Chemistry & Biology Interface, 2019, 9, 3, 163-171

RESEARCH PAPER ISSN: 2249 –4820

CHEMISTRY & BIOLOGY INTERFACE

An official Journal of ISCB, Journal homepage; www.cbijournal.com

Antimicrobial, cytotoxic and haemolytic activity of newly synthesized Benzotriazole substituted 1,2,4-triazoles

DINESH R. GODHANI[a]*, VISHAL B. MULANI[a], JIGNASU P. MEHTA[a]

[a]*Department of Chemistry (UGC NON-SAP & DST-FIST sponsored Department), Mahatma Gandhi Campus, Maharaja Krishnakumarsinhji Bhavnagar University, Bhavnagar 364002, INDIA*
Corresponding author : Tel : + 91 9687615488, email : drgodhani@yahoo.com
Received 30 May 2019; Accepted 30 June 2019

Abstract: A one-pot reaction leading to 3,5-dibenzotriazole-4-benzylideneamino-1,2,4-triazoles was synthesized by cyclization of Schiff base by phosphoryl chloride and acetonitrile in acidic condition. The structures were recognized on the basis of spectral tools and their purity by elemental analysis. All the compounds were preliminary evaluated for their *in vitro* antimicrobial activities against 5 bacterial strains *viz* [*Staphylococcus aureus* (MRSA; ATCC 43300), *Klebsiella pneumoniae* (ATCC 700603), *Escherichia coli* (ATCC 25922), *Acinetobacter baumannii*(ATCC 19606), *Pseudomonas aeruginosa* (ATCC 27853)] and 2 fungi Strains *viz.* [*Candida albicans*(ATCC 90028), *Cryptococcus neoformans var. grubii* (H99; ATCC 208821)]. Some compounds displayed significant antimicrobial activity, out of 15 compounds, 11 compounds indicated promising antifungal activities without any indications of human cells cytotoxic [Hk: Human Embryonic Kidney cells (ATCC CRL-1573)] and haemolytic activity [RBC (ARCBS 5400 00150)].

Keywords: 1,2,4-triazole; benzotriazole; cytotoxicity; haemolysis; antimicrobial activity

INTRODUCTION

Benzotriazole is an enormously valuable and synthetically explore dauxiliary.Various heterocyclic compounds were synthesized by using this synthetic scaffold including diazepanes, benzoxazines, oxadiazines, quinazolines, hydantoins, triazinetriones and many other heterocyclic compounds. A huge contribution regarding the development of benzotriazole chemistry has been laid by Katritzky and co-workers. *Via* developing an organised benzotriazole methodology [1–6].

The medicinal significance of benzotriazole derivativeshas been deeply explored over the last decade and Benzotriazole derivatives have demonstrated their efficiency to treat several kinds of disease such as psychotropic disorders, cancers, microbial infections, antiprotozoal [7], plant growth regulator [8], antibacterial [9], choleretic [10], and antiviral

activity [11]. Many benzotriazole-based pharmaceutically active molecules (**Figure 1**) are under clinical trial such as alizapride (1) [12], 1-isopropylbenzotriazole-5-carboxylic acid (2) and vorozole (3) [13]. While, vorozole has been withdrawn from clinical trial in the US Phase III trial notwithstanding similar effectiveness to other aromatase inhibitors [14, 15].

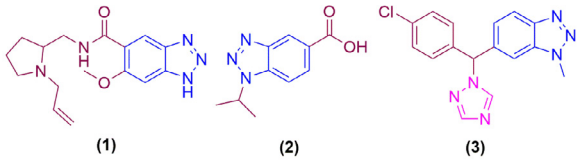
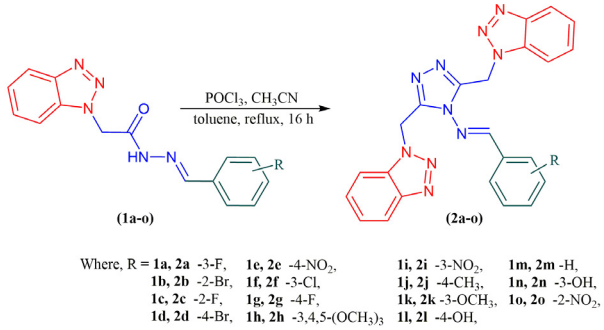


Figure 1. Benzotriazole containing molecules under clinical trial.

1,2,4-Triazole and its derivatives are an imperative type of compounds which destroys the consistency of the fungal cell wall and prevents the progress and reproduction of fungi [16] Furthermore, it shows antimicrobial [17-19], and antifungal [20] activities.

A search of the literature revealed that very few published reports define the route of cyclization of Schiff base to 3,5-dibenzotriazole-4-benzylideneamino-1,2,4-triazole nucleus (Zielinski *et al*.) [21]. Utilizing a similar procedure and diverse approach, we have incorporated fifteen Benzotriazole substituted 1,2,4-triazole derivatives. As outlined in **Scheme 1.** Furthermore, their antimicrobial, cytotoxic and haemolytic activities were determined.



Scheme 1. Synthetic track for the preparation of title compounds (2a-o)

Results and Discussion

Chemistry

In this work, we have synthesized novel 3,5-dibenzotriazole-4-benzylideneamino-1,2,4-triazole derivatives **(2a-o)** are set up according to standard method with minor modifications as displayed in **Scheme 1**. Starting hydrazonyl chlorides were synthesized *in situ* from Schiff base and $POCl_3$. Chloro-diazabutadienes were found to undergo condensation *in situ* with unreacted Schiff baseto 3,5-dibenzotriazole-4-benzylideneamino-1,2,4-triazolesin boiling toluene in the presence of acetonitrile [21]. Acetonitrile facilitates the formation of carbocation by increasing the polarity of the reaction media.

Biological evaluation

Antimicrobial studies[22-24]

The biological evolution of synthesized compounds was assessed against varied bacterial and fungal strains by a conventional broth-dilution method. The active compounds were further screened for cytotoxicity against human embryonic kidney cell line, HEK293. The compounds were also screened for haemolysis of human blood cells. Fluconazole was used as a positive fungal inhibitor standard for fungi. Colistin was employed as positive bacterial inhibitor standards for Gram-negative and Vancomycin for Gram-positive bacteria. Melittin and tamoxifen were employed as a positive heamolytic and cytotoxicity standard, respectively. Each antibiotic standard was provided in 4 concentrations, with 2 above and 2 below its MIC value, and plated into the first 8 wells of column 23 of the 384-well NBS plates. Tamoxifen and melittin were used in 8 concentrations in 2-fold serial dilutions with 50 µg/mL highest concentration.

The quality control (QC) of the assays was determined by Z'-Factor, calculated from the Negative (media only) and Positive Controls (bacterial, fungal or cell culture without inhibitor), and the Standards. Plates with a Z'-Factor of ≥ 0.4 and Standards active at the highest and inactive at the lowest concentration, were accepted for further data analysis.

The results of these studies were represented in **Table 1**. Samples with inhibition value above 80% were classed as actives. Samples with inhibition values between 50 - 80% were classed as partial actives. In Cytotoxic and Haemolysis assay, samples were flagged as partial cytotoxic if $D_{Max} \geq 50\%$.

Single point bacterial inhibition assay

The primary bacteria panel, including *E. coli*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *S. aureus* were cultured in Cation-adjusted Muller–Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh MHB and incubated at 37 °C for 1.5–3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD_{600}), then added to each well of the compound containing plates, giving a cell density of $5x10^5$ CFU/mL and a total volume of 50 µL. All the plates were covered and incubated at 37 °C for 18 h without shaking.

Fungi strains (*C. albicans* and *C. neoformans*) were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of $1x10^6$ to $5x10^6$ CFU/mL (as determined by OD_{530}) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of $2.5x10^3$ CFU/mL and a total volume of 50 µL. All plates were covered and incubated at 35 °C for 36 h without shaking.

Cytotoxicity Assay

HEK293 cells were counted manually in a Neubauer haemocytometer and then plated in the 384-well plates containing the compounds to give a density of 5000 cells/well in a final volume of 50 µL. DMEM supplemented with 10% FBS was used as growth media and the cells were incubated together with the compounds for 20 h at 37 °C in 5% CO_2.

Haemolysis Assay

Human whole blood was washed three times with 3 volumes of 0.9% NaCl and then resuspended in the same to a concentration of $0.5x10^8$ cells/mL, as determined by manual cell count in a Neubauer haemocytometer. The washed cells were then added to the 384-well compound-containing plates for a final volume of 50 µL. After a 10 min shake on a plate shaker the plates were then incubated for 1 h at 37 °C. After incubation, the plates were centrifuged at 1000g for 10 min to pellet cells and debris, 25 µL of the supernatant was then transferred to a polystyrene 384-well assay plate.

The results of the antimicrobial screening (at 32 µg/mL) revealed that compound 2b, 2c, 2d, 2e, 2f, 2g, 2h, 2i, 2k, 2n and 2o had high activity against *Cryptococcus neoformans.* Furthermore, compound 2f, 2g and 2n exhibited substantial activity against *Candida albicans*. No other significant activity was observed for the other derivatives which were tested at same concentration intensities and with the uniform bacterial and fungal strains verified. Every compound which were tested verified to no noticeable haemolytic activity against human red blood cells and no cytotoxicity against the human embryonic kidney cell line, HK293.

Experimental

Material and Methods[22]

Table 1. Percentage inhibition for compounds **2a-o** at 32µg/mL.

No.	-R	Antibacterial					Antifungal		Cytotoxicity	Haemolysis
		Sa	***Ec***	***Kp***	***Pa***	***Ab***	***Ca***	***Cn***	**Hk, D_{Max}**	**RBC,D_{Max}**
2a	-3-F	-35.1; -36.2	-1.4; -3.6	16.3; 8.1	12.3; 14.3	12.7; 17.9	1.6; 9.8	28.8; 33.2	NT	NT
2b	-2-Br	-25.6; -28.2	-3.7; -5.7	6.4; 7.0	10.1; 7.8	11.0; 15.1	48.1; 49.3	**100.0; 95.5**	**-1.8; 11.3**	**-1.8; 0.5**
2c	-2-F	-15.9; -21.6	-2.7; 0.3	10.6; 5.9	6.6; 9.9	10.0; 12.3	3.6; 4.3	**108.2; 110.4**	**10.3; 40.7**	**3.2; 6.6**
2d	-4-Br	-33.6; -50.8	-7.8; 0.7	16.4; 3.1	11.5; 7.7	18.7; 8.1	32.6; 33.7	**113.7; 120.1**	**-2.3; 31.7**	**-1.9; 2.7**
2e	-4-NO_2	-27.8; -52.2	-5.0; 1.3	11.1; 8.8	6.9; 9.3	14.6; 15.6	25.8; 3.9	**108.9; 115.7**	**12.3; 17.2**	**-2.1; 1.1**
2f	-3-Cl	-52.7; -65.7	-1.3; -8.3	0.4; 8.2	5.9; 8.0	6.4; 8.2	40.2; 70.7	**112.6; 116.0**	**-1.5; 33.7**	**-1.2; -2.6**
2g	-4-F	-38.9; -47.7	-8.0; 0.4	14.9; 3.4	6.0; 8.9	12.6; 15.7	45.3; 58.9	**112.4; 124.1**	**21.2; 5.9**	**-1.8; 0.0**
2h	-3,4,5-OH	-18.6; -36.6	-0.7; -7.1	0.0; 7.1	4.6; 8.2	11.0; 13.3	2.1; 8.0	**117.0; 123.6**	**29.8; 4.2**	**-2.4; -3.4**
2i	-3-NO_2	-23.8; -9.6	-2.7; -8.8	-0.7; 9.2	4.1; 7.5	6.6; 8.1	-0.9; 2.1	**116.1; 124.1**	**37.2; 4.7**	**-1.9; -2.6**
2j	-4-CH_3	-18.0; -18.4	-5.9; -8.9	1.1; 4.0	4.9; 7.1	5.6; 7.4	0.2; 4.3	-44.7; -93.2	NT	NT
2k	-4-OCH_3	-15.4; -7.0	-10.9; -5.1	1.6; 2.6	4.9; 8.5	1.8; 3.6	-0.8; -0.9	**108.9; 112.4**	**13.9; 40.1**	**2.0; 3.5**
2l	-4-OH	-41.7; -45.2	-10.6; -14.7	-1.3; 5.7	2.1; 2.3	-1.9; -6.9	13.4; 2.7	-55.8; -56.1	NT	NT
2m	-H	-25.0; -31.7	-12.4; -12.6	3.3; 8.9	-0.7; 1.6	-14.9; -20.2	13.9; 7.5	-13.7; -32.7	NT	NT
2n	-3-OH	-19.0; -20.9	-11.1; -16.8	13.1; 6.9	5.6; 6.5	-11.3; -2.3	47.0; 64.3	**115.8; 123.9**	**14.4; 36.4**	**-0.6; 0.8**
2o	-2-NO_2	-10.4; -15.9	-8.8; 3.7	18.2; 9.3	6.8; 7.6	-2.3; 6.0	-3.2; 5.6	**121.8; 127.7**	**32.7; 7.8**	**-2.0; 2.9**

Sa: *Staphylococcus aureus* (MRSA; ATCC 43300), *Ec*: *Escherichia coli* (ATCC 25922), *Kp*:*Klebsiella pneumoniae* (ATCC 700603), *Pa*: *Pseudomonas aeruginosa* (ATCC 27853), *Ab*: *Acinetobacter baumannii* (ATCC 19606), *Ca*: *Candida albicans* (ATCC 90028), *Cn*: *Cryptococcus neoformans* (ATCC 208821), Hk: Human Embryonic Kidney cells (ATCC CRL-1573), RBC: Human red blood cells (ARCBS 5400 00150), NT: Not Tested. Data with bold fonts are active compounds.

The starting materials were obtained from commercial providers and utilized with or without purification as required. The melting point was checked through an open capillary technique on a 'Toshvin melting point' apparatus and are uncorrected. TLC on silica gel plates (Merck, 60, F_{254}) was utilized for purity checking and reaction monitoring. Flash chromatography with silica gel (Merck, 70-230 mesh and 230-400 mesh ASTH) was valuable when essential to isolate and purify the reaction products. ^{1}H NMR spectra were recorded on a Bruker Advance II 400 MHz and ^{13}C NMR spectra on Varian Mercury-400, 100 MHz in DMSO-d_6 as a solvent and tetramethylsilane (TMS) as an internal standard. IR spectra were obtained from a Shimadzu Prestige-21 FT-IR spectrophotometer using ATR assembly. Mass spectra were acquired by Shimadzu LC-MS 2010 spectrometer. Elemental analysis (C, H, N) was completed by a Perkin-Elmer 2400 CHN analyser and found inside ±0.4% of theoretical values.

General procedure for synthesis of *N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-phenylmethanimine, (2a–o):

An equimolar mixture of corresponding compounds (1a-o) and $POCl_3$ in toluene (60 ml) was added followed by acetonitrile (5 ml). The reaction mixture was refluxed for 14-16 hours with exclusion of moisture then the completed reaction mixture was cooled and poured into crushed ice. The resultant solid precipitate was filtered, dried and crystallized from $CHCl_3$.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(3-fluorophenyl)-methanimine, (2a):**

Dark oak color powder; Yield 84%; m.p. 223-225 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1666 (C=N stretching), 1074 (Ar-F); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.67 (s, 1H, –N=CH-), 7.38-8.03 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 164.8, 162.6, 151.6 (2), 145.7 (2), 133.2, 132.4, 130.4, 126.8, 126.2, 124.7 (2), 119.4 (2), 117.8 (2), 114.1 (2), 109.6 (2), 48.3 (2); LCMS: *m/z* 491.5 (M^+K). Anal. Calcd. For $C_{23}H_{17}FN_{10}$: C, 61.06; H, 3.79; N, 30.96. Found: C, 60.84; H, 3.41; N, 30.61%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(2-bromophenyl)methanimine, (2b):**

Dark yellow powder, Yield 86%. m.p. 224-226 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1668 (C=N stretching), 634 (Ar-Br); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.61 (s, 1H, –N=CH-), 7.32-7.96 (m, 12H, Ar-H), 6.04 (s, 2H, CH_2), 5.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.5, 152.0 (2), 145.4 (2), 134.5, 132.8, 132.3, 131.2, 130.1 (2), 127.8 (2), 126.6, 126.5, 121.4 (2), 119.4 (2), 109.9 (2), 48.3 (2); LCMS: *m/z* 512.0 (M^+). Anal. Calcd. For $C_{23}H_{17}BrN_{10}$: C, 53.81; H, 3.34; N, 27.28. Found: C, 53.35; H, 2.13; N, 27.04%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(2-fluorophenyl)methanimine, (2c):**

Cream color powder Yield 83%. m.p. 218-220 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1662 (C=N stretching), 1134 (Ar-F); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.65 (s, 1H, –N=CH-), 7.08-7.91 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.4, 159.4, 151.9 (2), 145.6 (2), 132.7, 132.6, 130.2 (2), 126.6, 126.5, 124.2 (2), 119.4 (2), 118.4 , 115.6 (2), 109.9 (2), 48.3 (2); LCMS: *m/z* 452.1 (M^+). Anal. Calcd. For $C_{23}H_{17}FN_{10}$: C, 61.06; H, 3.79; N, 30.96. Found: C, 60.94; H, 3.51; N, 30.56%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(4-**

bromophenyl)methanimine, (2d):
Yellowish orange powder, Yield 81%. m.p. 230-232 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1668 (C=N stretching), 638 (Ar-Br); ^1H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.69 (s, 1H, –N=CH-), 7.36-7.88 (m, 12H, Ar-H), 6.04 (s, 2H, CH_2), 5.60 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.5, 152.0 (2), 145.4 (2), 132.5, 131.7, 131.5 (2), 129.6 (2), 128.4 (2), 126.6 (2), 125.6 (2) , 119.4 (2), 109.9 (2), 48.3 (2); LCMS: *m/z* 512.1 (M^+). Anal. Calcd. For $C_{23}H_{17}BrN_{10}$: C, 53.81; H, 3.34; N, 27.28. Found: C, 53.33; H, 3.71; N, 26.96%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(4-nitrophenyl)-methanimine, (2e):**
Gray powder, Yield 92%. m.p. 278-280 °C. IR (λ_{max}, cm^{-1},KBr): 3223 (C-H, aromatic), 3099 (-C-H-, CH_2), 1672 (C=N stretching), 1354, 1533 (Ar-NO_2); ^1H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.71 (s, 1H, –N=CH-), 7.42-8.07 (m, 12H, Ar-H), 6.07 (s, 2H, CH_2), 5.62 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 165.4, 151.6 (2), 149.8, 145.4 (2), 133.1, 132.9, 129.3 (2), 128.6, 127.5 (2), 121.6 (2), 119.4 (2), 111.4 (2), 109.5 (2), 48.3 (2); LCMS: *m/z* 479.1 (M^+). Anal. Calcd. For $C_{23}H_{17}N_{11}O_2$: C, 57.62; H, 3.57; N, 32.14. Found: C, 57.28; H, 3.32; N, 32.22%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(3-chlorophenyl)-methanimine, (2f):**
Turmeric yellow powder, Yield 85%. m.p. 225-227 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1662 (C=N stretching), 748 (Ar-Cl); ^1H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.22 (s, 1H, –N=CH-), 7.36-8.03 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.58 (s, 2H, CH_2);^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.4, 152.0 (2), 145.4 (2), 134.4, 132.8 (2), 132, 131.2 (2), 130.4, 129.3 (2), 127.2, 126.5 (2) , 119.4 (2), 109.9 (2), 48.3 (2); LCMS: *m/z* 507.3 (M^+K). Anal. Calcd. For $C_{23}H_{17}ClN_{10}$: C, 58.91; H, 3.65; N, 29.87. Found: C, 58.36; H, 3.46; N, 29.81%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(4-fluorophenyl)methanimine, (2g):**
Buff color powder, Yield 89%. m.p. 223-225 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1662 (C=N stretching), 1148 (Ar-F); ^1H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.57 (s, 1H, –N=CH-), 7.14-7.86 (m, 12H, Ar-H), 6.04 (s, 2H, CH_2), 5.58 (s, 2H, CH_2);^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 165.4, 162.2, 151.9 (2), 145.6 (2), 132.7 (2), 131.4 (2), 130.8, 126.6 (2), 124.8, 119.4 (2), 117.6, 115.6 (2), 114, 109.9 (2), 48.3 (2); LCMS: *m/z* 452.1 (M^+). Anal. Calcd. For $C_{23}H_{17}FN_{10}$: C, 61.06; H, 3.79; N, 30.96. Found: C, 61.26; H, 3.42; N, 30.63%.

5-(((3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)imino)methyl)benzene-1,2,3-triol, (2h):
Light brown powder, Yield 92%. m.p. 243-245 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 2952, 2843 (C-H, OCH_3), 1674 (C=N stretching); ^1H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.61 (s, 1H, –N=CH-), 7.03-7.96 (m, 10H, Ar-H), 6.05 (s, 2H, CH_2), 5.63 (s, 2H, CH_2), 3.73-3.76 (m, 3H, OH); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.2, 153.4, 151.7 (2), 145.1 (2), 141.5 (2), 133.0 (2), 126.4 (2), 124.8 (2), 119.4 (2), 110.2 (2), 103.4, 60.5, 56.4, 48.3 (2); LCMS: *m/z* 482.1 (M^+). Anal. Calcd. For $C_{23}H_{18}N_{10}O_3$: C, 57.26; H, 3.76; N, 29.03. Found: C, 57.12; H, 3.57; N, 29.36%.

***N*-(3,5-bis((1*H*-benzo[*d*][1,2,3]triazol-1-yl)methyl)-4*H*-1,2,4-triazol-4-yl)-1-(3-nitrophenyl)methanimine, (2i):**
Pale yellow powder, Yield 87%. m.p. 275-277 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1671 (C=N stretching),

1348, 1532 (Ar-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.58 (s, 1H, –N=CH-), 7.52-8.76 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.58 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.3, 151.7 (2), 148.2, 145.4, 135.3 (2), 132.8 (2), 131.5 (2), 129.7 (2), 126.6 (2), 123.9 (2), 119.4 (2), 110.1 (2), 48.3 (2); LCMS: *m/z* 479.1 (M^+). Anal. Calcd. For $C_{23}H_{17}N_{11}O_2$: C, 57.62; H, 3.57; N, 32.14. Found: C, 57.13; H, 3.32; N, 31.96%.

_N_-(3,5-bis((1_H_-benzo[_d_][1,2,3]triazol-1-yl) methyl)-4_H_-1,2,4-triazol-4-yl)-1-(p-tolyl) methanimine, (2j):

fuller earth color powder, Yield 77%. m.p. 272-274 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 2848 (C-H, CH_3), 1663 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.71 (s, 1H, –N=CH-), 7.22-7.98 (m, 12H, Ar-H), 6.05 (s, 2H, CH_2), 5.61 (s, 2H, CH_2), 2.41-2.47 (m, 3H, CH_3);^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.2, 151.7 (2), 145.1, 140.7 , 132.5 (2), 129.8 (2), 129.4 (2), 127.5 (2), 126.4 (2), 126.3 (2), 119.4 (2), 110.2 (2), 48.3 (2), 21.3; LCMS: *m/z* 448.1 (M^+). Anal. Calcd. For $C_{24}H_{20}N_{10}$: C, 64.27; H, 4.50; N, 31.23. Found: C, 63.95; H, 4.12; N, 31.02%.

_N_-(3,5-bis((1_H_-benzo[d][1,2,3]triazol-1-yl)methyl)-4_H_-1,2,4-triazol-4-yl)-1-(4-methoxyphenyl)methanimine, (2k):

Thunder grey color powder, Yield 89%. m.p. 269-271 °C. IR (λ_{max}, cm^{-1},KBr): 3224 (C-H, aromatic), 3099 (-C-H-, CH_2), 2922 (C-H, OCH_3), 1662 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.28 (s, 1H, –N=CH-), 7.63-8.11 (m, 12H, Ar-H), 6.04 (s, 2H, CH_2), 5.47 (s, 2H, CH_2), 3.86 (m, 3H, OCH_3);^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 165.2, 160.7, 151.7 (2), 145.1 (2), 132.6 (2), 130.4 (2), 126.3 (2), 122.7 (2), 119.4 (2), 114.6, 114.4 (2), 110.2 (2), 48.3 (2), 56.4; LCMS: *m/z* 464.1 (M^+).Anal. Calcd. For $C_{24}H_{20}N_{10}O$: C, 62.06; H, 4.34; N, 30.16. Found: C, 61.78; H, 4.17; N, 30.02%.

4-(((3,5-bis((1_H_-benzo[_d_][1,2,3]triazol-1-yl) methyl)-4_H_-1,2,4-triazol-4-yl)imino)methyl) phenol, (2l):

Dark brown powder, Yield 88%. m.p. 246-248 °C. IR (λ_{max}, cm^{-1},KBr): 3486 (OH, Ar-OH), 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1668 (C=Nstretching); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.37 (s, 1H, –N=CH-), 7.36-8.07 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.62 (s, 1H, Ar-OH), 5.47 (s, 2H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.2, 160.7, 151.7 (2), 145.1 (2), 132.6, 130.6 (2), 126.6 (2), 126.4 (2), 123.4 (2), 119.4 (2), 116.2 (2), 110.1 (2), 48.3 (2); LCMS: *m/z* 450.1 (M^+). Anal. Calcd. For $C_{23}H_{18}N_{10}O$: C, 61.33; H, 4.03; N, 31.09. Found: C, 61.11; H, 3.91; N, 30.92%.

_N_-(3,5-bis((1_H_-benzo[_d_][1,2,3]triazol-1-yl)methyl)-4_H_-1,2,4-triazol-4-yl)-1-phenylmethanimine, (2m):

Brown powder, Yield 89%. m.p. 231-233 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1667 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.22 (s, 1H, –N=CH-), 7.36-8.02 (m, 13H, Ar-H), 6.03 (s, 2H, CH_2), 5.58 (s, 2H, CH_2);^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.5, 151.8 (2), 145.2 (2), 132.9 (2), 131.2 (2), 130.4 (2), 129.2 (2), 128.6 (2), 126.3 (2), 119.4 (2), 109.8 (2), 48.3 (2); LCMS: *m/z* 434.1 (M^+).Anal. Calcd. For $C_{23}H_{18}N_{10}$: C, 63.58; H, 4.18; N, 32.24. Found: C, 63.15; H, 3.94; N, 31.87%.

3-(((3,5-bis((1_H_-benzo[_d_][1,2,3]triazol-1-yl) methyl)-4_H_-1,2,4-triazol-4-yl)imino)methyl) phenol, (2n):

light yellow powder; Yield 83%; m.p. 241-243 °C. IR (λ_{max}, cm^{-1},KBr): 3492 (OH, Ar-OH), 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1669 (C=N stretching); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.35 (s, 1H, –N=CH-), 7.32-8.06 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.69 (s, 1H, Ar-OH), 5.48 (s, 2H, CH_2); ^{13}C

NMR (100 MHz, DMSO-d_6, ppm): δ = 162.2, 156.6, 151.8 (2), 145.1 (2), 132.6, 132.0, 130.2, 126.4 (2), 121.8 (2), 119.4 (2), 118.2 , 114.7 (2), 110.1 (2), 48.3 (2); LCMS: *m/z* 450.4 (M^+). Anal. Calcd. For $C_{23}H_{18}N_{10}O$: C, 61.33; H, 4.03; N, 31.09. Found: C, 60.92; H, 3.87; N, 30.82%.

_N_-(3,5-bis((_1H_-benzo[_d_][1,2,3]triazol-1-yl)methyl)-4_H_-1,2,4-triazol-4-yl)-1-(2-nitrophenyl)methanimine, (2o):

Yellow powder, Yield 87%. m.p. 269-271 °C. IR (λ_{max}, cm^{-1},KBr): 3227 (C-H, aromatic), 3099 (-C-H-, CH_2), 1670 (C=N stretching), 1352, 1542 (Ar-NO_2); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 8.71 (s, 1H, –N=CH-), 7.37-8.24 (m, 12H, Ar-H), 6.03 (s, 2H, CH_2), 5.58 (s, 2H, CH_2);^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 162.3, 151.7 (2), 147.6, 145.4, 135.1, 132.8 (2), 131.7 (2), 130.3 (2), 126.6 (2), 123.9 (2), 119.4 (2), 110.1 (2), 48.3 (2); LCMS: *m/z* 479.4 (M^+). Anal. Calcd. For $C_{23}H_{17}N_{11}O_2$: C, 57.62; H, 3.57; N, 32.14. Found: C, 57.36; H, 3.49; N, 31.93%.

Conclusion

The cyclization of Schiff base to 1,2,4-triazole derivatives with benzotriazole skeleton was incorporated and assessed for their *in vitro* antimicrobial, cytotoxic and haemolytic activities. Preliminary conclusions unveiled that some of the compounds displayed substantial antimicrobial activity. 11 Compounds demonstrated the best inhibitory activity against *Candida albicans*and *Cryptococcus neoformans* fungi with no noticeable haemolytic activity against human red blood cells and no cytotoxicity against the human embryonic kidney cell line, HK293. This exertion might be helpful to contend drug-resistant infections.

Acknowledgements

Authors are grateful to the UGC, New Delhi and Department of Science and Technology, New Delhi for NON-SAP and DST-FIST programs respectively. Antimicrobial screening was done in collaboration with CO-ADD (The Community for Antimicrobial Drug Discovery), funded by the Wellcome Trust (UK) and The University of Queensland (Australia).

References

1. B. Zorc, Z. RajićDžolić and I. Butula, CroaticaChemicaActa. 85(4), 2012, 595. https://doi.org/10.5562/cca2124
2. S. Panda, C. Hall, E. Scriven and A.R.Katritzky, AldrichimicaActa, 46(2), 2013, 43. https://doi.org/10.1002/chin.201441274
3. J. Monbaliu and A.R.Katritzky, Chemical Communications, 48(95), 2012, 11601. https://doi.org/10.1039/c2cc34434c
4. A.R.Katritzky and S. Rachwal, Chemical Reviews, 110(3), 2010, 1564. https://doi.org/10.1021/cr900204u
5. A.R.Katritzky and S. Rachwal, Chemical Reviews, 111(11), 2011, 7063. https://doi.org/10.1021/cr200031r
6. A.R.Katritzky and B. Rogovoy, Chemistry - A European Journal, 9(19), 2003, 4586. https://doi.org/10.1002/chem.200304990
7. F. López-Vallejo, R. Castillo, L. Yépez-Mulia and J.L. Medina-Franco, Journal of Biomolecular Screening, 16, 2011, 862. https://doi.org/10.1177/1087057111414902
8. F. Sparatore, M. la Rotonda, E. Ramundo, C. Silipo and A. Vittoria, Farmaco, EdizioneScientifica, 33(12), 1978, 924.
9. D.R. Godhani, V.B. Mulani, J.P. Mehta and N.B. Kukadiya, World Scientific News, 100, 2018, 51.
10. G. Paglietti, P. Sanna, A. Carta, F. Sparatore, I. Vazzana, A. Peana and M. Satta, Farmaco, 49(11), 1994, 693.
11. P. Borowski, J. Deinert, S. Schalinski, M. Bretner, K. Ginalski, T. Kulikowski and D. Shugar, European Journal of Biochemistry, 270(8), 2003, 1645. https://doi.org/10.1046/j.1432-1033.2003.03540.x
12. A. Kauste, M. Tuominen, H. Heikkinen, A. Gordon and K. Korttila, Survey of Anesthesiology, 30, 1986, 281. https://doi.org/10.1097/00132586-198610000-00029
13. P. Carlini, E. Bria, D. Giannarelli, A. Felici, P. Papaldo, A. Fabi, E.M. Ruggeri, M. Milella, C. Nuzzo, F.L. Cecere, A. Gelibter, M.S. Pino, C. Nisticò, F. Cuppone, G. Metro, E. Terzoli, F. Cognetti and G. Ferretti, Expert Opinion on Investigational Drugs, 16, 2007, 1023. https://doi.org/10.1517/13543784.16.7.1023
14. G. Semple, P.J. Skinner, M.C. Cherrier, P.J. Webb, C.R. Sage, S.Y. Tamura, R. Chen, J.G. Richman and D.T. Connolly, Journal of Medicinal Chemistry. 49, 2006, 1227. https://doi.org/10.1021/jm051099t
15. Y. Ren, Medicinal Chemistry. 4, 2014. https://doi.org/10.4172/2161-0444.1000207
16. B. Črešnar and Š. Petrič, Biochimica et

BiophysicaActa(BBA) - Proteins and Proteomics, 1814, 2011, 29. https://doi.org/10.1016/j.bbapap.2010.06.020
17. D.R. Godhani, A.A. Jogel, A.M. Sanghani and J.P. Mehta, Indian Journal of Chemistry, 54B, 2015, 556.
18. D.R. Godhani, D.B. Sanja, A.M. Sanghani and P.B. Dobariya, Indian Journal of Heterocyclic Chemistry, 22(3), 2013, 249.
19. D.R. Godhani, D.B. Sanja and A.M. Sanghani, Journal of Chemical and Pharmaceutical Research, 5(1), 2013, 240.
20. D.R. Godhani, D.B. Sanja, A.M. Sanghani and P.B. Dobariya, Indian Journal of Heterocyclic Chemistry, 20(4), 2011, 411.
21. W. Zielinski and W. Czardybon, Chemistry of Heterocyclic Compounds. 37, 2001, 1107. https://doi.org/10.1023/a:1013227632521
22. D.R. Godhani, V.B. Mulani and J.P. Mehta, World Scientific News, 124(2), 2019, 304.
23. M.A.T. Blaskovich, J. Zuegg, A.G. Elliott and M.A. Cooper, ACS Infectious Diseases, 1(7), 2015, 285. https://doi.org/10.1021/acsinfecdis.5b00044
24. D.R. Godhani, V.B. Mulani and J.P. Mehta, Chemistry & Biology Interface, 9(2), 2019, 120.