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Synthesis and antimicrobial evolution of some new benzotriazole substituted 1,3,4-thiadiazoles

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ABSTRACT

A series of novel benzotriazole-substituted 1,3,4-thiadiazole-2(3*H*)-thione derivatives was synthesized by cyclization of Schiff base. The structures were recognized on the foundation of spectral tools and their purity by elemental analysis. All the compounds were preliminary evaluated for their *in vitro* antimicrobial activities against five 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 two fungi Strains *viz.* [*Candida albicans* (ATCC 90028), *Cryptococcus neoformans* (ATCC 208821)]. Several compounds exhibited remarkable antimicrobial activity, out of 15 compounds, three compounds *viz.* 5a, 5g and 5i showed promising antifungal activities with no signs of human cells cytotoxic [HEK293: Human Embryonic Kidney cells (ATCC CRL-1573)] and haemolytic activity [Whole blood (ARCBS 5400 00150)].

Keywords: benzotriazole, thiadiazole, schiff base, cytotoxicity, haemolysis, antimicrobial activity

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1. INTRODUCTION

The dealing with microbial and fungus-related diseases remains an important question because of a combination of aspects including emerging newer infectious diseases and spreading a number of multi-drug resistant microbial pathogens. This crisis is particularly pronounced for the fungus [1-3]. The therapeutic problem is an important part of hospitalized patients, immunosuppressed patients with AIDS and those undergoing organ transplants or chemo therapy. Even though thousands of antibiotics and chemotherapeutics available for medical purpose, the emerging resistance to old and new antibiotics has created a substantial requirement for new classes of antifungal agents A possible approach to defeat the problem of antibiotic resistance is to design novel agents with various mode of action so as to, no cross resistance to present drugs can come [4-6].

A different kind of biological activities is conducted for 1,3,4-thiadiazoles and their sulfur analogs 1,3,4-thiadiazole-2(3*H*)-thione. This includes bactericidal [7,8], fungicidal [9,10], anti-inflammatory [11], Anticonvulsant [12], herbicidal activities [13] and Anti-HIV [14] Some of them are also known to be strong inhibitors of monoamine oxidase, succinate dehydrogenase [12], phosphodiesterase [13], cyclooxygenase, lipoxygenase [15] and tyrosinase enzymes [16].

A search of the recent literature revealed that very few published reports describe the route of syntheses of various substituted 1,3,4-thiadiazole-2(3*H*)-thione nucleus [17] and (Bhole *et al*.) [18] reported the synthesis of such compounds by the cyclization reaction of a Schiff base with carbon disulfide under basic conditions and investigated the antihypertensive activity. Using similar methodology and different approach, we have synthesized fifteen 1,3,4-thiadiazole-2(3*H*)-thione derivatives formed by the reaction of Schiff base of benzotriazole and Carbon disulfide under basic condition. As outlined in **Scheme 1.**

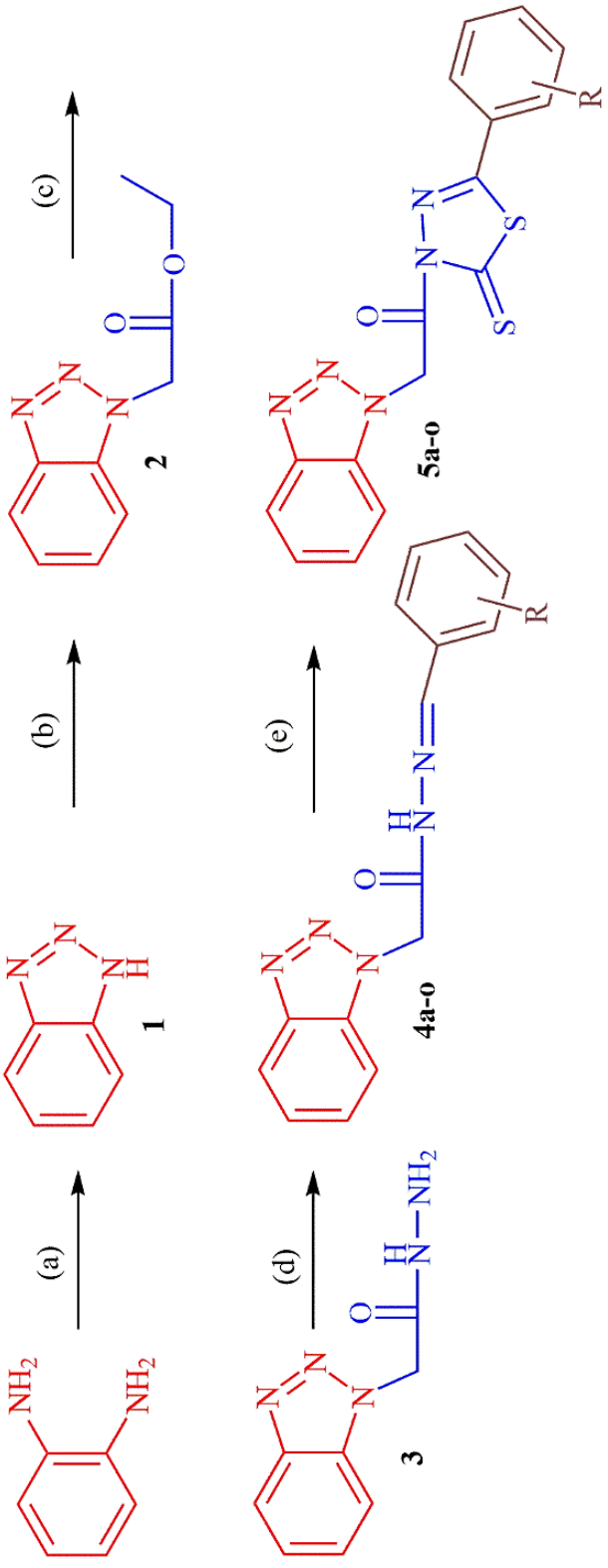
The compounds prescribed herein were achieved in good isolated yields, using simple routes which did not require any need for additional purification for the main products. In the extension of our previous work, we herein report the synthesis of some new 1,3,4-thiadiazole-2(3*H*)-thione derivatives and their antimicrobial, cytotoxic and haemolytic activities were also determined.

2. RESULTS AND DISCUSSION

2. 1. Chemistry

New benzotriazole-substituted 1,3,4-thiadiazole **(5a-5o)** derivatives are prepared as per standard protocols with minor modifications as exhibited in **Scheme 1**. Esterification of benzotriazole was carried out with reflux for 7 hours in presence of ethylbromoacetate to obtain corresponding ester with 84% yield. Hydrazinolysis of the ester in presence of hydrazine hydrate in ethanol at 100 °C for 6 hours gave 2-(1*H*-benzo[d][1,2,3]triazol-1-yl)acetohydrazide. Condensation of hydrazide with aromatic aldehydes in ethanol at 80 °C for 8 hours afford Schiff base derivatives.

Cyclization of Schiff base with CS_2 in alcoholic KOH afford 2-(1*H*-benzo[d][1,2,3] triazol-1-yl)-1-(5-(aryl)-2-thioxo-1,3,4-thiadiazol-3(2*H*)-yl)ethan-1-one, 5a-5o) derivatives in 77-92% yield. The structure and carbon numbering of compound (5a-5o) as an example is shown in **Figure 1.**



Where, R = **4a, 5a** -3-F, **4b, 5b** -2-Br, **4c, 5c** -2-F, **4d, 5d** -4-Br, **4e, 5e** -4-NO_2, **4f, 5f** -3-Cl, **4g, 5g** -4-F, **4h, 5h** -3,4,5-$(OCH_3)_3$ **4i, 5i** -3-NO_2, **4j, 5j** -4-CH_3, **4k,5k** -3-OCH_3, **4l, 5l** -4-OH, **4m,5m** -H, **4n, 5n** -3-OH, **4o, 5o** -2-NO_2,

Reagents and conditions: (a) $NaNO_2$ (10% aq.) solution, CH_3COOH, 2 h; (b) CH_3OH, $BrCH_2COOCH_2CH_3$, reflux, 7 h; (c) CH_3OH (99% aq.), $NH_2NH_2.H_2O$, reflux, 5–6 h; (d) R-CHO, CH_3OH, reflux, 6-8 h; (e) CS_2, KOH, reflux, 10–16 h.

Scheme 1. Synthetic track for the preparation of title compounds (5a-o).

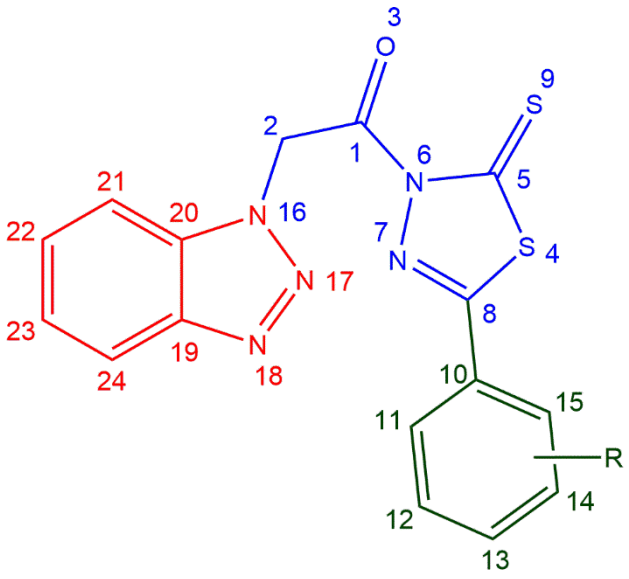


Figure 1. Carbon numbering of the final compounds (5a-5o).

Spectral techniques *viz.* ^{1}H NMR, ^{13}C NMR, IR and Mass spectrometry were used for possible structure determination of newly synthesized benzotriazole-substituted 1,3,4-thiadiazol compounds **(5a-o)**. As a representative example the ^{1}H NMR of 2-(1*H*-benzo[d] [1,2,3]triazol-1-yl)-1-(5-(3-fluorophenyl)-2-thioxo-1,3,4-thiadiazol-3(2*H*)-yl)ethan-1-one, (5a) is depicted here. The proton singlet at δ 6.03 and 5.58 ppm, due to presence of -CH_2 group and eight aromatic protons appeared as multiplet between δ 7.38-8.03 ppm.

Appearance of characteristic peak in ^{13}C NMR spectra at δ 191.48, 171.88 and 162.68 ppm pointed out the presence of -C=S, >C=O and C-F group in the final compound respectively. The IR spectra of **5a** showed distinct stretching frequencies at 1666 cm^{-1} and 1187 cm^{-1} corresponding to >C=O and -C=S respectively. The absorption band at 1107 cm^{-1} showed the presence of C-F stretching. Molecular ion peak at *m/z* 371.05 [M^{+}] in mass spectroscopic data was in accordance with molecular weight of compound **5a**. Similarly, the structural confirmation of the remaining benzotriazole-substituted 1,3,4-thiadiazol derivatives **(5a-o)** was carried out on the basis of the above description.

2. 2. Biological evaluation

2. 2. 1. Antimicrobial studies

The biological evolution of synthesized compounds were 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.

The results of these studies were represented in **Table 1**. Compounds were deemed to be active at a single concentration of 32 µg/mL, n = 2, where replicates showed inhibition of ≥ 30% and Z-Score ≥ 2.5.

Table 1. Percentage inhibition for compounds **5a-o** at 32µg/mL.

Entry	-R	Antibacterial					Antifungal		Cytotoxicity	Haemolysis
		Sa	***Ec***	***Kp***	***Pa***	***Ab***	***Ca***	***Cn***	**HEK293, D_{Max}**	**Whole blood, D_{Max}**
5a	-3-F	23.7; 32.2	-1.9; -5.5	10.5; 15.3	12.1; 8.5	1.3; 34	12.9; 41.4	43.7; 86.2	20.7; 39.8	3.1; 3.6
5b	-2-Br	0.0; 6.5	-12.1; -15.9	6.0; 9.8	10.6; 3.9	13.6; 7.8	2.3; 2.5	-74.2; -95.4	NT	NT
5c	-2-F	-12.1; -9.1	-12.2; -12.4	-0.5; -4.7	4.8; 4.9	-5.6; 8.0	-0.5; 1.0	-35.6; -36.6	NT	NT
5d	-4-Br	27.1; 46.7	-14; -9.2	11.3; 3.6	2.6; 9.0	23.6; 25.7	10.6; 11.5	-46.4; -54.1	NT	NT
5e	-4-NO_2	0.3; 2.7	-4.7;-5.3	-2.3; 0.8	12.6; 8.2	-4.6; -6.6	3.7; 3.9	-31.8; -34.2	NT	NT
5f	-3-Cl	22.0; 24.8	-7.8; -7.8	0.2; 0.2	1.4; 4.2	-7.9; 27.2	18.2; 3.7	-155.3; -219.0	NT	NT
5g	-4-F	25.9; 29.6	-3.2; -5.4	5.9; 6.7	-2.0; 0.6	-9.5; 28.3	17.0; 17.1	101.0; 86.5	22.5; 34.6	7.1; 8.8
5h	-3,4,5-$(OCH_3)_3$	20.9; 24.0	-0.8; -10.3	2.6; 6.5	0.6; 5.6	-19.3; 15.2	1.0; 9.3	-175.4; -196.7	NT	NT
5i	-3-NO_2	42.4; 44.9	-5.6; 2.4	10.5; 9.7	6.0; 7.5	0.4; 34.3	17.9; 33.2	-159.9; 97.3	19.3; 26.9	5.3; 9.3
5j	-4-CH_3	8.8; 9.8	-12.4; -2.9	3.1; 6.7	12.1; 5.4	-5.5; 25.3	0.9; 5.5	-31.4; -38.3	NT	NT

5k	-4-OCH_3	8.6; 9.7	-10.0; -2.3	10.1; 4.3	14.0; 3.5	-4.4; 23.8	-1.3; 9.7	-32.7; -41.6	NT	NT
5l	-4-OH	11.0; 8.3	-11.4; -6.4	3.5; 7.9	2.5; 6.8	-25.2; 10.9	11.9; 6.7	-102.8; -90.2	NT	NT
5m	-H	26.7; 9.8	-0.6; -1.9	1.3; 2.4	14.0; 8.5	-0.2; 24.8	30.2; 55.1	-45.0; -73.4	NT	NT
5n	-3-OH	0.5; 4.9	-20.4; 50.9	-33.1;-43.8	-3.0; 9.9	-12.8; -66.7	40.1; 5.2	-41.3; -54.4	NT	NT
5o	-2-NO_2	19.6; 21.9	-7.4; 1.9	11.2; 9.8	13.7; 15.6	17.5; 44.5	2.1; 5.7	-63.9; -87.5	NT	NT

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), HEK293: Human Embryonic Kidney cells (ATCC CRL-1573), Whole blood: (ARCBS 5400 00150), NT: Not Tested.

The results of the antimicrobial screening (at 32 μg/mL) revealed that compound **5g** and **5a** had high activity against *Cryptococcus neoformans*, ranging from (mean 65-94% inhibition) whereas its ortho derivative **5c**, showed no comparable activity against any of the fungi. Furthermore, compound **5m** exhibited substantial activity (mean 42% inhibition) against *Candida albicans*. In Gram- positive, Compound **5i** and **5d** had significant activity (mean 36-43% inhibition) against *Staphylococcus aureus* bacteria and in Gram- negative, compound **5o** showed significant activity (mean 31% inhibition) against *Acinetobacter baumannii* bacteria. No other important activity was observed for the other compounds which were tested at same concentration levels and with the equal bacterial and fungal strains tested.

All of the compounds which were tested verified to no observable cytotoxicity against the human embryonic kidney cell line, HEK293 and no haemolytic activity observed against human whole blood cells.

3. EXPERIMENTAL

3. 1. Material and methods

The starting materials were obtained from commercial providers and used with or without purification as needed. Melting point was checked by an open capillary method on a 'Toshvin melting point' apparatus and are uncorrected. TLC on silica gel plates (Merck, 60, F_{254}) was used for purity checking and reaction monitoring. Flash chromatography with silica gel (Merck, 70-230 mesh and 230-400 mesh ASTH) was useful when essential to separate and refine 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 as a solvent and tetramethylsilane (TMS) as an internal standard.

IR spectra was obtained from a Perkin-Elmer FT-IR spectrophotometer in KBr. Mass spectra were obtained by Shimadzu LC-MS 2010 spectrometer. Elemental analysis (C, H, N) was carried out by a Perkin-Elmer 2400 CHN analyser and found within ±0.4% of theoretical values.

3. 2. Synthesis of 2-(1*H*-benzo[d][1,2,3]triazol-1-yl)acetohydrazide, (3)

2-(1*H*-benzo[d][1,2,3]triazol-1-yl)acetohydrazide was prepared according to the literature method [19].

3. 3. General procedure for synthesis of 2-(1*H*-benzo[d][1,2,3]triazol-1-yl)-*N*'-(aryl)acetohydrazide, (4a–4o)

An equimolar mixture of compound 3 and substituted aromatic aldehydes was refluxed for 6-8 h. Then the reaction mixture was cooled and poured into crushed ice. The resultant solid was filtered, washed with H_2O and recrystallized from MeOH to give the Schiff bases.

3. 3. 1. 2-(1*H*-benzo[d][1,2,3]triazol-1-yl)-*N*'-(3-fluorobenzylidene)acetohydrazide, (4a)

Cream color powder, Yield 86%; mp 196-198 °C; IR (λ_{max}, cm^{-1}, KBr): 3029 (C-H, aromatic), 1727 (C=O stretching), 1658 (C=N stretching), 1373 (C-N), 1097 (Ar-F); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 10.57 (s, 1H, -NH-N-), 8.22 (s, 1H, -CH=N-), 7.36-8.06 (m, 8H, Ar-H), 5.17 (s, 1H, CH_2), 5.08 (s, 1H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 172.87 (C, C-1), 162.74 (C-F, C-12), 146.72 (C, C-8), 144.63 (C, C-19), 137.51 (C, C-10), 133.44 (CH, C-20), 130.45 (C, C-14), 126.54 (CH, C-23), 126.22 (CH, C-22), 124.74 (CH, C-15), 119.41 (CH, C-24), 117.87 (CH, C-13), 114.12 (CH, C-11), 109.63 (CH, C-21), 62.87 (CH_2, C-2); LC-MS (m/z): 297.12 [M^+]; Anal. Calcd. For $C_{15}H_{12}FN_5O$: C, 60.60; H, 4.07; N, 23.56. Found: C, 60.14; H, 3.91; N, 23.41%.

3. 4. General procedure for synthesis 2-(1*H*-benzo[d][1,2,3]triazol-1-yl)-1-(5-(aryl)-2-thioxo-1,3,4-thiadiazol-3(2*H*)-yl)ethan-1-one, (5a–5o)

To a mixture of corresponding compounds 4a-4o (1 mmol) in ethanol (50 mL), a solution of potassium hydroxide (3 mmol) in ethanol was added followed by carbon disulphide (3 mmol). The reaction mixture was refluxed for 10-16 h then it was concentrated and poured into crushed ice. Acidify with dilute HCl solution then precipitated solid was filtered, dried and recrystallized from MeOH.

3. 4. 1. 2-(1*H*-benzo[d][1,2,3]triazol-1-yl)-1-(5-(3-fluorophenyl)-2-thioxo-1,3,4-thiadiazol-3(2*H*)-yl)ethan-1-one, (5a)

Dark oak color powder, Yield 84%; mp 214-216 °C; IR (λ_{max}, cm^{-1}, KBr): 3072 (C-H, aromatic), 1666 (C=O stretching), 1187 (C=S stretching), 1107 (Ar-F); ^{1}H NMR (400 MHz, DMSO-d_6, ppm): δ = 7.38-8.03 (m, 8H, Ar-H), 6.03 (s, 1H, CH_2), 5.58 (s, 1H, CH_2); ^{13}C NMR (100 MHz, DMSO-d_6, ppm): δ = 191.48 (C, C-5), 171.88 (C, C-1), 162.68 (C-F, C-12), 145.78 (C, C-19), 143.68 (C, C-8), 133.26 (C, C-10), 132.40 (CH, C-15), 130.41 (C, C-20),

126.83 (CH, C-23), 126.25 (CH, C-22), 124.70 (CH, C-14), 119.41 (CH, C-11), 117.80 (CH, C-13), 114.15 (CH, C-24), 109.61 (CH, C-21), 60.31 ($\underline{C}H_2$, C-2); LC-MS (m/z): 371.05 [M^+]; Anal. Calcd. For $C_{16}H_{10}FN_5OS_2$: C, 51.74; H, 2.71; N, 18.86. Found: C, 50.84; H, 3.41; N, 18.16%.

4. CONCLUSION

A series of thiadiazole derivatives with the benzotriazole skeleton were synthesized and evaluated for their *in vitro* antimicrobial activities. Preliminary outcomes disclosed that some of the compounds displayed substantial antimicrobial activity. Compounds **5a**, **5g** and **5m** showed the most effective inhibitory activity against *Candida albicans* and *Cryptococcus neoformans* fungi. Furthermore, compound **5d, 5i** and **5o** showed significant antibacterial activity with no observable cytotoxicity against HEK293 and haemolytic against whole blood. This effort might be helpful to contend drug-resistant infections.

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