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SHORT REPORT

Synthesis and Evaluation of Antifungal Activity of Benzotriazole Derivatives

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ABSTRACT

The present research work is related to synthesis of different derivatives of benzotriazole at the laboratory scale. By reacting substituted orthochloronitrobenzene with hydrazine hydrate in the presence of sodium carbonate gives substituted 1-hydroxy benzotriazole. Further First series of 1-(benzoyloxy) benzotriazole have been synthesized by esterification reaction of 1-hydroxy-bezotriazole with substituted benzoic acid by using dicyclohexylcarbdiimide (DCC) as a catalyst, second series of 1-(benzoyloxy)-6-chloro benzotriazole have been synthesized by esterification reaction of 1-hydroxy-6-chloro benzotriazole with substituted benzoic acid by using dicyclohexylcarbdiimide (DCC) as a catalyst, and third series of 1-(benzoyloxy)-6-nitro benzotriazole have been synthesized by esterification reaction of 1-hydroxy-6-nitro benzotriazole with substituted benzoic acid by using dicyclohexylcarbdiimide (DCC) as a catalyst All the compounds have been evaluated for *in-vitro* antifungal activity (MIC) against *Trichophyton rubrum, Epidermophyton floccosum* and *Malassazia furfur* by using tube dilution method & activity was compared with Ketoconazole. In the primary screening some of the compounds exhibited appreciable activity. The purity of synthesized compounds was checked by using TLC & structure of the synthesized compounds is confirmed on the basis of spectral data.

Keywords: Benzotriazole; antifungal, *in-vitro* antifungal activity.

1. INTRODUCTION

The increasing incidence of fungal infection associated with unsatisfactory therapeutic treatment in immunocompromized patients and the emergence of azole-resistant fungal strains have stimulated the search for alternative antifungal drugs with higher potency and broader spectrum of activity against resistant fungal strains along with a greater metabolic stability. From a clinical stand point, candidiasis and aspergillosis are the most common fungal infections affecting immunocompromized individuals [1, 2].

Triazole may be considered as a bioisostere of imidazole which is incorporated into the structures of many antifungal compounds [3].With the aim of obtaining new antifungal compounds, we synthesized a series of benzotriazole derivatives.

CYP51 is an essential enzyme in the sterol biosynthetic pathway in eukaryotes, where inhibition by azole drugs in fungi leads to a depletion of ergosterol [4]. The key interactions in the active site are these components: (i) the amidine nitrogen atom (N-3 in the imidazoles, N-4 in the triazoles) to bind to the heme iron of enzyme; (ii) aromatic rings; (iii) the large nonpolar portion of molecule [5].

2. MATERIALS AND METHODS

Chemistry

1-(Benzoyloxy)-(1H-Benzo[d] [1,2,3]triazol-1-yl) derivatives B1-B30 were prepared according to the procedure depicted in **Scheme-1**. The precursor 1-hydroxy-(1H-Benzo[d] [1,2,3]triazol-1-yl) **1, 2 and 3** was prepared according to a previously reported method [6] by reaction of substituted Ortho chloro nitro benzene with hydrazine

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hydrate in the presence of sodium carbonate. When **1, 2 and 3** are reacted with different substituted benzoic acid in presence of dicyclohexylcarbdiimide as catalyst
afforded the target compounds B1-B30 in good yield. The substitution data is given in **table 1**.

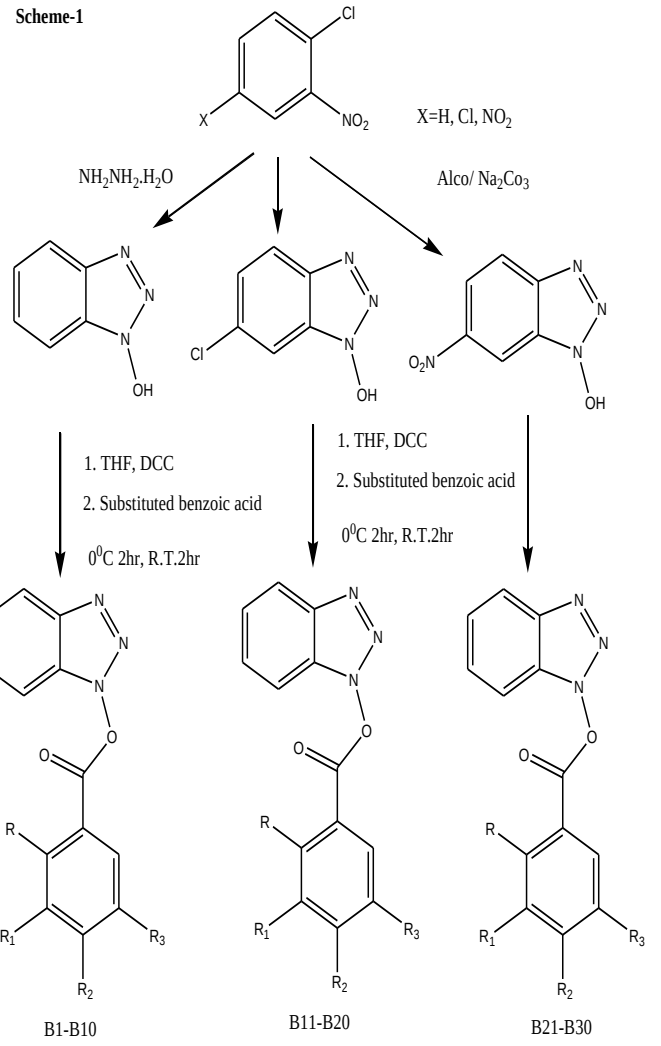
Sr.No.	Code	X	R	R1	R2	R3
1	B1	H	H	H	H	H
2	B2	H	H	H	NO_2	H
3	B3	H	H	NO_2	H	H
4	B4	H	Cl	H	H	H
5	B5	H	H	H	Cl	H
6	B6	H	H	NO_2	H	NO_2
7	B7	H	H	CH_3	H	H
8	B8	H	CH_3	H	H	H
9	B9	H	H	H	CH_3	H
10	B10	H	H	H	OCH_3	H
11	B11	Cl	H	H	H	H
12	B12	Cl	H	H	NO_2	H
13	B13	Cl	H	NO_2	H	H
14	B14	Cl	Cl	H	H	H
15	B15	Cl	H	H	Cl	H
16	B16	Cl	H	NO_2	H	NO_2
17	B17	Cl	H	CH_3	H	H
18	B18	Cl	CH_3	H	H	H
19	B19	Cl	H	H	CH_3	H
20	B20	Cl	H	H	OCH_3	H
21	B21	NO_2	H	H	H	H
22	B22	NO_2	H	H	NO_2	H
23	B23	NO_2	H	NO_2	H	H
24	B24	NO_2	Cl	H	H	H
25	B25	NO_2	H	H	Cl	H
26	B26	NO_2	H	NO_2	H	NO_2
27	B27	NO_2	H	CH_3	H	H
28	B28	NO_2	CH_3	H	H	H
29	B29	NO_2	H	H	CH_3	H
30	B30	NO_2	H	H	OCH_3	H

Table No. 1: Substitution data of the compounds (B1-B30)

Melting points of all the synthesized compounds are uncorrected. The purity of synthesized compounds was checked by thin-layer chromatography. The IR spectra have been recorded on FT IR spectrophotometer Shamadzu using Nujol. ^{1}H NMR spectra were scanned at 300 MHz Varian-NMR-Mercury 300 FT NMR spectrophotometer in $CDCl_3$ using TMS as an internal standard. Physicochemical and spectral analysis data is given in **table 2**. [7, 8]

General

Procedure for the synthesis of derivatives of 1-hydroxy-(1H-Benzo[d] [1,2,3]triazol-1-yl): Ortho chloro nitro benzene (15.75gm, 0.1mol), was refluxed with hydrazine hydrate (10gm, 0.2mol) in ethanol (50ml) in the presence of sodium carbonate (10.6gm, 0.13mol) for 24hrs. After completion of reaction, mixture was diluted with ice-cold water and acidified with dilute HCl. The precipitated product was filtered. The precipitate was washed with cold water. The product was recrystallized from hot water. The precipitated product was filtered. The precipitate was washed with cold water. The product was recrystallized from hot water. This was obtained as a white solid in 62.96% yield. R*f*= 0.72 (95:5 EtOAc:MeOH); mp 158–161oC; IR (KBr) cm-1 3500-3300 (O-H), 1160 (N-O) ;1H-NMR (300 MHz, CDCl3+ (CD3)2 CO) δ): 5.34 (1H, s, OH), 7.86 (1H,d, J =9.0 Hz, Bt-4), 7.35 (1H, d, J = 7.8 Hz, Bt-5),7.38 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7).



Where; X=H, Cl, NO_2. R, R_1, R_2, R_3= H, NO_2, Cl, CH_3, OCH_3.

Scheme 1: The synthetic pathway of compounds B1-B30.

Procedure for the synthesis of derivatives of 1-hydroxy-6-chloro-(1H-Benzo[d] [1,2,3]triazol-1-yl): 2,5-dichloro nitro benzene (19.1gm, 0.1mol), was refluxed with hydrazine hydrate (10gm, 0.2mol) in ethanol (50ml) in the presence of sodium carbonate (10.6gm, 0.13mol) for 24hrs. After

completion of reaction, mixture was diluted with ice-cold water and acidified with dilute HCl. The precipitated product was filtered. The precipitate was washed with cold water. The product was recrystallized from hot water. This was obtained as a white solid in 53.25% yield. R*f*= 0.62 (95:5 EtOAc:MeOH); mp 190–192oC; IR (KBr) cm-1 3500-3300 (O-H), 1160 (N-O) ;1H-NMR (300 MHz, CDCl3+ (CD3)2 CO) δ): 2.58 (1H, s, OH), 7.30 (1H,d, J =10 Hz, Bt-4), 7.84 (1H, d, J = 9 Hz, Bt-5), 7.61 (1H, s, Bt-7).

Procedure for the synthesis of derivatives of 1-hydroxy-6-nitro-(1H-Benzo[d] [1,2,3]triazol-1-yl): 2-chloro-1,5-dinitro benzene (19.1gm, 0.1mol), was refluxed with hydrazine hydrate (10gm, 0.2mol) in ethanol (50ml) in the presence of sodium carbonate (10.6gm, 0.13mol) for 24hrs. After completion of reaction, mixture was diluted with ice-cold water and acidified with dilute HCl. The precipitated product was filtered. The precipitate was washed with cold water. The product was recrystallized from hot water. This was obtained as a white solid in 53.25% yield. R*f*= 0.62 (95:5 EtOAc:MeOH); mp 190–192oC; IR (KBr) cm-1 3500-3300 (O-H), 1580 (N=O), 1310 (N=O),1160 (N-O) ;1H-NMR (300 MHz, CDCl3+ (CD3)2 CO) δ): 2.58 (1H, s, OH), 7.30 (1H,d, J =10 Hz, Bt-4), 7.84 (1H, d, J = 9 Hz, Bt-5), 7.61 (1H, s, Bt-7).

Sr. No.	Code	Molecular formula	Molecular weight	M.P. (^{o}c)	R_f value	Spectral analysis data
1	B1	$C_{13}H_9O_2N_3$	239	76-78	0.76[1]	IR (KBr) cm^{-1} 1780 (C=O), 1240 (C-O), 1600 (C=C), 1160 (N-O), 730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.86 (1H,d, J =9.0 Hz, Bt-4), 7.35 (1H, d, J = 7.8 Hz, Bt-5),7.38 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 8.13 (1H,d, J =11 Hz, Be-2^1), 7.47 (1H, d, J = 8 Hz, Be-3^1),7.58 (1H, d, J =14 Hz, Be-4^1), 7.45 (1H, d, J= 7.2 Hz, Be-5^1). 8.12 (1H, d, J= 9.2 Hz, Be-6^1).
2	B2	$C_{13}H_8O_4N_4$	284	142-145	0.81[2]	IR (KBr) cm^{-1} 1800 (C=O), 1650 (C=C), 1540 (C-NO_2), 1350 (C-NO_2),730 (C-H) 1250 (C-O), 1160 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.98 (1H,d, J =9.0 Hz, Bt-4), 7.45 (1H, d, J = 7.8 Hz, Bt-5),7.42 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 8.39 (1H,d, J =11 Hz, Be-2^1), 8.47 (1H, d, J = 8 Hz, Be-3^1), 8.45 (1H, d, J= 7.2 Hz, Be-5^1). 8.32 (1H, d, J= 9.2 Hz, Be-6^1).
3	B3	$C_{13}H_8O_4N_4$	284	148-149	0.84[3]	IR (KBr) cm^{-1} 1793 (C=O), 1632 (C=C), 1538 (C-NO_2), 1347 (C-NO_2),728 (C-H) 1246 (C-O), 1164 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.85 (1H,d, J =9.0 Hz, Bt-4), 7.38 (1H, d, J = 7.8 Hz, Bt-5),7.34 (1H, d, J =14 Hz, Bt-6), 7.84 (1H, d, J= 7.2 Hz, Bt-7), 8.82 (1H,d, J =11 Hz, Be-2^1), 8.58 (1H, d, J =14 Hz, Be-4^1), 7.73 (1H, d, J= 7.2 Hz, Be-5^1). 8.52 (1H, d, J= 9.2 Hz, Be-6^1).
4	B4	$C_{13}H_8O_2N_3Cl$	273	61-64	0.87[3]	IR (KBr) cm^{-1} 1804 (C=O), 1628 (C=C), 1592 (C-C), 1251-1229 (C-O), 1105 (N-O), 818 (C-Cl), 722 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.86 (1H,d, J =9.0 Hz, Bt-4), 7.35 (1H, d, J = 7.8 Hz, Bt-5),7.38 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 7.49 (1H, d, J = 8 Hz, Be-3^1),7.54 (1H, d, J =14 Hz, Be-4^1), 7.35 (1H, d, J= 7.2 Hz, Be-5^1). 8.07 (1H, d, J= 9.2 Hz, Be-6^1).
5	B5	$C_{13}H_8O_2N_3Cl$	273	127-129	0.65[3]	IR (KBr) cm^{-1} 1800 (C=O), 1630 (C=C), 1580 (C-C), 1250-1230 (C-O), 1100 (N-O), 820 (C-Cl), 730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.88 (1H,d, J =9.2 Hz, Bt-4), 7.37 (1H, d, J = 7.7 Hz, Bt-5),7.38 (1H, d, J =12 Hz, Bt-6), 7.88 (1H, d, J= 7.1 Hz, Bt-7), 8.07 (1H,d, J =11 Hz, Be-2^1), 7.5 (1H, d, J = 8 Hz, Be-3^1), 7.46 (1H, d, J= 7.2 Hz, Be-5^1). 7.94 (1H, d, J= 9.7 Hz, Be-6^1).
6	B6	$C_{13}H_7O_6N_5$	329	140-142	0.70[4]	IR (KBr) cm^{-1} 1787 (C=O), 1629 (C=C), 1541 (C-NO_2), 1342 (C-NO_2),731 (C-H) 1252 (C-O), 1154 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.52 (1H,d, J =8.7 Hz, Bt-4), 7.48 (1H, d, J = 7.8 Hz, Bt-5),7.32 (1H, d, J =9.8 Hz, Bt-6), 7.78 (1H, d, J= 7.2 Hz, Bt-7), 9.23 (1H,d, J =11 Hz, Be-2^1), 9.46 (1H, d, J =14 Hz, Be-4^1), 9.27 (1H, d, J= 11 Hz, Be-6^1).
7	B7	$C_{14}H_{11}O_2N_3$	253	113-115	0.65[4]	IR (KBr) cm^{-1} 1800 (C=O), 1630 (C=C), 1580 (C-C), 1250-1230 (C-O), 1100 (N-O), 730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.91 (1H,d, J =9.0 Hz, Bt-4), 7.37 (1H, d, J = 7.8 Hz, Bt-5),7.41 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 7.84 (1H,d, J =11 Hz, Be-2^1), 2.10 (3H, d, J = 4.5 Hz, Be-3^1 CH_3), 7.4 (1H, d, J =14 Hz, Be-4^1), 7.35 (1H, d, J= 7.2 Hz, Be-5^1). 7.94 (1H, d, J= 9.2 Hz, Be-6^1).
8	B8	$C_{14}H_{11}O_2N_3$	253	155-158	0.97[3]	IR (KBr) cm^{-1} 1795 (C=O), 1638 (C=C), 1594 (C-C), 1252-1232 (C-O), 1110 (N-O), 735 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.86 (1H,d, J =9.0 Hz, Bt-4), 7.35 (1H, d, J = 7.8 Hz, Bt-5),7.38 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 2.21 (3H, d, J = 4.5 Hz, Be-2^1 CH_3), 7.27 (1H, d, J = 8 Hz, Be-3^1),7.48 (1H, d, J =14 Hz, Be-4^1), 7.28 (1H, d, J= 7.2 Hz, Be-5^1). 8.01 (1H, d, J= 9.2 Hz, Be-6^1).
9	B9	$C_{14}H_{11}O_2N_3$	253	206-208	0.81[3]	IR (KBr) cm^{-1} 1802 (C=O), 1631 (C=C), 1587 (C-C), 1248-1227 (C-O), 1100 (N-O), 730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.89 (1H,d, J =9.0 Hz, Bt-4), 7.45 (1H, d, J = 7.8 Hz, Bt-5),7.40 (1H, d, J =14 Hz, Bt-6), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 8.01 (1H,d, J =11 Hz, Be-2^1), 7.27 (1H, d, J = 8 Hz, Be-3^1), 2.28 (3H, d, J = 4.5 Hz, Be-4^1 CH_3), 7.28 (1H, d, J= 7.2 Hz, Be-5^1). 8.14 (1H, d, J= 9.2 Hz, Be-6^1).
10	B10	$C_{14}H_{11}O_3N_3$	269	115-118	0.90[2]	IR (KBr) cm^{-1} 1730 (C=O), 1610 (C=C), 1580 (C-C), 1260 (C-O), 1170 (N-O), 830 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.98 (1H,d, J =9.0 Hz, Bt-4), 7.45 (1H, d, J = 7.8 Hz, Bt-5),7.47 (1H, d, J =14 Hz, Bt-6), 7.93 (1H, d, J= 7.2 Hz, Bt-7), 8.02 (1H,d, J =11 Hz, Be-2^1), 6.98 (1H, d, J = 8 Hz, Be-3^1), 3.73 (3H, d, J = 4.5 Hz, Be-4^1 CH_3), 6.98 (1H, d, J= 7.2 Hz, Be-5^1). 8.02 (1H, d, J= 9.2 Hz, Be-6^1).
11	B11	$C_{13}H_8O_2N_3Cl$	273	80-83	0.80[5]	IR (KBr) cm^{-1} 1780 (C=O), 1230 (C-O), 1620 (C=C), 1160 (N-O), 790 (C-Cl) 730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.92 (1H,d, J =10 Hz, Bt-4), 7.84 (1H, d, J = 9 Hz, Bt-5), 7.91 (1H, s, Bt-7), 8.13 (1H,d, J =11 Hz, Be-2^1), 7.47 (1H, d, J = 8 Hz, Be-3^1),7.58 (1H, d, J =14 Hz, Be-4^1), 7.45 (1H, d, J= 7.2 Hz, Be-5^1). 8.12 (1H, d, J= 9.2 Hz, Be-6^1).

12	B12	$C_{13}H_7O_4N_4Cl$	318	150-154	0.85[5]	IR (KBr) cm^{-1} 1800 (C=O), 1650 (C=C), 1540 (C-NO_2), 1350 (C-NO_2), 810 (C-Cl), 730 (C-H) 1250 (C-O), 1160 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.92 (1H,d, J =9.0 Hz, Bt-4), 7.46 (1H, d, J = 7.8 Hz, Bt-5), 7.98 (1H, d, J= 7.2 Hz, Bt-7), 8.39 (1H,d, J =11 Hz, Be-2^1), 8.47 (1H, d, J = 8 Hz, Be-3^1), 8.45 (1H, d, J= 7.2 Hz, Be-5^1). 8.32 (1H, d, J= 9.2 Hz, Be-6^1).
13	B13	$C_{13}H_7O_4N_4Cl$	318	160-162	0.78[3]	IR (KBr) cm^{-1} 1796 (C=O), 1653 (C=C), 1539 (C-NO_2), 1356 (C-NO_2), 813 (C-Cl), 731 (C-H) 1248 (C-O), 1161 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.95 (1H,d, J =9.0 Hz, Bt-4), 7.42 (1H, d, J = 7.8 Hz, Bt-5), 7.93 (1H, d, J= 7.2 Hz, Bt-7), 8.82 (1H,d, J =11 Hz, Be-2^1), 8.58 (1H, d, J =14 Hz, Be-4^1), 7.73 (1H, d, J= 7.2 Hz, Be-5^1). 8.52 (1H, d, J= 9.2 Hz, Be-6^1).
14	B14	$C_{13}H_7O_2N_3Cl_2$	307	80-83	0.90[5]	IR (KBr) cm^{-1} 1781 (C=O), 1618 (C=C), 1574 (C-C), 1254-1231 (C-O), 1106 (N-O), 816 (C-Cl), 723 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.86 (1H,d, J =9.0 Hz, Bt-4), 7.35 (1H, d, J = 7.8 Hz, Bt-5), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 7.49 (1H, d, J = 8 Hz, Be-3^1),7.54 (1H, d, J =14 Hz, Be-4^1), 7.35 (1H, d, J= 7.2 Hz, Be-5^1). 8.07 (1H, d, J= 9.2 Hz, Be-6^1).
15	B15	$C_{13}H_7O_2N_3Cl_2$	307	135-138	0.75[5]	IR (KBr) cm^{-1} 1800 (C=O), 1630 (C=C), 1580 (C-C), 1250-1230 (C-O), 1100 (N-O), 820 (C-Cl), 730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.88 (1H,d, J =9.2 Hz, Bt-4), 7.37 (1H, d, J = 7.7 Hz, Bt-5), 7.88 (1H, d, J= 7.1 Hz, Bt-7), 8.07 (1H,d, J =11 Hz, Be-2^1), 7.5 (1H, d, J = 8 Hz, Be-3^1), 7.46 (1H, d, J= 7.2 Hz, Be-5^1). 7.94 (1H, d, J= 9.7 Hz, Be-6^1).
16	B16	$C_{13}H_6O_6N_5Cl$	363	151-153	0.78[5]	IR (KBr) cm^{-1} 1811 (C=O), 1662 (C=C), 1543 (C-NO_2), 1349 (C-NO_2), 812 (C-Cl), 741 (C-H) 1261 (C-O), 1146 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.92 (1H,d, J =8.7 Hz, Bt-4), 7.48 (1H, d, J = 7.8 Hz, Bt-5), 7.98 (1H, d, J= 7.2 Hz, Bt-7), 9.23 (1H,d, J =11 Hz, Be-2^1), 9.46 (1H, d, J =14 Hz, Be-4^1), 9.27 (1H, d, J= 11 Hz, Be-6^1).
17	B17	$C_{14}H_{10}O_2N_3Cl$	287	146-150	0.73[4]	IR (KBr) cm^{-1} 1786 (C=O), 1624 (C=C), 1579 (C-C), 1247-1228 (C-O), 1104 (N-O), 842 (C-Cl), 728 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.91 (1H,d, J =9.0 Hz, Bt-4), 7.37 (1H, d, J = 7.8 Hz, Bt-5), 7.98 (1H, d, J= 7.2 Hz, Bt-7), 7.84 (1H,d, J =11 Hz, Be-2^1), 2.10 (3H, d, J = 4.5 Hz, Be-3^1 CH_3), 7.4 (1H, d, J =14 Hz, Be-4^1), 7.35 (1H, d, J= 7.2 Hz, Be-5^1). 7.94 (1H, d, J= 9.2 Hz, Be-6^1).
18	B18	$C_{13}H_{10}O_2N_3Cl$	287	190-192	0.76[4]	IR (KBr) cm^{-1} 1798 (C=O), 1645 (C=C), 1591 (C-C), 1255-1240 (C-O), 1118 (N-O), 851 (C-Cl), 726 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.86 (1H,d, J =9.0 Hz, Bt-4), 7.35 (1H, d, J = 7.8 Hz, Bt-5), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 2.21 (3H, d, J = 4.5 Hz, Be-2^1 CH_3), 7.27 (1H, d, J = 8 Hz, Be-3^1),7.48 (1H, d, J =14 Hz, Be-4^1), 7.28 (1H, d, J= 7.2 Hz, Be-5^1). 8.01 (1H, d, J= 9.2 Hz, Be-6^1).
19	B19	$C_{14}H_{10}O_2N_3Cl$	287	215-217	0.85[3]	IR (KBr) cm^{-1} 1800 (C=O), 1630 (C=C), 1580 (C-C), 1250-1230 (C-O), 1100 (N-O), 848 (C-Cl),730 (C-H). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.89 (1H,d, J =9.0 Hz, Bt-4), 7.45 (1H, d, J = 7.8 Hz, Bt-5), 7.88 (1H, d, J= 7.2 Hz, Bt-7), 8.01 (1H,d, J =11 Hz, Be-2^1), 7.27 (1H, d, J = 8 Hz, Be-3^1), 2.28 (3H, d, J = 4.5 Hz, Be-4^1 CH_3), 7.28 (1H, d, J= 7.2 Hz, Be-5^1). 8.14 (1H, d, J= 9.2 Hz, Be-6^1).
20	B20	$C_{14}H_{10}O_3N_3Cl$	303	80-83	0.84[1]	IR (KBr) cm^{-1} 1780 (C=O), 1650 (C=C), 1600 (C-C), 1270-1250 (C-O), 1180-1150 (N-O), 850 (C-Cl). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 7.98 (1H,d, J =9.0 Hz, Bt-4), 7.45 (1H, d, J = 7.8 Hz, Bt-5), 7.93 (1H, d, J= 7.2 Hz, Bt-7), 8.02 (1H,d, J =11 Hz, Be-2^1), 6.98 (1H, d, J = 8 Hz, Be-3^1), 3.73 (3H, d, J = 4.5 Hz, Be-4^1 CH_3), 6.98 (1H, d, J= 7.2 Hz, Be-5^1). 8.02 (1H, d, J= 9.2 Hz, Be-6^1).
21	B21	$C_{13}H_8O_4N_4$	284	153-155	0.88[2]	IR (KBr) cm^{-1} 1785 (C=O), 1648 (C=C), 1542 (C-NO_2), 1348 (C-NO_2), 731 (C-H) 1252 (C-O), 1157 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.24 (1H,d, J =10 Hz, Bt-4), 8.38 (1H, d, J = 9 Hz, Bt-5), 8.91 (1H, s, Bt-7), 8.13 (1H,d, J =11 Hz, Be-2^1), 7.47 (1H, d, J = 8 Hz, Be-3^1),7.58 (1H, d, J =14 Hz, Be-4^1), 7.45 (1H, d, J= 7.2 Hz, Be-5^1). 8.12 (1H, d, J= 9.2 Hz, Be-6^1).
22	B22	$C_{13}H_7O_6N_5$	329	208-210	0.91[3]	IR (KBr) cm^{-1} 1791 (C=O), 1642 (C=C), 1546 (C-NO_2), 1351 (C-NO_2), 728 (C-H) 1244 (C-O), 1146 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.12 (1H,d, J =9.0 Hz, Bt-4), 8.46 (1H, d, J = 7.8 Hz, Bt-5), 8.88 (1H, d, J= 7.2 Hz, Bt-7), 8.39 (1H,d, J =11 Hz, Be-2^1), 8.47 (1H, d, J = 8 Hz, Be-3^1), 8.45 (1H, d, J= 7.2 Hz, Be-5^1). 8.32 (1H, d, J= 9.2 Hz, Be-6^1).
23	B23	$C_{13}H_7O_6N_5$	329	140-143	0.35[5]	IR (KBr) cm^{-1} 1801 (C=O), 1650 (C=C), 1548 (C-NO_2), 1355 (C-NO_2), 734 (C-H) 1240 (C-O), 1156 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.25 (1H,d, J =9.0 Hz, Bt-4), 8.42 (1H, d, J = 7.8 Hz, Bt-5), 8.83 (1H, d, J= 7.2 Hz, Bt-7), 8.82 (1H,d, J =11 Hz, Be-2^1), 8.58 (1H, d, J =14 Hz, Be-4^1), 7.73 (1H, d, J= 7.2 Hz, Be-5^1). 8.52 (1H, d, J= 9.2 Hz, Be-6^1).
24	B24	$C_{13}H_7O_4N_4Cl$	318	190-192	0.35[4]	IR (KBr) cm^{-1} 1800 (C=O), 1650 (C=C), 1540 (C-NO_2), 1350 (C-NO_2), 810 (C-Cl), 730 (C-H) 1250 (C-O), 1160 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.26 (1H,d, J =9.0 Hz, Bt-4), 8.35 (1H, d, J = 7.8 Hz, Bt-5), 8.87 (1H, d, J= 7.2 Hz, Bt-7), 7.49 (1H, d, J = 8 Hz, Be-3^1),7.54 (1H, d, J =14 Hz, Be-4^1), 7.35 (1H, d, J= 7.2 Hz, Be-5^1). 8.07 (1H, d, J= 9.2 Hz, Be-6^1).
25	B25	$C_{13}H_7O_4N_4Cl$	318	228-230	0.80[1]	IR (KBr) cm^{-1} 1788 (C=O), 1649 (C=C), 1543 (C-NO_2), 1353 (C-NO_2), 818 (C-Cl), 733 (C-H) 1246 (C-O), 1166 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.78 (1H,d, J =9.2 Hz, Bt-4), 8.37 (1H, d, J = 7.7 Hz, Bt-5), 8.91 (1H, d, J= 7.1 Hz, Bt-7), 8.07 (1H,d, J =11 Hz, Be-2^1), 7.5 (1H, d, J = 8 Hz, Be-3^1), 7.46 (1H, d, J= 7.2 Hz, Be-5^1). 7.94 (1H, d, J= 9.7 Hz, Be-6^1).
26	B26	$C_{13}H_6O_8N_6$	374	208-210	0.90[3]	IR (KBr) cm^{-1} 1790 (C=O), 1643 (C=C), 1551 (C-NO_2), 1341 (C-NO_2), 732 (C-H) 1238 (C-O), 1161 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.22 (1H,d, J =8.7 Hz, Bt-4), 8.48 (1H, d, J = 7.8 Hz, Bt-5), 8.94 (1H, d, J= 7.2 Hz, Bt-7), 9.23 (1H,d, J =11 Hz, Be-2^1), 9.46 (1H, d, J =14 Hz, Be-4^1), 9.27 (1H, d, J= 11 Hz, Be-6^1).

27	B27	$C_{14}H_{10}O_4N_4$	298	198-200	0.70[3]	IR (KBr) cm^{-1} 1798 (C=O), 1664 (C=C), 1557 (C-NO_2), 1352 (C-NO_2), 733 (C-H) 1245 (C-O), 1148 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.21 (1H,d, J =9.0 Hz, Bt-4), 8.37 (1H, d, J = 7.8 Hz, Bt-5), 8.84 (1H, d, J= 7.2 Hz, Bt-7), 7.84 (1H,d, J =11 Hz, Be-2[1]), 2.10 (3H, d, J = 4.5 Hz, Be-3[1] CH_3), 7.4 (1H, d, J =14 Hz, Be-4[1]), 7.35 (1H, d, J= 7.2 Hz, Be-5[1]). 7.94 (1H, d, J= 9.2 Hz, Be-6[1]).
28	B28	$C_{14}H_{10}O_4N_4$	298	184-187	0.84[1]	IR (KBr) cm^{-1} 1801 (C=O), 1650 (C=C), 1548 (C-NO_2), 1355 (C-NO_2), 734 (C-H) 1240 (C-O), 1156 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.26 (1H,d, J =9.0 Hz, Bt-4), 8.35 (1H, d, J = 7.8 Hz, Bt-5), 8.93 (1H, d, J= 7.2 Hz, Bt-7), 2.21 (3H, d, J = 4.5 Hz, Be-2[1] CH_3), 7.27 (1H, d, J = 8 Hz, Be-3[1]),7.48 (1H, d, J =14 Hz, Be-4[1]), 7.28 (1H, d, J= 7.2 Hz, Be-5[1]). 8.01 (1H, d, J= 9.2 Hz, Be-6[1]).
29	B29	$C_{14}H_{10}O_4N_4$	298	225-227	0.84[1]	IR (KBr) cm^{-1} 1801 (C=O), 1650 (C=C), 1548 (C-NO_2), 1355 (C-NO_2), 734 (C-H) 1240 (C-O), 1156 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.29 (1H,d, J =9.0 Hz, Bt-4), 8.45 (1H, d, J = 7.8 Hz, Bt-5), 8.86 (1H, d, J= 7.2 Hz, Bt-7), 8.01 (1H,d, J =11 Hz, Be-2[1]), 7.27 (1H, d, J = 8 Hz, Be-3[1]), 2.28 (3H, d, J = 4.5 Hz, Be-4[1] CH_3), 7.28 (1H, d, J= 7.2 Hz, Be-5[1]). 8.14 (1H, d, J= 9.2 Hz, Be-6[1]).
30	B30	$C_{14}H_{10}O_5N_4$	314	188-190	0.85[1]	IR (KBr) cm^{-1} 1810 (C=O), 1662 (C=C), 1555 (C-NO_2), 1351 (C-NO_2), 735 (C-H) 1239 (C-O), 1156 (N-O). ^{1}H-NMR (300 MHz, $CDCl_3$+ $(CD_3)_2$ CO) δ): 8.28 (1H,d, J =9.0 Hz, Bt-4), 8.45 (1H, d, J = 7.8 Hz, Bt-5), 8.93 (1H, d, J= 7.2 Hz, Bt-7), 8.02 (1H,d, J =11 Hz, Be-2[1]), 6.98 (1H, d, J = 8 Hz, Be-3[1]), 3.73 (3H, d, J = 4.5 Hz, Be-4[1] CH_3), 6.98 (1H, d, J= 7.2 Hz, Be-5[1]). 8.02 (1H, d, J= 9.2 Hz, Be-6[1]).

Table No. 2: Physicochemical and Spectral data of synthesized compounds (B1-B30)

Mobile Phase: Benzene[1], Benzene: Acetone 10:0.5[2], Benzene: Acetone 10:1[3], Chloroform: Methanol 10:1[4], Chloroform: Acetone 10:1[5], Chloroform[6].

Procedure for the synthesis of derivatives of 1-(Benzoyloxy)-(1H-Benzo[d] [1,2,3]triazol-1-yl): substituted benzoic acid (0.01mol, 1.22gm) and substituted 1-hydroxy benzotriazole (0.01mol, 1.35gm) were dissolved in dried tetra hydra furan (THF, 40ml) in two-neck round bottom flask with stirrer. The solution was cooled to 0-5^{o}C. to it 2.26gm (0.011mol) of dicyclohexylcarbdiimide (DCC) was added with stirring. The mixture was stirred for 2hrs, and then allowed to warm to room temperature and stirred for an additional 2hrs. The precipitate of dicyclohexylurea was removed by filtration. THF was evaporated by vacuum distillation. The residue was dissolved in100ml of solvent ether (diethyl ether) and separated ethereal layer was washed with sodium carbonate ($NaHCO_3$) solution (20ml x 3times) to neutralize unreacted acid, then with water, and was dried with anhydrous sodium sulphate. The ether was removed on water bath, and the residue is collected and recrystallized from pet-ether at 40-46^{o}C.

Determination of antifungal activity

The antifungal activity was evaluated by tube dilution method (Turbidimetric method) [9] one ml of sterilized media (Sabourand's glucose broth) was poured into sterilized test tubes. 1ml of 1µm/ml test solution was transferred in one tube and serially diluted to give a concentration of 0.5, 0.25, 0.125, 0.0625, 0.0312 & 0.01561µm/ml. To all the tubes 0.1ml of suspension of fungal in saline was added and the tubes were incubated at 30^{0}C (*T. rubrum and M. furfur)* and at 25^{0}C (*E. floccosum*) for 72 hrs. The growth in the tubes was observed visually for turbidity and inhibition was determined by absence of growth. MIC was determined by the lowest concentration of sample that prevented the development of turbidity. Activity was compared with Ketoconazole

3. RESULTS AND DISCUSSION:

The in vitro antifungal activities of compounds B1-B30, using the tube dilution method (turbidometric method). The minimum inhibitory concentration (MIC) values (mg/ml) obtained from triplicate assay (three or two test tubes with identical results were taken as MICs) against *Epidermophyton floccosum, Trychophyton rubrum and Malassazia furfur are* compared with Ketoconazole. The MIC values are presented in the **Table 3**.

Sr. No.	Compound Code	*Epidermophyton floccosum*	*Trychophyton rubrum*	*Malassazia furfur*
1	B1	0.125	0.0625	0.125
2	B2	0.125	0.0625	0.125
3	B3	0.125	0.125	0.125
4	B4	0.125	0.25	0.125
5	B5	0.25	0.25	0.125
6	B6	0.5	0.125	0.125
7	B7	0.25	0.125	0.125
8	B8	0.125	0.5	0.25
9	B9	0.25	0.0625	0.25
10	B10	1.506	0.125	0.25
11	B11	0.0625	1.506	0.125

12	B12	0.5	0.125	0.125
13	B13	0.0625	0.125	0.125
14	B14	0.25	0.125	0.125
15	B15	0.5	0.125	0.125
16	B16	0.0625	0.0625	0.125
17	B17	0.125	0.125	0.25
18	B18	0.0625	0.25	0.25
19	B19	0.25	0.125	0.25
20	B20	0.25	0.25	0.25
21	B21	0.25	0.25	0.25
22	B22	0.5	0.5	0.5
23	B23	0.25	0.25	0.125
24	B24	0.25	0.125	0.125
25	B25	0.25	0.5	0.25
26	B26	0.5	0.125	0.125
27	B27	0.125	0.0625	0.125
28	B28	0.25	0.125	0.125
29	B29	0.25	0.125	0.125
30	B30	0.125	0.0625	0.125
31	Ketoconazole	**0.0156**	**0.0156**	**0.0156**

Table No. 3: *In vitro* antifungal activity of compounds B1-B30.

From antifungal screening it was observed that the compounds B11, B13, B16 and B18 showed MIC values comparable to that of Ketoconazole on *E.floccosum*, compound B1, B2, B9, B16, B27 and B30 showed good activity against *T.rubrum,* and no compound was found to be effective against *M.furfur*. Introduction of electron withdrawing group chloro at 6-position on the benzotriazole ring in compound 2g-j considerably reduced the compounds activities against *T.rubrum*.

4. CONCLUSION:

In summary, a close examination of *in vitro* antifungal activities of variously substituted 1-hydroxy-(1H-Benzo[d] [1,2,3]triazol-1-yl) against the fungal strains provide a better structure activity correlation of the tested compounds, those with Chloro group at C-6 exerted lowest level of antifungal activity. And there is need to synthesize more compounds with varied substitutions on benzotriazole & phenyl ring, which can advances the SAR on benzotriazole for further optimization of antifungal benzotriazole for greater potency and broader spectrum of activity.

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Conflict of Interest: None Declared

