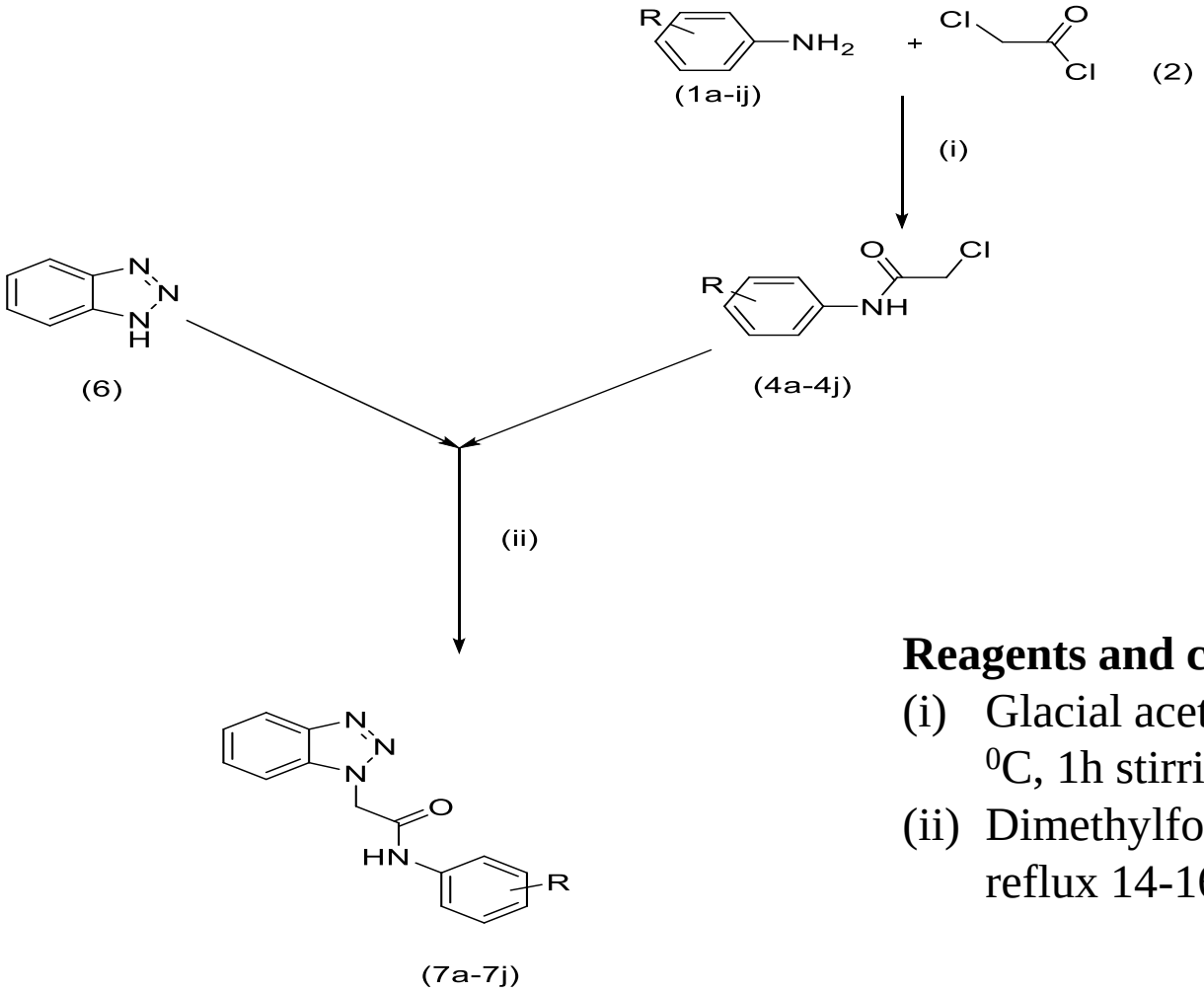
Analogs of type (4a-4j) were prepared according to method reported for choloroanilides [16-18]. Appropriate substituted anilines (1a-1j) were treated with 2-chloroacetyl chloride (2) resulting the formation of corresponding chloroanilides as shown in **table 1.**



S. No.	R	S. No.	R
1a	H	1f	2,5-di- CH_3
1b	o-CH_3	1g	3,4-di- CH_3
1c	m-CH_3	1h	o-Cl
1d	p-CH_3	1i	m-Cl
1e	2,4-di- CH_3	1j	p-Cl

Table 1: Types of substituent.

General synthetic scheme for compounds (7a-7j)

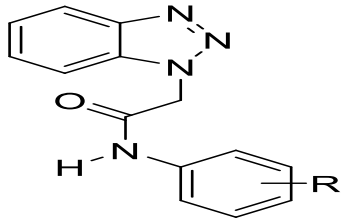


Reagents and conditions:

(i) Glacial acetic acid (GAA), 0-5 ^{0}C, 1h stirring,

(ii) Dimethylformamide (DMF), reflux 14-16 h.

Physical and Preparative Characteristic data of Benzotriazole derivatives. (7a-7j)



COMP.	R	Yield (%)	M.p (^{0}C)	Mol.formula	Mol.wt
7a	H	78	212-214	$C_{14}H_{12}N_4O$	252.28
7b	2-CH_3	82	196-198	$C_{15}H_{14}N_4O$	266.30
7c	3-CH_3	84	194-196	$C_{15}H_{14}N_4O$	266.30
7d	4-CH_3	80	192-194	$C_{15}H_{14}N_4O$	266.30
7e	2,4-di-CH_3	72	208-210	$C_{16}H_{16}N_4O$	280.33
7f	2,5-di-CH_3	76	218-220	$C_{16}H_{16}N_4O$	280.33
7g	3,4-di-CH_3	72	210-212	$C_{16}H_{16}N_4O$	280.33
7h	2-Cl	69	198-200	$C_{14}H_{11}ClN_4O$	286.72
7i	3-Cl	62	197-199	$C_{14}H_{11}ClN_4O$	286.72
7j	4-Cl	62	228-230	$C_{14}H_{11}ClN_4O$	286.72

COMP.	IR (KBr, cm^{-1})	MASS
7a	3279.10 (N-H stretching), 1667.77 (C=O stretching), 1448.59 (C-CH_3 stretching), 1317.43 (C-N stretching)	251.13
7b	3265.59 (N-H stretching), 1666.55 (C=O stretching), 1454.38 (C-CH_3 stretching), 1363.72 (C-N stretching)	265.27
7c	3279.10 (N-H stretching), 1668.48 (C=O stretching), 1448.59 (C-CH_3 stretching), 1224.84 (C-N stretching)	265.27
7d	3276.25 (N-H stretching), 1656.42 (C=O stretching), 1462.63 (C-CH3 stretching), 1324.26 (C-N stretching)	265.27
7e	3263.66 (N-H stretching), 1656.42 (C=O stretching), 1410.01 (C-CH_3 stretching), 1273.06 (C-N stretching).	279.18
7f	3261.74 (N-H stretching), 1668.48 (C=O stretching), 1411.94 (C-CH_3 stretching), 1226.77 (C-N stretching).	279.18
7g	3258.57 (N-H stretching), 1686.21 (C=O stretching), 1424.06 (C-CH_3 stretching), 1271.42 (C-N stretching).	279.18
7h	3304.45 (N-H stretching), 1684.02 (C=O stretching), 1356.21 (C-CH_3 stretching), 1282.35 (C-N stretching)	285.10
7i	3261.74 (N-H stretching), 1685.84 (C=O stretching), 1411.94 (C-CH_3 stretching), 1243.48 (C-N stretching)	285.10
7j	3333.10 (N-H stretching), 1683.91 (C=O stretching), 1386.86 (C-CH_3 stretching), 1232.55 (C-N stretching)	285.10

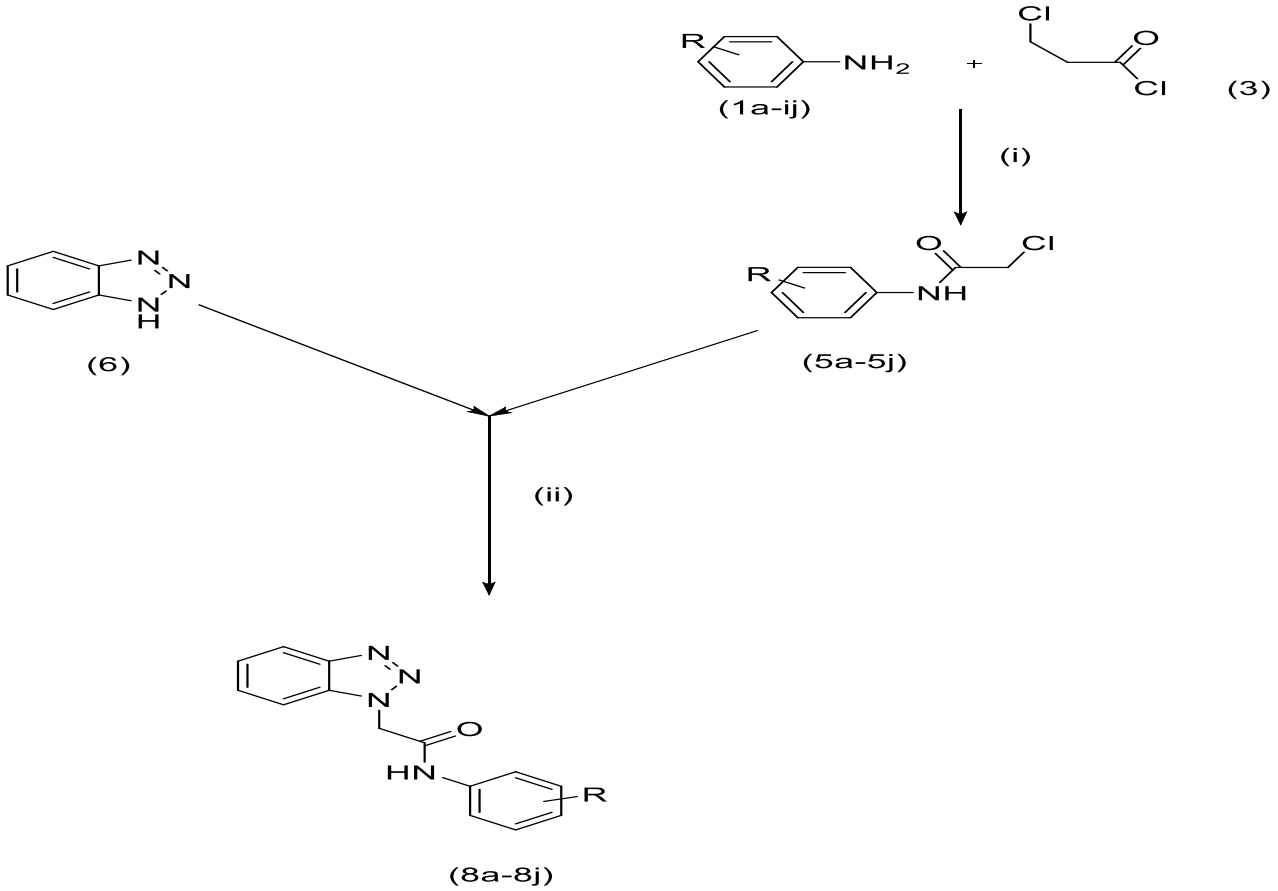
^{1}H and ^{13}C NMR of Synthesized Compounds.

COMP.	^{1}H NMR (DMSO-d_6, 400MHz)	^{13}C NMR(DMSO-d_6, 100MHz)
7a.	11.180 (s; 1H; N<u>H</u>-CO-CH_2), 8.220- 7.370 (m; 9H; Ar-<u>H</u>), 5.747 (s; 2H; NH-CO-C<u>H_2</u>).	50.994, 111.499, 118.428, 119.779, 124.321, 127.157, 127.934, 129.448, 134.412, 138.887, 144.531, 145.604, 164.904.
7b.	9.897 (s; 1H; N<u>H</u>-CO-CH_2), 8.045- 7.065 (m; 8H; Ar-<u>H</u>), 5.709 (s; 2H; NH-CO-C<u>H_2</u>), 2.230 (s; 3H; C<u>H_3</u>).	18.393, 50.726, 111.461, 118.428, 119.597, 124.426, 125.337, 126.180, 126.602, 127.119, 127.905, 130.981, 132.198, 134.354, 136.089, 144.541, 144.633, 165.096.
7c.	10.503 (s; 1H; N<u>H</u>-CO-CH_2), 8.044- 6.857 (m; 8H; Ar-<u>H</u>), 5.647 (s; 2H; NH-CO-C<u>H_2</u>), 2.228 (s; 3H; C<u>H_3</u>).	21.680, 51.013, 111.509, 117.029, 118.418, 119.578, 120.373, 124.417, 125.021, 127.138, 129.275, 134.412, 138.666, 144.431, 145.604, 164.827.
7d.	10.490 (s; 1H; NH-CO-CH2), 8.041- 7.080 (m; 8H; Ar-H), 5.636 (s; 2H; NH-CO-CH2), 2.208 (s; 3H; CH3).	20.971, 50.965,111.499, 118.418, 119.788, 124.426, 127.138, 127.915, 129.812, 133.300, 134.412, 136.453, 144.521, 145.595, 163.869, 164.636.
7e.	9.838 (s; 1H; N<u>H</u>-CO-CH_2), 8.041- 7.003 (m; 7H; Ar-<u>H</u>), 5.680 (s; 2H; NH-CO-C<u>H_2</u>), 2.140 (s; 6H; C<u>H_3</u>).	18.278, 21.000, 50.697,111.461, 118.428, 119.587, 124.417, 125.375, 127.090, 127.886, 131.479, 132.150, 133.482, 134.345, 135.312, 144.521, 145.623, 165.028.

^{1}H and ^{13}C NMR of Synthesized Compounds.

COMP.	^{1}H NMR (DMSO-d_6, 400MHz)	^{13}C NMR(DMSO-d_6, 100MHz)
7f.	9.832 (s; 1H; N<u>H</u>-CO-CH_2), 8.042- 7.059 (m; 7H; Ar-<u>H</u>), 5.710 (s; 2H; NH-CO-C<u>H_2</u>), 2.182 (s; 6H; C<u>H_3</u>).	17.905, 21.067, 50.726,111.471, 118.428, 119.587, 124.517, 125.806, 126.755, 127.110, 127.895, 129.026, 130.770, 134.354, 135.792, 144.531, 145.623, 134.319, 165.038.
7g.	10.322 (s; 1H; N<u>H</u>-CO-CH_2), 8.040- 7.030 (m; 7H; Ar-<u>H</u>), 5.729 (s; 2H; NH-CO-C<u>H_2</u>), 2.181 (s; 6H; C<u>H_3</u>).	18.126, 21.224, 50.722,111.498, 118.412, 119.576, 124.509, 125.812, 126.761, 127.116, 127.889, 129.012, 130.765, 134.348, 135.786, 144.528, 145.618, 134.315, 165.032.
7h.	9.342 (s; 1H; N<u>H</u>-CO-CH_2), 8.076- 7.048 (m; 8H; Ar-<u>H</u>), 5.924 (s; 2H; NH-CO-C<u>H_2</u>).	51.016, 111.474, 118.264, 119.418, 124.015, 127.119, 131.142, 133.720, 134.404, 140.512, 144.548, 145.609, 164.365, 165.405.
7i.	8.045 (s; 1H; N<u>H</u>-CO-CH_2), 8.023- 7.092 (m; 8H; Ar-<u>H</u>), 5.691 (s; 2H; NH-CO-C<u>H_2</u>).	51.003,111.499, 118.255, 119.405, 124.005, 127.117, 131.144, 133.722, 134.402, 140.506, 144.541, 145.604, 164.363, 165.402.
7j.	10.149 (s; 1H; N<u>H</u>-CO-CH_2), 7.990- 7.269 (m; 8H; Ar-<u>H</u>), 4.949 (s; 2H; NH-CO-C<u>H_2</u>).	51.012, 111.506, 118.275, 119.422, 124.010, 127.123, 131.148, 133.726, 134.408, 140.509, 144.545, 145.608, 164.366, 165.406.

General synthetic scheme for compounds (8a-8j)

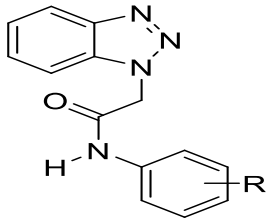


Reagents and conditions:

(i) Glacial acetic acid (GAA), 0-5 ^{0}C, 1h stirring,

(ii) Dimethylformamide (DMF), reflux 14-16 h.

Physical and Preparative Characteristic data of Benzotriazole derivatives. (8a-8j)



COMP.	R	Yield (%)	M.p (^{0}C)	Mol.formula	Mol.wt
8a	H	81	202-204	$C_{15}H_{14}N_4O$	266.30
8b	2-CH_3	82	198-200	$C_{16}H_{16}N_4O$	280.33
8c	3-CH_3	77	146-148	$C_{16}H_{16}N_4O$	280.33
8d	4-CH_3	85	162-164	$C_{16}H_{16}N_4O$	280.33
8e	2,4-di-CH_3	76	155-157	$C_{17}H_{18}N_4O$	294.36
8f	2,5-di-CH_3	79	154-156	$C_{17}H_{18}N_4O$	294.36
8g	3,4-di-CH_3	82	160-162	$C_{17}H_{18}N_4O$	294.36
8h	2-Cl	64	192-194	$C_{15}H_{12}ClN_4O$	300.75
8i	3-Cl	62	197-199	$C_{15}H_{12}ClN_4O$	300.75
8j	4-Cl	67	228-230	$C_{15}H_{12}ClN_4O$	300.75

COMP.	IR (KBr, cm^{-1})	MASS
8a	3305.63 (N-H stretching), 1656.22 (C=O stretching), 1367.38 (C-CH_3 stretching), 1211.49 (C-N stretching).	267.26
8b	3230.18 (N-H stretching), 1660.84 (C=O stretching), 1458.12 (C-CH_3 stretching), 1263.29 (C-N stretching).	279.25
8c	3216.27 (N-H stretching), 1659.88 (C=O stretching), 1412.74 (C-CH_3 stretching), 1284.34 (C-N stretching).	279.25
8d	3242.38 (N-H stretching), 1652.74 (C=O stretching), 1437.48 (C-CH_3 stretching), 1237.86 (C-N stretching).	279.25
8e	3220.48 (N-H stretching), 1650.13 (C=O stretching), 1440.36 (C-CH_3 stretching), 1245.37 (C-N stretching).	294.15
8f	3221.22 (N-H stretching), 1652.15 (C=O stretching), 1441.83 (C-CH_3 stretching), 1244.24 (C-N stretching).	294.15
8g	3222.45 (N-H stretching), 1650.26 (C=O stretching), 1440.82 (C-CH_3 stretching), 1242.35 (C-N stretching)	294.15
8h	3265.57 (N-H stretching), 1670.73 (C=O stretching), 1373.62 (C-CH_3 stretching), 1262.68 (C-N stretching).	299.09
8i	3311.57 (N-H stretching), 1678.58 (C=O stretching), 1296.48 (C-CH_3 stretching), 1262.94 (C-N stretching).	299.09
8j	3222.65 (N-H stretching), 1668.04 (C=O stretching), 1280.72 (C-CH_3 stretching), 1254.62 (C-N stretching)	299.09

^{1}H and ^{13}C NMR of Synthesized Compounds.

COMP.	^{1}H NMR (DMSO-d_6, 400MHz)	^{13}C NMR(DMSO-d_6, 100MHz)
8a.	10.001 (s; 1H; N<u>H</u>-CO-CH_2), 7.991- 6.974 (m; 9H; Ar-<u>H</u>), 4.968-4.935 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.077-3.044 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$).	36.668, 44.200, 111.403, 118.313, 119.587, 123.746, 124.455, 126.851, 127.656, 129.237, 133.463, 139.404, 144.119, 145.604, 168.737.
8b.	10.150 (s; 1H; N<u>H</u>-CO-CH_2), 7.989- 7.329 (m; 8H; Ar-<u>H</u>), 4.962-4.929 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.081-3.048 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$), 2.456 (s; 3H; C<u>H</u>$_3$).	21.667, 36.677, 44.094, 111.365, 115.362, 118.313, 119.539, 121.434, 124.465, 126.860, 127.665, 132.073, 133.453, 138.753,145.495, 168.967.
8c.	9.920 (s; 1H; N<u>H</u>-CO-CH_2), 7.991- 6.801 (m; 8H; Ar-<u>H</u>), 4.960-4.927 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.069-3.036 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$), 2.195 (s; 3H; C<u>H</u>$_3$).	21.680, 36.658, 44.219, 111.384, 116.818, 119.530, 120.143, 124.493, 127.646, 129.055, 133.663, 138.388, 139.337, 145.614, 168.651.
8d.	9.989 (s; 1H; N<u>H</u>-CO-CH_2), 7.945- 6.821 (m; 8H; Ar-<u>H</u>), 4.964-4.932 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.072-3.045 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$), 2.224 (s; 3H; C<u>H</u>$_3$).	21.672, 36.651, 44.214, 111.376, 116.811, 119.525, 120.138, 124.488, 127.639, 129.050, 133.658, 138.380, 139.332, 145.610, 168.647.
8e.	9.260 (s; 1H; N<u>H</u>-CO-CH_2), 8.004- 6.868 (m; 7H; Ar-<u>H</u>), 4.968-4.935 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.058-3.025 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$), 2.163,1.859 (s; 6H; C<u>H</u>$_3$).	17.953, 20.962, 36.236, 44.573, 111.471, 119.501, 124.507, 125.653, 126.851, 127.579, 131.230, 132.246, 133.463, 133.885, 134.852, 145.643, 168.708.

^{1}H and ^{13}C NMR of Synthesized Compounds.

COMP.	^{1}H NMR (DMSO-d_6, 400MHz)	^{13}C NMR(DMSO-d_6, 100MHz)
8f.	9.258 (s; 1H; N<u>H</u>-CO-CH_2), 8.001- 6.852 (m; 7H; Ar-<u>H</u>), 4.928-4.936 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.042-3.012 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$), 2.154,1.846 (s; 6H; C<u>H</u>$_3$).	19.265, 20.109, 36.629, 44.276, 111.394, 117.201, 119.520, 120.881, 124.436, 127.627, 130.042, 131.498, 133.453, 136.740, 137.152, 168.392.
8g.	9.237 (s; 1H; N<u>H</u>-CO-CH_2), 7.993- 6.825 (m; 7H; Ar-<u>H</u>), 4.939-4.902 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.040-3.010 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$), 2.159,1.844 (s; 6H; C<u>H</u>$_3$).	19.945, 20.952, 36.247, 44.569, 111.463, 119.505, 124.502, 125.648, 126.854, 127.576, 131.235, 132.242, 133.459, 133.880, 134.841, 145.639, 168.702.
8h.	10.212 (s; 1H; N<u>H</u>-CO-CH_2), 7.994- 7.242 (m; 8H; Ar-<u>H</u>), 4.968-4.939 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.106-3.072 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$).	36.662, 44.054, 111.341, 117.942, 119.056, 119.545, 123.522, 124.461, 127.670, 130.957, 133.450, 140.798, 1456.600, 169.203.
8i.	10.206 (s; 1H; N<u>H</u>-CO-CH_2), 7.991- 7.253 (m; 8H; Ar-<u>H</u>), 4.966-4.935 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.098-3.065 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$).	36.668, 44.056, 111.346, 117.948, 119.060, 119.549, 123.526, 124.465, 127.675, 130.962, 133.453, 140.803, 1456.604, 169.207.
8j.	10.149 (s; 1H; N<u>H</u>-CO-CH_2), 7.990- 7.269 (m; 8H; Ar-<u>H</u>), 4.965-4.932 (t; 2H; NH-CO-C<u>H</u>$_2$-CH_2), 3.084-3.051 (t; 2H; NH-CO-CH_2-C<u>H</u>$_2$).	36.658, 44.113, 111.365, 118.313, 119.539, 121.111, 124.465, 126.851, 127.330, 127.665, 129.166, 133.453, 138.341, 145.595, 168.938.

Anti-HIV-1 Activity of Test Samples *in Vitro*

Testing Report

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Table 2. Cytotoxicity and AntiHIV activities of compound (7a-7j) and (8a-8j)

Compounds	Experiment	Method	CC_{50} (µM)	EC_{50} (µM)	Therapeutic Index (TI)
7a	Cytotoxicity assay	MTT	87.2	-	17.5
	Inhibition of syncytium formation	CPE	-	4.98	
7b	Cytotoxicity Assay	MTT	64.35	-	19.9
	Inhibition of syncytium formation	CPE	-	3.23	
7c	Cytotoxicity Assay	MTT	36.4	-	3.4
	Inhibition of syncytium formation	CPE	-	10.72	
7d	Cytotoxicity Assay	MTT	31.01	-	9.1
	Inhibition of syncytium formation	CPE	-	3.4	
7e	Cytotoxicity Assay	MTT	59.23	-	17.8
	Inhibition of syncytium formation	CPE	-	3.37	
7f	Cytotoxicity Assay	MTT	27.14	-	8.1
	Inhibition of syncytium formation	CPE	-	3.39	
7g	Cytotoxicity Assay	MTT	109.83	-	8.02
	Inhibition of syncytium formation	CPE	-	13.7	
7h	Cytotoxicity Assay	MTT	20.91	-	5.5
	Inhibition of syncytium formation	CPE	-	3.77	
7i	Cytotoxicity Assay	MTT	25.21	-	9.1
	Inhibition of syncytium formation	CPE	-	2.77	
7j	Cytotoxicity Assay	MTT	19.91	-	1.6
	Inhibition of syncytium formation	CPE	-	12.43	

8a	Cytotoxicity Assay	MTT	31.69	-	2.7
	Inhibition of syncytium formation	CPE	-	11.75	
8b	Cytotoxicity Assay	MTT	42.03	-	2.8
	Inhibition of syncytium formation	CPE	-	15.09	
8c	Cytotoxicity Assay	MTT	>200	-	>22.4
	Inhibition of syncytium formation	CPE	-	8.92	
8d	Cytotoxicity Assay	MTT	80.00	-	31.1
	Inhibition of syncytium formation	CPE	-	2.57	
8e	Cytotoxicity Assay	MTT	104.63	-	30.5
	Inhibition of syncytium formation	CPE	-	3.43	
8f	Cytotoxicity Assay	MTT	100.9	-	51.5
	Inhibition of syncytium formation	CPE	-	1.96	
8g	Cytotoxicity Assay	MTT	7.00	-	15.7
	Inhibition of syncytium formation	CPE	-	4.97	
8h	Cytotoxicity Assay	MTT	88.11	-	6.8
	Inhibition of syncytium formation	CPE	-	13.00	
8i	Cytotoxicity Assay	MTT	117.39	-	34.6
	Inhibition of syncytium formation	CPE	-	3.39	
8j	Cytotoxicity Assay	MTT	25.14	-	11.4
	Inhibition of syncytium formation	CPE	-	2.2	
AZT	Cytotoxicity Assay	MTT	1291.00	-	514342.6
	Inhibition of syncytium formation	CPE	-	0.00251	

Antibacterial Activity

Strain	Gram-positive bacteria: *Staphylococcus aureus* NCIM 2122 , *Bacillus subtilis* MTCC 121. Gram-negative bacteria: *Escherichia coli* MTCC 118, *Pseudomonas aeruginosa* MTCC 647, *Salmonella typhi* NCIM 2501, *Klebsiella pneumoniae* MTCC 3384.
Medium	Double strength nutrient broth
Method	Two fold serial dilution
Culture used	10^8 -10^7 CFU/mL
Test compounds	1-60
Standard	Ciprofloxacin
Incubation Condition	35 - 37 ° C for 24 h
Growth assessment	Visual Observation
MIC	Lowest concentration tested that completely inhibited growth.

Table 3: Minimum Inhibitory Concentration (MIC) of test compounds (7a-7j) and (8a-8j) against *Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas aeruginosa, Salmonella typhi and Klebsiella Pneumoniae*.

Test compound	MIC(µg/ml)					
	Gram +ve bacteria		*Gram -ve bacteria*			
	***S. aureus* (NCIM 2122)**	***B. subtilis* (MTCC 121)**	***E. coli* (MTCC118)**	***P. aeruginosa* (MTCC 647)**	***S. typhi* (NCIM 2501)**	***K. pneumoniae* (MTCC 3384)**
7a	3.125	6.25	12.5	12.5	3.125	3.125
7b	6.25	3.125	12.5	12.5	6.25	6.25
7c	6.25	3.125	6.25	6.25	25	12.5
7d	3.125	6.25	6.25	3.125	12.5	25
7e	6.25	3.125	25	6.25	3.125	12.5
7f	6.25	3.125	6.25	3.125	25	12.5
7g	3.125	6.25	25	6.25	6.25	12.5
7h	3.125	3.125	3.125	3.125	3.125	6.25
7i	3.125	6.25	6.25	6.25	3.125	6.25
7j	3.125	6.25	3.125	6.25	6.25	3.125
8a	6.25	6.25	25	25	6.25	6.25
8b	6.25	6.25	6.25	6.25	12.5	12.5
8c	6.25	3.125	6.25	6.25	12.5	12.5
8d	3.125	6.25	6.25	3.125	25	12.5
8e	6.25	6.25	12.5	6.25	6.25	12.5
8f	6.25	6.25	6.25	6.25	12.5	25
8g	6.25	3.125	12.5	3.125	6.25	12.5
8h	3.125	6.25	3.125	6.25	12.5	3.125
8i	3.125	6.25	6.25	6.25	6.25	6.25
8j	6.25	3.125	6.25	3.125	3.125	6.25
Ciprofloxacin	≤1	≤1	≤1	≤1	≤1	≤1

Antifungal Activity

Strain	*Candida albicans* MTCC 227 and *Aspergillus niger* NCIM 1026
Medium	Double strength malt yeast extract broth
Method	Two fold serial dilution
Culture used	10^8 -10^7 CFU/mL
Test compounds	1-60
Standard	Fluconazole
Incubation Condition	25 - 27 ° C for 48 h
Growth assessment	Visual Observation
MIC	Lowest concentration tested that completely inhibited growth.

Table 4: Minimum Inhibitory Concentration (MIC) of test compounds (7a-7j) and (8a-8j) against *Candida albicans* and *Aspergillus niger.*

Test compound	MIC(µg/ml)	
	C.albicans **(MTCC 227)**	***A.niger*** **(NCIM 1056)**
7a	12.5	12.5
7b	12.5	12.5
7c	6.25	12.5
7d	12.5	12.5
7e	3.125	6.25
7f	3.125	6.25
7g	12.5	25
7h	6.25	12.5
7i	6.25	6.25
7j	3.125	3.125
8a	12.5	12.5
8b	12.5	12.5
8c	6.25	6.25
8d	3.125	6.25
8e	6.25	6.25
8f	6.25	12.5
8g	3.125	3.125
8h	3.125	3.125
8i	6.25	6.25
8j	12.5	25
Fluconazole	12.5	12.5

Anthelmintic Activity

Earthworm species	Pheretima Posthuma
Medium	2% v/v Tween80 and normal saline
Method	Two fold serial dilution
Paralysis and Death assessment	Paralysis- When worms do not revive even in normal saline Death-When worms lost their motility followed with fading away of their body color
Test compounds	1-60
Standard	Albendazole
PT	Paralysis Time (Time taken to paralysis)
LT	Lethal Time (Time taken to death)

Table 5: Anthelmentic activity of test compounds 7a-7j and 8a-8j against *Pheretima Posthuma.*

Test Compound	Time Taken for Paralysis (P) and Death (D)					
	Paralysis time(PT)			Lethal time (LT)		
	5mg	10 mg	20mg	5mg	10 mg	20mg
7a	20.96±0.47	15.96±0.61	12.45±0.45	24.73±0.55	19.40±0.71	11.95±0.33
7b	14.21±0.55	9.06±0.47	5.93±0.55	19.33±0.55	14.66±0.37	8.70±0.60
7c	12.56±0.47	10.53±0.47	6.73±0.70	16.50±0.56	13.55±0.71	9.30±0.62
7d	18.57±0.57	19.64±0.68	8.60±0.45	21.63±0.41	23.86±0.68	11.96±0.58
7e	14.06±0.51	10.66±0.26	7.10±0.32	20.40±1.15	16.06±0.31	11.75±0.11
7f	20.00±0.70	17.67±0.50	12.3±0.20	26.10±0.42	20.70±0.61	14.35±0.34
7g	18.03±0.47	15.73±0.36	14.56±0.6	24.70±0.60	20.93±0.37	17.46±0.60
7h	29.96±0.41	20.75±0.52	14.7±0.43	35.96±0.51	24.35±0.80	17.00±0.50
7i	11.63±0.51	10.9±0.71	6.1±0.43	15.70±0.70	12.60±0.62	8.90±0.62
7j	19.87±0.31	14.90±0.41	9.26±0.18	29.60±0.36	20.00±0.35	12.2±0.21
8a	10.5±0.51	9.66±0.61	6.34±0.10	15.93±0.26	12.86±0.68	9.96±0.49
8b	17.66±0.47	13.63±0.47	8.67±0.66	23.73±0.47	18.00±0.55	10.26±0.60
8c	22.43±0.22	18.13±0.51	8.63±0.55	26.33±0.58	23.90±0.65	15.34±0.15
8d	09.80±0.52	6.60±0.43	4.35±0.20	13.06±0.51	9.53±0.73	6.66±0.37
8e	25.75±0.41	17.63±0.51	12.63±0.66	24.63±0.47	20.80±0.55	16.03±0.55
8f	23.57±0.73	18.26±0.85	8.43±0.05	33.56±0.43	22.90±0.42	12.23±0.11
8g	20.96±0.47	15.96±0.61	7.45±0.45	24.73±0.55	19.40±0.71	11.95±0.33
8h	10.21±0.55	8.06±0.47	4.93±0.55	16.33±0.55	12.66±0.37	7.70±0.60
8i	12.56±0.47	10.53±0.47	6.73±0.70	16.50±0.56	13.55±0.71	9.30±0.62
8j	17.57±0.57	19.64±0.68	8.6±0.45	21.63±0.41	23.86±0.68	11.96±0.58
Albendazole		05.32±0.72			08.35±0.54	

Conclusions

➢ Compounds containing benzotriazole nucleus, exhibited a wide variety of activities such as anti-HIV, antibacterial, antifungal, anthelmentic, antiprotozoal, antiviral, anticancer, antihistaminic, antiulcer, antipshycotic and various other biological activities.

➢ All the test compounds (7a-7j) and (8a-8j) were evaluated for anti-HIV, antibacterial, antifungal and anthelmintic activities.

➢ All the test compounds from (7a-7j) and (8a-8j) were screened for anti-HIV activities. Compounds 7a, 7b, 7e, 7i, 8c, 8d, 8e, 8f and 8i showed a significant degree of anti-HIV activity compared to zidovudine (AZT).

➢The test compounds 7h, 7i, 7j, 8h, 8i and 8j exhibited very high activity against all the strains of Gram (+) ve and Gram (-) ve bacteria.

➢The test compounds 7a, 7b, 7c, 7d, 7e, 7f, 7g, 7h, 7i, 7j, 8a, 8b, 8c, 8d, 8e, 8f, 8g, 8h, 8i and 8j were found to be very active against both the above fungal strains.

➢Test compounds 7b, 7e, 7i, 8a, 8d, 8h and 8i were found very active at all concentrations of dose which was comparable to the standard dose of albendazole.

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Thank
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