

Synthesis, Antimicrobial and Cytotoxic Activities of Some Bio-isosteres of 1, 3, 4-Oxadiazoles

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Abstract

In the present study, 5-(4-substituted phenyl)-1, 2, 4-triazole-3-thiol (5a-e) were synthesized from 5-(4-substituted phenyl)-1, 3, 4-oxadiazole-2-thiol (4a-e) and their structures were confirmed by IR, NMR, Mass spectral and elemental analysis. These synthesized compounds were screened for the antimicrobial and cytotoxic activities. The results indicated that, compound 5(a) and 5(b) showed the highest antibacterial activity. Compound 5(d) and 5(e) were observed to be the most potent antifungal agents. The structure activity relationship discovered that the presence of electron withdrawing and electron donating substituents at para position of phenyl ring of synthesized compounds enhance the antibacterial and antifungal activities, respectively. Further, the results of cytotoxicity studies on breast cancer cell line (MCF-7) suggested that active antimicrobial agents 5(a), 5(b), 5(c), 5(d) and 5(e) are accompanied with low cytotoxicity. The biological profiles of these 1, 2, 4-triazoles would be a fruitful matrix for further development of safe and efficacious compounds.

Keywords: Antimicrobial; Breast cancer cell line; Cytotoxic; Oxadiazole; Triazole

Introduction

The microbial infections are serious problem to human being and clinically useful antimicrobial drugs prevent the growth of bacteria and infections. The factors are important to select the therapy based on known characteristics of organisms and particular pharmacological features of antimicrobial agents [1]. The various antifungals like fluconazole, voriconazole containing triazole nucleus and antibacterial heterocycles such as azetidinones and penicillins, are reported as clinically useful compounds [2]. Therefore, 1, 2, 4-triazoles have been expected to be of considerable importance due to their precious biological significance. Though, these reported drugs have been used clinically but they pose limitations due to their toxicity, drug resistance and pharmacokinetic deficiencies. There is an urgent need of newer antimicrobial agents to rectify these above-mentioned problems. In the literature, the substituted 1, 3, 4-oxadiazoles have been reported to possess antimicrobial [3-5], anticonvulsant [6,7], antitubercular [8,9], anticancer [10] and anti-inflammatory activities [11,12]. Similarly, 1, 2, 4-

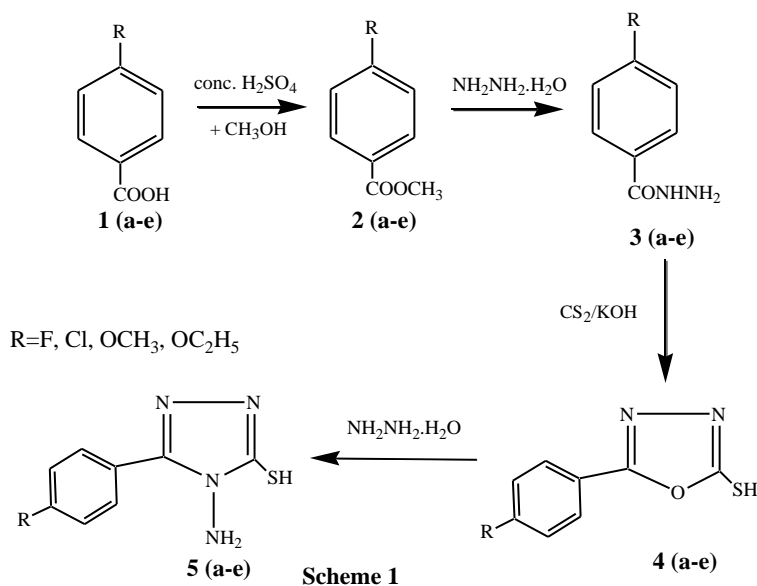
triazole derivatives are reported to impart antitumor [13,14], antimicrobial [15-17], antitubercular [18,19], antioxidant [20,21] and anti-inflammatory activities [22,23]. From these published research work, it is proved that 1, 2, 4-triazole-3-thiol and their analogs can be considered as promising antimicrobial agents due to the presence of toxicophoric part $-N=C-N-$ in them [24].

Thus, a look into the structures of 1, 2, 4-triazole and 1, 3, 4-oxadiazole, it can be said that it is only a matter of bio-isoteric replacement of oxygen of 1, 3, 4-oxadiazole with nitrogen of 1, 2, 4-triazole nucleus. This may be attributed to the promising biological activities of both the nuclei impart their appreciable difference in chemical properties. However, the conversion of 1, 3, 4-oxadiazoles to 1, 2, 4-triazoles were reported only in few research papers [25]. Therefore, it would be worthwhile to synthesize 1, 2, 4-triazoles from 1, 3, 4-oxadiazoles and evaluate them for antimicrobial and cytotoxic activities.

Experimental

All chemicals and reagents were procured from Qualigens, Spectrochem (P) Ltd. and Rankem Chemicals, from Mumbai, India. The melting points of compounds were determined in open capillaries and are uncorrected. The purity of the compounds was checked by performing thin layer chromatography (TLC) on silica gel G coated glass plates using iodine vapors as detecting agent and chloroform and methanol solvent system.

IR spectra (in KBr) were recorded on a Shimadzu IR Affinity-1 FTIR spectrophotometer. ^{13}C NMR spectra in Dimethyl Sulfoxide (DMSO) were recorded on a Bruker-400 NMR spectrometer using TMS as a reference standard (chemical shifts expressed in parts per million (ppm) on δ scale). Mass spectra were scanned on a Shimadzu LC-MS 2010 Spectrometer. Elemental analysis was produced using Perkin Elmer 2400 analyzer. The elemental analysis of C, H, N and S% established for the synthesized compounds were found to be within $\pm 0.5\%$ limits of theoretical values. The synthesized compounds were recrystallized from various dilutions of ethanol in distilled water. All the 1, 2, 4-triazoles, 5(a-e) were prepared according to method outlined in the SCHEME 1.



SCHEME 1. Synthesis of 5-(4-substituted phenyl)-1, 2, 4-triazole-3-thiole, 5(a-e).

Synthesis of methyl 4-substituted benzoate, 2(a-e)

The methyl benzoates, 2(a-e) were prepared according to reported method [26].

Synthesis of 4-substituted benzoic acid hydrazide, 3(a-e)

The acid hydrazides, 3(a-e) were prepared according to reported procedure [27] with some modifications. The ester [2(a-e), 0.1 mol] dissolved in methanol (100 ml) and was taken in round bottomed flask fitted with a reflux condenser. Hydrazine hydrate (99%, 25 ml) was added and the reaction mixture refluxed for 6 h. The excess of hydrazine hydrate was distilled off under reduced pressure. The crude product was washed with distilled water and recrystallized from aqueous ethanol.

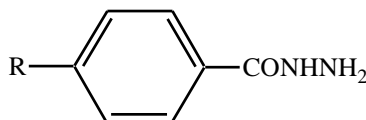
Synthesis of 5-(4-substituted phenyl)-1, 3, 4-oxadiazole-2-thiol, 4(a-e) [28,29]

In a round bottomed flask, the acid hydrazide [3(a-e), 0.1 mol], carbon disulphide (0.12 mol), potassium hydroxide (0.1 mol) were taken in methanol (250 ml) and stirred well. The reaction mixture was refluxed in the fume cupboard. Heating was continued till evolution of hydrogen sulphide ceased. The excess of methanol was then distilled off under reduced pressure. The product was washed with distilled water and precipitated by addition of dilute hydrochloric acid. It was then filtered, dried and recrystallized from ethanol.

Synthesis of 5-(4-substituted phenyl)-1, 2, 4-triazole-3-thiol, 5(a-e) [30,31]

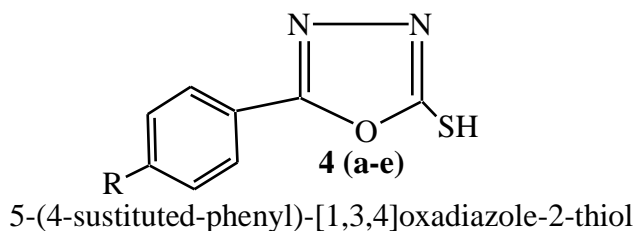
The substituted 1, 3, 4-oxadiazole-2-thiol [4(a-e), 0.02 mol] was dissolved in 50 ml of methanol in round bottomed flask and hydrazine hydrate (99%; 0.08 mol) was added. This reaction mixture was refluxed for about 8 h. The excess of methanol and hydrazine hydrate were removed under reduced pressure. On addition of ice-cold distilled water, the product separates out. The separated product was washed with cold water; dried and recrystallized from ethanol.

TABLE 1. Physical data of methyl 4-substituted benzoic acid hydrazide, 3(a-e).



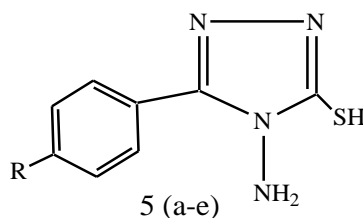
Compound Code	R	Yield (%)	M.P. (°C)
3(a)	-F	91.3	158-60
3(b)	-Cl	93.4	161-63
3(c)	-OH	94.1	125-27
3(d)	-OCH ₃	94.6	131-33
3(e)	-OC ₂ H ₅	94.0	141-43

TABLE 2. Physical data of 5-(4-substituted phenyl)-1,3,4-oxadiazole-2-thiol, 4(a-e).



Compound code	R	Molecular formula	Molecular weight	Yield (%)	MP (°C)	R _f value
4(a)	-F	C ₈ H ₅ FN ₂ OS	196.20	71.2	174-76	0.74
4(b)	-Cl	C ₈ H ₅ ClN ₂ OS	212.66	78.5	180-82	0.82
4(c)	-OH	C ₈ H ₆ N ₂ O ₂ S	194.20	80.6	184-86	0.86
4(d)	-OCH ₃	C ₉ H ₈ N ₂ O ₂ S	208.23	84.8	202-04	0.82
4(e)	-OC ₂ H ₅	C ₁₀ H ₁₀ N ₂ O ₂ S	222.26	87.8	206-08	0.86

TABLE 3. Physical Data of 4-Amino-5-(4-substituted phenyl)-4H- [1, 2, 4] triazole-3-thiol, 5(a-e).



Compound code	R	Molecular formula	Molecular weight	Yield (%)	MP (°C)	R _f value
5(a)	-F	C ₈ H ₇ FN ₄ S	210.23	70.4	203-05	0.70
5(b)	-Cl	C ₈ H ₇ ClN ₄ S	226.69	76.8	209-11	0.74
5(c)	-OH	C ₈ H ₈ N ₄ OS	218.24	74.9	207-09	0.78
5(d)	-OCH ₃	C ₉ H ₁₀ N ₄ OS	222.27	80.1	216-18	0.76
5(e)	-OC ₂ H ₅	C ₁₀ H ₁₂ N ₄ OS	236.29	81.6	221-23	0.80

4-Amino-5-(4-Fluoro-phenyl)-4H- [1,2,4] triazole-3-thiol (5a)

IR (KBr v, cm⁻¹): 3392 (N-H stretching), 3078 (C-H str, aromatic), 2560 (S-H str), 1647 (C=N str, ring) 1566-1490 (C=C str, aromatic), 1208 (C-N str), 1035 (C-F str); ¹³C NMR 400 MHz, (DMSO-d₆, δ, ppm): 166.8 (C-3, C-SH), 162.6 (C-4'), 151.2

(C-5), 128.7 (C-2',C-6'), 125.4 (C-1'), 114.7 (C-3',C-5'); LC-MS: m/z 210 (M⁺); Elemental analysis (C₈H₇FN₄S), Found % (calculated %): C, 45.58 (45.70); H, 3.30 (3.36); N, 26.52 (26.65); S, 15.18 (15.25).

4-Amino-5-(4-Chloro-phenyl)-4H- [1,2,4] triazole-3-thiol (5b)

IR (KBr v, cm⁻¹): 3396 (N-H stretching), 3090 (C-H str, aromatic), 2568 (S-H str), 1660 (C=N str, ring), 1574-1492 (C=C str, aromatic), 1216 (C-N str), 782 (C-Cl str); ¹³C NMR 400 MHz, (DMSO-d₆, δ, ppm): 166.1 (C-3, C-SH), 150.6 (C-5), 135.2 (C-4'), 128.9 (C-3',C-5'), 128.1 (C-2',C-6'), 126.7 (C-1'); LC-MS: m/z 226 (M⁺); Elemental analysis (C₈H₇ClN₄S), Found % (calculated %): C, 42.23 (42.39); H, 3.07 (3.11); N, 24.58 (24.72); S, 14.07 (14.14).

4-Amino-5-(4-Hydroxy-phenyl)-4H- [1,2,4] triazole-3-thiol (5c)

IR (KBr v, cm⁻¹): 3485 (O-H str), 3392 (N-H stretching), 3096 (C-H str, aromatic), 2902 (C-H str, aliphatic), 2570 (S-H str), 1662 (C=N str, ring), 1572-1490 (C=C str, aromatic), 1368 (C-H bend, aliphatic), 1204 (C-N str), 1171-1096 (C-O str); ¹³C NMR 400 MHz, (DMSO-d₆, δ, ppm): 167.8 (C-3, C-SH), 159.2 (C-4'), 152.1 (C-5), 130.9 (C-2',C-6'), 124.3 (C-1') 117.2 (C-3',C-5'); LC-MS: m/z 218 (M⁺); Elemental analysis (C₈H₈N₄OS), Found % (calculated %): C, 46.02 (46.14); H, 3.71 (3.87); N, 26.70 (26.91); S, 15.32 (15.40).

4-Amino-5-(4-Methoxy-phenyl)-4H- [1,2,4] triazole-3-thiol (5d)

IR (KBr v, cm⁻¹): 3402 (N-H stretching), 3098 (C-H str, aromatic), 2906 (C-H str, aliphatic), 2572 (S-H str), 1664 (C=N str, ring), 1578-1494 (C=C str, aromatic), 1372 (C-H bend, aliphatic), 1210 (C-N str), 1161-1090 (C-O str); ¹³C NMR 400 MHz, (DMSO-d₆, δ, ppm): 167.2 (C-3, C-SH), 161.2 (C-4'), 150.9 (C-5), 131.3 (C-2',C-6'), 123.1 (C-1') 115.2 (C-3',C-5'), 55.6 (Ar-O-CH₃); LC-MS: m/z 222 (M⁺); Elemental Analysis (C₉H₁₀N₄OS), Found % (calculated %): C, 48.49 (48.64); H, 4.47 (4.53); N, 25.17 (25.21); S, 14.36 (14.42).

4-Amino-5-(4-Ethoxy-phenyl)-4H- [1,2,4] triazole-3-thiol (5e)

IR (KBr v, cm⁻¹): 3404 (N-H stretching), 3104 (C-H str, aromatic), 2916 (C-H str, aliphatic), 2574 (S-H str), 1669 (C=N str, ring), 1582-1498 (C=C str, aromatic), 1365 (C-H bend, aliphatic), 1202 (C-N str), 1157-1084 (C-O str); ¹³C NMR 400 MHz, (DMSO-d₆, δ, ppm): 166.9 (C-3, C-SH), 160.6 (C-4'), 149.4 (C-5), 130.8 (C-2',C-6'), 122.7 (C-1'), 114.5 (C-3',C-5'), 63.7 (Ar-O-CH₂-CH₃), 14.8 (Ar-O-CH₂-CH₃); LC-MS: m/z 236 (M⁺); Elemental analysis (C₁₀H₁₂N₄OS), Found % (calculated %): C, 50.64 (50.83); H, 5.01 (5.12); N, 23.58 (23.70); S, 13.48 (13.57).

Antimicrobial studies

The synthesized compounds 5(a-e) were evaluated for the antibacterial activities against *Staphylococcus aureus* (MTCC7443; Gram +ve), *Bacillus subtilis* (MTCC121; Gram +ve), *Escherichia coli* (MTCC118; Gram -ve) and *Pseudomonas aeruginosa* (MTCC424; Gram-ve) bacterial strains [32,33]. For antifungal activity, two fungal strains, *Aspergillus niger* and *Candida albicans* were taken using disc diffusion technique [33,34]. Fluconazole and Ciprofloxacin (20 µg/ml) were taken as standard drugs for antifungal activity and antibacterial activity, respectively. Test compounds (100 µg/ml) were made in N, N-dimethyl formamide (DMF).

Sabouraud dextrose medium and Nutrient Agar medium were used for antifungal and antibacterial activity, respectively. The nutrient broth having composition as beef extract (1.0 g), yeast extract (2.0 g), peptone (5.0 g) and sodium chloride (5.0 g) which were dissolved in distilled water and volume made up to 1000 ml. The Nutrient Agar medium was prepared by adding 2.0% w/v of agar to nutrient broth and the pH adjusted to 7.4. For antifungal studies, Sabouraud's dextrose medium with peptone (10.0 g), dextrose (40.0 g) in 1000 ml of distilled water was used; having pH to 5.7. This medium was solidified by adding 1.5% w/v of agar to it.

Whatman no. 1 filter paper was used to prepare about 6 mm in diameter paper discs. The media and discs were sterilized by autoclaving at 121°C and 15 lb/inch pressure for 20 min. Molten agar medium was poured on petriplate aseptically. A standard inoculum (5×10^5 c.f.u./ml) was properly distributed onto the surface of both sterile nutrient agar and dextrose medium petriplates. Sterile discs soaked with the standard and test solutions were kept at centre on the agar culture plates. The bacterial and fungal plates were incubated at $37 \pm 1^\circ\text{C}$ for 24h and $25 \pm 1^\circ\text{C}$ for 72 h respectively. The zone of inhibition and percentage of inhibition were observed and compared with standard drugs (TABLE 4).

Minimum inhibitory concentrations (MIC) were determined by disc diffusion method for all the synthesized compounds. The various concentrations of test compounds in DMF like 90, 80, 70, 60, 50, 40, 30 and 20 $\mu\text{g/ml}$ were prepared in DMF and observed for microbial inhibition. The concentrations showing inhibition or that not showing were taken and further diluted and experiment performed similarly. This was continued till the actual inhibitory concentration was reached (TABLE 5).

Cytotoxicity studies

The *in vitro* cytotoxic activities of the synthesized compounds were evaluated against human breast cell line (MCF-7) using Sulfo Rhodamine-B dye (SRB) assay [35,36]. The cell suspensions were prepared in the appropriate growth medium to produce about 100 μl volume with the cell density of 1×10^4 cells/well. The 100 μl aliquots of cell suspensions were transferred into 96-well micro-titer plates which were incubated for 120 h. The cell lines were kept at 37°C in a 5% v/v CO_2 with 95% humidity. Cultures were developed within a period of 7 days and culture medium changed at least one time in the 5 days. The 100 μl of culture with optimal cell density was taken in each well of 96-well titer plates. The test compounds were properly dissolved in dimethyl sulphoxide (DMSO) which was diluted to obtain various concentrations of 10, 20, 40 and 80 $\mu\text{g/ml}$. 100 μl of each concentration was added to the wells containing the cell suspension and 100 μl of DMSO solvent to control cells. The cells with the test compound were incubated for 48 h and fixed using 100 μl of cold 40% w/v of trichloroacetic acid at 4°C for 1 h. Thereafter, the sufficient volume of cold distilled water was added to wash the plates for five times. Aliquots of 50 μl of 0.4% w/v of SRB dye in 1% v/v acetic acid solution was added to each well of dried 96 well-plates and then cells were kept with dye stain for about 30 min. The dye was removed readily by washing the plates with 50 ml of 1% v/v acetic acid and rinsed 4-5 times till dye bonded with cells only, was retained. The 100 μl of 10 mM Tris base maintaining pH 10.5 were added in each well of the dried plates to solubilize the contents of dye. A shaker was generally used to tremble the treated plates smoothly for 20 min and therefore, in each well the absorbance was read on a plate reader at 492 nm. The same operation was done for all the samples and got the observations in triplicate as well as LC_{50} , (drug concentration that kill the cell growth), TGI, (drug concentration that inhibit total cell growth) and GI_{50} values in $\mu\text{g/ml}$ (drug concentration that inhibit 50% of cell growth) were determined by comparing with doxorubicin used as standard drug (TABLE 6).

Results and Discussion

The 5-(4-substituted phenyl)-4H- [1, 2, 4] triazole-3-thiols, 5(a-e) were synthesized from 1, 3, 4-oxadiazoles, 4(a-e) by treating with hydrazine hydrate as per SCHEME 1. The 1, 3, 4-oxadiazoles were obtained from benzoic acid hydrazides by treating with carbon disulphide and alcoholic potassium hydroxide. The structures were characterized on the basis of spectral data (IR, NMR and Mass spectra) and elemental analysis.

TABLE 4. Data for Antimicrobial Activity of 4-Amino-5-(4-substituted phenyl)-4H- [1,2,4] triazole-3-thiol, (5a-e)

Compounds (100 µg/ml)	Diameter of zone of inhibition ± SD (mm) [@]					
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
5 (a)	15.17 ± 0.29 (75.2%)	15.67 ± 0.29 (73.1%)	15.83 ± 0.58 (74.2%)	13.67 ± 0.29 (67.2%)	14.00 ± 0.50 (63.1%)	13.50 ± 0.87 (65.9%)
5 (b)	13.67 ± 0.29 (67.7%)	15.50 ± 0.50 (71.5%)	14.83 ± 0.58 (69.5%)	13.17 ± 0.29 (64.8%)	13.00 ± 0.50 (58.6%)	12.50 ± 0.87 (60.9%)
5 (c)	12.67 ± 0.29 (62.8%)	13.83 ± 0.58 (63.8%)	13.67 ± 0.29 (64.1%)	12.50 ± 0.50 (61.5%)	13.83 ± 0.76 (62.4%)	14.33 ± 0.58 (65.0%)
5 (d)	12.50 ± 0.50 (61.9%)	13.17 ± 0.29 (60.7%)	13.50 ± 0.50 (63.3%)	13.83 ± 0.76 (68.0%)	16.83 ± 0.29 (75.9%)	15.50 ± 0.50 (75.6%)
5 (e)	13.00 ± 0.50 (64.5%)	12.67 ± 0.29 (58.5%)	14.00 ± 0.50 (65.6%)	13.00 ± 0.50 (63.9%)	17.67 ± 0.29 (79.7%)	16.50 ± 0.50 (80.5%)
Ciprofloxacin (20 µg/ml)	20.17 ± 0.29 (100%)	21.67 ± 0.58 (100%)	21.33 ± 0.76 (100%)	20.33 ± 0.58 (100%)	--	--
Fluconazole (20 µg/ml)	--	--	--	--	22.17 ± 0.29 (100%)	20.50 ± 0.50 (100%)
DMF	--	--	--	--	--	--
<p>@ The percentage (%) zone of inhibition of test compounds was calculated against various microbial strains with reference to standard and solvent control.</p> <p>'--' indicates the zone of inhibition of control (DMF) was considered as negligible.</p>						

A perusal of the TABLE 4, show that the compound 5(a) and 5(b) were prominently active (with 67.7 to 75.1% inhibition) against bacterial strains studied except *P. aeruginosa*. Rest of the compounds i.e., 5(c), 5(d) and 5(e) showed moderate activities comparing with Ciprofloxacin.

Out of the five compounds studied, it is observed from the TABLE 4 that compounds 5(d) and 5(e) were found to be prominent antifungal in nature showing 75.6 to 80.5% inhibition when compared with Fluconazole. All other compounds i.e., 5(a), 5(b) and 5(c) showed moderate fungal toxicities.

Since, the solutions of all test compounds and standard drugs were prepared in DMF and the zone of inhibition of DMF solvent control was found to be negligible.

The MIC values of all synthesized compounds were found to be 30 to 42 µg/ml, 32 to 44 µg/ml, 34 to 46 µg/ml and 42 to 52 µg/ml for *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* respectively. Compounds 5(a) and 5(b) were found to be most active antibacterial agents.

Similarly, MIC values were found to be 28 to 48 µg/ml for *Aspergillus niger* and 30 to 46 µg/ml for *Candida albicans* in the antifungal studies. Out of five synthesized compounds; 5(d) and 5(e) were most potent antifungal agents as shown in TABLE 5.

From the above result, it was observed that the synthesized compound 5(a) (R=4-fluorophenyl on C-5 position of triazole nucleus) produced highest antimicrobial activity against microbial strains while compound 5(b) (R=4-chlorophenyl) possess significant antibacterial and moderate antifungal activity and the compound 5(c) (R=4-hydroxy phenyl) exhibited moderate antibacterial as well as antifungal activities. In contrast to this, the compounds 5(d) (R=4-methoxy phenyl) and 5(e) (R=4-ethoxy phenyl) showed moderate antibacterial but prominent antifungal activities.

Thus, it can be said that electron density on 4-substituents of aromatic ring at C-5 position of 1, 2, 4-triazole nucleus determine antibacterial and antifungal activities [37]. The enhancement in the antibacterial activity of these compounds may be due to electron withdrawing ability of 4-substituents ((F and Cl atoms)) of phenyl group while antifungal activity most likely depends on electron donating capability of 4-substituents ((methoxy and ethoxy groups)) of phenyl group at C-5 position. Therefore, electron withdrawing and electron donating groups are responsible for potency of antibacterial and antifungal agents, respectively. In addition to state above, -N=C-N- toxicophoric part of triazole ring; play a crucial role in retaining the antimicrobial profile because of structural similarities with standard drug.

TABLE 5. Minimum inhibitory concentrations of 4-Amino-5-(4-substituted phenyl)-4H-[1,2,4]triazole-3-thiol, 5(a-e)

Compounds	#Minimum inhibitory concentrations (MICs), µg/ml					
	<i>S. aureus</i>	<i>B. subtilis</i>	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>A. niger</i>	<i>C. albicans</i>
5 (a)	30	32	34	42	48	46
5 (b)	36	34	38	44	ND	ND
5 (c)	42	44	46	52	42	40
5 (d)	ND	ND	ND	ND	28	26
5 (e)	ND	ND	ND	ND	32	30
Ciprofoxacin	18	20	20	24	ND	ND
Fluconazole	ND	ND	ND	ND	26	24

ND indicates the MIC of those compounds were not determined.

The cytotoxicity of the synthesized compounds was evaluated by determining LC₅₀, TGI, and GI₅₀ values in µg/ml using sulforhodamine-B dye method. The compounds 5(a) possessing LC₅₀ value >80; TGI value >80 and GI₅₀=48.6 µg/ml and 5(b) with LC₅₀ value >80; TGI value >80 and GI₅₀=46.3 µg/ml exhibited low cytotoxicity while compounds 5(c), 5(d) and 5(e) exhibited very low cytotoxicity with same values of LC₅₀>80; TGI values >80 but they have the GI₅₀ values 59.4, 53.0 and 51.0 µg/ml respectively.

In accordance, with the data obtained from *in vitro* cytotoxicity studies (TABLE 6), all the synthesized compounds are categorized as very low to low cytotoxic when compared with doxorubicin as a standard drug. The compounds 5(c), 5(d) and 5(e) have shown comparatively very low cytotoxicity, while 5(a) and 5(b) are categorized as compounds with low cytotoxicity. It was also indicated that the bioactive compounds possessing log P values between 1.6 to 2.2 showed very low cytotoxicity.

TABLE 6. Cytotoxicity of 4-Amino-5-(4-substituted phenyl)-4H-[1,2,4]triazole-3-thiol (5a-e).

S.No.	Compound Code	logP	^a LC ₅₀	TGI	GI ₅₀	^b Cytotoxicity
1	5 (a)	2.22	>80	>80	48.6	low
2	5 (b)	2.62	>80	>80	46.3	low
3	5 (c)	1.67	>80	>80	59.4	very low
4	5 (d)	1.94	>80	>80	53	very low
5	5 (e)	2.27	>80	>80	51	very low
Standard	Doxorubicin	2.82	>80	<10	<10	high
Control	DMSO	--	--	--		--
^a LC ₅₀ =drug concentration that kill 50% cell growth in µg/ml; TGI=drug concentration that inhibit total cell growth in µg/ml; GI ₅₀ =drug concentration that inhibit 50% cell growth in µg/ml ^b Cytotoxicity categorizes to high, low and very low cytotoxicity of active antimicrobial agents; '--' indicates the activity of control was considered as negligible.						

In this study, a series of 4-Amino-5-(4-substituted phenyl)-4H-[1,2,4]triazole-3-thiol were synthesized for evaluating inhibitory action against the growth of cultured human breast cell line [37,38]. When an electron releasing group like hydroxy group (compound 5c) was substituted at 4-position in the phenyl ring which possess very low cytotoxicity. The replacement of the hydroxy group by less electron-releasing groups like methoxy (compound 5d) and ethoxy (compound 5e) increase the cytotoxicity. From above discussion, it may be concluded that the bulkiness of the substituent results to enhanced cytotoxicity except compound, 5(a).

Conclusion

In summary, SCHEME 1 represents a simple synthetic route of 4-substituted 5-phenyl-1, 2, 4-triazole-3-thiol, 5(a-e) and synthesized compounds were evaluated for antimicrobial activity and cytotoxic activity on breast cancer cell line (MCF-7). The compounds 5(a) and 5(b) exhibited good bacterial inhibition whereas compounds 5(d) and 5(e) showed prominent fungal

inhibition comparing with standard drugs. The biological profiles of newly synthesized 1, 2, 4-triazoles would represent a fruitful matrix for further development of newer compounds that could generate more safe and efficacious agents.

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