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Research Article

SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF SOME NOVEL BIS-HETEROCYCLES ENCOMPASSING PYRROLE

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ABSTRACT

A series of bis heterocycles comprising of bis-1,2,4-triazoles and 1,3,4-thiadiazoles were synthesized from 3, 5-dimethyl-1H-pyrrole-2,4-dicarbohydrazide in two steps via respective thiosemicarbazide intermediates. The compounds **3a-g** and **4a-g** were evaluated for their anti fungal activity against a panel of four pathogenic fungal strains namely, *Aspergillus niger, Aspergillus flavus, Aspergillus terrius* and *Candida albicans* by two fold serial dilution method. The cytotoxic effect of these compounds was also studied against human lung carcinoma cells (A-549) using MTT assay method.

Keywords: Bis-triazole, Bis-thiadiazole, Antifungal activity, Cytotoxic activity, MTT assay.

INTRODUCTION

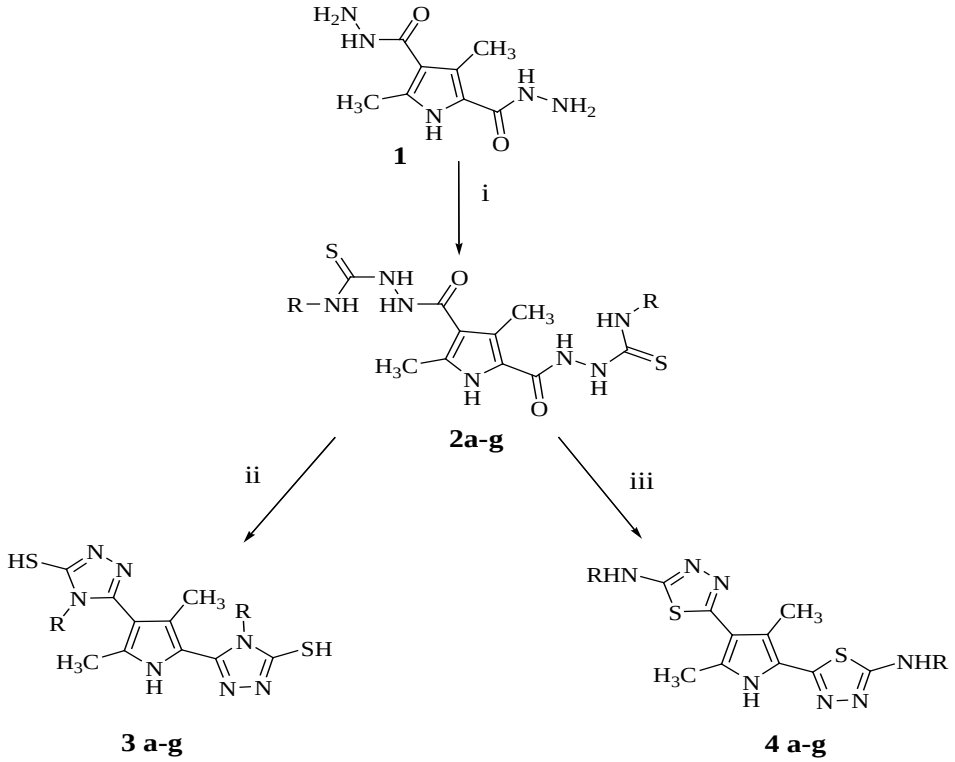
Bis-heterocyclic compounds are gaining increased interest in the recent past as the dimeric analogues have proven to be having better and potent biological activity than the corresponding monomer[1-3]. Many of the bis-1, 2, 4-triazoles [4-6] and bis-thiadiazoles[7-9] have also been reported to possess wide spectrum of biological activity.

The triazoles such as fluconazole and terconazole are also being used clinically as potent antifungal agents. We have reported earlier the synthesis and cytotoxicity study of certain bis 1,2,4-triazole systems from our lab.[10-12] It has been observed that the lipophilicity of the molecules seems to be important for the higher cytotoxic activity as evident from the lower IC_{50} values. Further the higher lipophilicity is also of importance to exert antifungal activity as high lipophilic compounds can easily penetrate through the fungal cell membrane. Keeping these observations in mind we report herein the synthesis and *in vitro* cytotoxic as well as antifungal activity of certain novel bis 1,2,4-triazoles and 1,3,4-thiadiazoles encompassing pyrrole.

MATERIALS AND METHODS

The melting points were determined in open glass capillaries and are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8400-S spectrophotometer by KBr pellet technique. ^{1}H-NMR and ^{13}C-NMR spectra were recorded on AMX-400 NMR spectrophotometer at 400 MHz using DMSO-d_6 as the solvent and tetra methyl silane (TMS) as internal standard. The chemical shifts are expressed in δ ppm. The splitting patterns were designated as follows; s: singlet; d: doublet; q: quartet; m: multiplet. LCMS were recorded by using Shimadzu LCMS-2010A instrument by ESI. Molecular ion (M^+) value in m/z units is provided along with percent relative abundance in parenthesis.

Synthesis of the intermediate and target compounds was accomplished according to the steps depicted in **Scheme 1.**



Scheme 1

Reagents and conditions: i) RNCS/EtOH and DMF reflux 4-6 h ii) 2N NaOH, heat for 4h neutralize with dil. HCl iii) dissolve in 5 ml Conc. H_2SO_4 stir below 5°C for 2h and neutralize with $NaHCO_3$

Preparation of 3, 5-dimethyl-1H-pyrrole-2,4-dicarbohydrazide (2):

To the suspension of 0.01 mol of diethyl 3,5-dimethyl-1H-pyrrole-2,4-dicarboxylate[12] in 50 ml of absolute ethanol, 0.06 mol of hydrazine hydrate (99%) was added slowly. The mixture was heated to dissolve the solid separated. Ethanol was added to affect the dissolution. The solution was further refluxed for 10 h. The excess of the solvent was removed by distillation under reduced pressure. The solid obtained after cooling was collected and crystallized with dichloromethane.

Yield- 60%; MP: 195^{0}C; IR (ν cm^{-1},KBr): 3234, 1710; ^{1}H-NMR (DMSO-*d*6, δ ppm): 10.5 (s,2H, CONH), 8.0 (s,1H, NH), 4.3 (s, 4H, NH_2), 2.5 (s, 3H, CH_3), 2.4 (s, 3H, CH_3); ^{13}C-NMR (DMSO-*d*6, δ ppm): 163.7, 161.9, 137.0, 124.1, 120.7, 14.3, 13.6; LCMS m/z: 211 (M$^+$, 15).

General method for the preparation of bis-thiosemicarbazides (2a-g):

A mixture of 0.005 mol of **2** and 0.01 mol of appropriate isothiocyantes in 20 ml of absolute ethanol was refluxed for 8 h on boiling water bath. The resulting solution was concentrated and cooled to get the bis-thiosemicarbazides in quantitative yield. The colourless solid thus obtained were washed with a mixture of DMF and ethanol and were used without further purification.

General method for the preparation of 2, 4 bis (5-mercapto 4-substituted-1,2,4 triazol-3-yl) 3,5 dimethyl 1H pyrrole (3a-g):

A solution of 0.005 mol of appropriate thiosemicarbazide (**2a-g)** in 10ml of 2N NaOH was refluxed for 4-6 h with constant stirring. The reaction mixture was cooled, filtered and the filtrate was acidified with cold dilute HCl to get the solid triazole. Recrystalization was done by ethanol.

2, 4 bis (5-mercapto 4-phenyl 1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3a)

The above method was followed using 0.005 mol **2a.**

Yield- 80%; MP: 225^{0}C; IR (ν cm^{-1},KBr): 3215, 3050; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.3 (s,2H, SH), 8.1 (s,1H, NH), 6.8-7.3 (m, 10H, Ar-H), 2.4 (s, 3H, CH_3), 2.25 (s, 3H, CH_3); ^{13}C-NMR (DMSO-*d*6, δ ppm): 169.7, 153.9, 149.7, 147.5, 138.0, 129.4, 128.2, 125.6, 124.9, 122.2, 120.5, 14.2, 13.1; LCMS m/z: 446 (M$^+$, 55).

2, 4 bis (5-mercapto 4-(p-tolyl) 1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3b)

The above method was followed using **2b** (0.005 mol).

Yield- 85%; MP: 256^{0}C; IR (ν cm^{-1},KBr): 3210, 3045; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.2 (s,2H, SH), 8.12 (s,1H, NH), 6.6-7.5 (m, 8H, Ar-H), 2.35 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 2.12 (s, 6H, tolyl CH_3); LCMS m/z: 474 (M$^+$, 75).

2, 4 bis (5-mercapto 4-(4-ethoxy phenyl) 1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3c)

The above method was followed using 0.005 mol **2c.**

Yield- 70%; MP: 234^{0}C; IR (ν cm^{-1},KBr): 3212, 3070, 2980; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.17 (s,2H, SH), 8.0 (s,1H, NH), 6.5-7.4 (m, 8H, Ar-H), 4.1 (q, 4H, OCH_2), 2.31 (s, 3H, CH_3), 2.23 (s, 3H, CH_3), 1.1(t, 6H, CH_3); ^{13}C-NMR (DMSO-*d*6, δ ppm): 168.3, 153.7, 148.9, 147.35, 139.20, 130.4, 128.12, 125.6, 124.9, 122.2, 120.5, 59.2, 14.5, 13.11, 10.34; LCMS m/z: 534 (M$^+$, 40).

2, 4 bis (5-mercapto 4-cyclohexyl-1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3d)

The above method was followed using 0.005 mol of **2d.**

Yield- 65%; MP: 205^{0}C; IR (ν cm^{-1},KBr): 3230, 2975; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.05 (s,2H, SH), 8.15 (s,1H, NH), 3.65 (s, 2H,C_1 protons of cyclohexyl), 2.32 (s, 3H, CH_3), 2.19 (s, 3H, CH_3), 2.10 (m, 8H, C_2 and C_6 protons of cyclohehyl), 1.70 (m, 8H C_3 and C_5 protons of cyclohehyl), 1.5 (m, 4H, C_4 protons of cyclohehyl); LCMS m/z: 458 (M$^+$, 45).

2, 4 bis (5-mercapto 4-(n-butyl)-1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3e)

The above method was followed using 0.005 mol of **2e.**

Yield- 60%; MP: 185^{0}C; IR (ν cm^{-1},KBr): 3233, 2985; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.15 (s,2H, SH), 8.13 (s,1H, NH), 3.87 (s, 2H,C_1 protons of butyl), 2.40 (s, 3H, CH_3), 2.27 (s, 3H, CH_3), 2.18 (m, 4H, C_2 protons of butyl), 1.90 (m, 4H C_3 protons of butyl), 1.2 (m, 6H, C_4 protons of butyl); LCMS m/z: 406 (M$^+$, 40).

2, 4 bis (5-mercapto 4-(4-methoxy phenyl) 1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3f)

The above method was followed using 0.005 mol of **2f.**

Yield- 66%; MP: 212^{0}C; IR (ν cm^{-1},KBr): 3225, 3065, 2978; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.32 (s,2H, SH), 8.14 (s,1H, NH), 6.6-7.4 (m, 8H, Ar-H), 3.7 (s, 6H, OCH_3), 2.4 (s, 3H, CH_3), 2.20 (s, 3H, CH_3); LCMS m/z: 506 (M$^+$, 65).

2, 4 bis (5-mercapto 4-(2-flouro phenyl) 1,2,4 triazol-3yl) 3,5 dimethyl 1H pyrrole (3g)

The above method was followed using 0.005 mol of **2g.**

Yield- 60%; MP: 210^{0}C; IR (ν cm^{-1},KBr): 3223, 3072; ^{1}H-NMR (DMSO-*d*6, δ ppm): 12.23 (s,2H, SH), 8.15 (s,1H, NH), 6.8-7.2 (m, 8H, Ar-H), 2.4 (s, 3H, CH_3), 2.25 (s, 3H, CH_3); LCMS m/z: 482 (M$^+$, 45).

General method for the preparation of 2, 4 bis (5-substituted-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4a-g)

A solution of 0.005 mol of appropriate thiosemicarbazide (**2a-g)** in minimum quantity of cold concentrated sulphuric acid (5ml) was stirred at ice cold condition for 2h. The resulting reaction mixture was kept overnight at room temperature. The solution was neutralized with saturated solution of sodium bicarbonate to obtain the solid thiadiazole derivatives (**4a-g**). Recrystallization was done by using a mixture of DMF and ethanol.

2, 4 bis (5-phenylamino-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4a)

Yield- 70%; MP: 197^{0}C; IR (ν cm^{-1},KBr): 3320, 3215, 3076; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.21 (s,1H, NH), 6.7-7.5 (m, 10H, Ar-H), 4.87 (s, 2H, NH), 2.34 (s, 3H, CH_3), 2.27 (s, 3H, CH_3); ^{13}C-NMR (DMSO-*d*6, δ ppm): 171.6, 155.3, 145.9, 132.4, 129.4, 127.8, 125.3, 120.2, 118.5, 116.2, 13.2, 11.1; LCMS m/z: 446 (M$^+$, 65).

2, 4 bis (5-(p-tolylamino)-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4b)

Yield- 65%; MP: 215^{0}C; IR (ν cm^{-1},KBr): 3323, 3210, 3065, 2980; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.18 (s,1H, NH), 6.5-7.4 (m, 8H, Ar-H), 4.85 (s, 2H, NH), 2.44 (s, 3H, CH_3), 2.29 (s, 3H, CH_3); LCMS m/z: 474 (M$^+$,60).

2, 4 bis (5-(4-ethoxyphenylamino)-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4c)

Yield- 62%; MP: 257^{0}C; IR (ν cm^{-1},KBr): 3345, 3221, 3078, 2987; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.1 (s,1H, NH), 6.8-7.3 (m, 8H, Ar-H), 4.90 (s, 2H, NH), 4.12 (q, 4H, OCH_2), 2.34 (s, 3H, CH_3), 2.22 (s, 3H, CH_3), 1.0 (t, 6H, CH_3); LCMS m/z: 534 (M$^+$, 50).

2, 4 bis (5-cyclohexylamino-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4d)

Yield- 55%; MP: 218^{0}C; IR (ν cm^{-1},KBr): 3337, 3215, 2950; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.14 (s,1H, NH), 4.68 (s, 2H, NH), 3.63 (s, 2H,C_1 protons of cyclohexyl), 2.42 (s, 3H, CH_3), 2.25 (s, 3H, CH_3), 2.13 (m, 8H, C_2 and C_6 protons of cyclohehyl), 1.65 (m, 8H C_3 and C_5 protons of cyclohehyl), 1.51 (m, 4H, C_4 protons of cyclohehyl); LCMS m/z: 458 (M$^+$, 50).

2, 4 bis (5-(n-butylamino)-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4d)

Yield- 60%; MP: 178^{0}C; IR (ν cm^{-1},KBr): 3330, 3214, 2980; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.15 (s,1H, NH), 4.66 (s, 2H, NH), 3.87 (s, 2H,C_1 protons of butyl), 2.43 (s, 3H, CH_3), 2.21 (s, 3H, CH_3), 2.10 (m, 4H, C_2 protons of butyl), 1.82 (m, 4H C_3 protons of butyl), 1.15 (m, 6H, C_4 protons of butyl); LCMS m/z: 406 (M^+, 35).

2, 4 bis (5-(4-methoxyphenyl amino)-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4f)

Yield- 70%; MP: 230^{0}C; IR (ν cm^{-1},KBr): 3342, 3230, 3068, 2986; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.21 (s,1H, NH), 6.4-7.3 (m, 8H, Ar-H), 4.88 (s, 2H,NH), 3.85 (s, 6H, OCH_3), 2.38 (s, 3H, CH_3), 2.23 (s, 3H, CH_3); LCMS m/z: 506 (M^+, 65).

2, 4 bis (5-(2-fluorophenyl amino)-1,3,4-thiadiazol-2-yl) 3,5 dimethyl 1H pyrrole (4g)

Yield- 50%; MP: 224^{0}C; IR (ν cm^{-1},KBr): 3342, 3220, 3050; ^{1}H-NMR (DMSO-*d*6, δ ppm): 8.21 (s,1H, NH), 6.4-7.6 (m, 8H, Ar-H), 4.85 (s, 2H,NH), 2.34 (s, 3H, CH_3), 2.22 (s, 3H, CH_3); LCMS m/z: 482 (M^+, 65).

Lipophilicity

The efficiency of the cytotoxicity of the drug depends on the accumulation of the compound into the cell and thus lipophilic character plays a major role in the cytotoxic effect of the compounds. The partition coefficient ($log_{10}P$) of the compounds which is a measure of lipophilicity was calculated using the software Bioloom (version 1) from Biobyte corp. (201, West 4th St. Suite 204, Claremont, CA 91711). The physical characteristics and C logP data of the compounds is presented in **Table 1.**

Table 1: List of compounds prepared

S. No.	Compound	R	Mol Wt	Mol formula	C log P
1	3ª	Phenyl	446	$C_{22}H_{19}N_7S_2$	4.60
2	3b	p-tolyl	474	$C_{24}H_{23}N_7S_2$	5.59
3	3c	p-ethoxy phenyl	534	$C_{26}H_{27}N_7O_2S_2$	5.61
4	3d	Cyclohexyl	458	$C_{22}H_{31}N_7S_2$	4.49
5	3e	n-butyl	406	$C_{18}H_{27}N_7S_2$	3.61
6	3f	p-methoxy phenyl	506	$C_{24}H_{23}N_7O_2S_2$	5.23
7	3g	2-fluoro phenyl	482	$C_{22}H_{17}F_2N_7S_2$	4.89
8	4a	Phenyl	446	$C_{22}H_{19}N_7S_2$	5.66
9	4b	p-tolyl	474	$C_{24}H_{23}N_7S_2$	6.62
10	4c	p-ethoxy phenyl	534	$C_{26}H_{27}N_7O_2S_2$	6.53
11	4d	Cyclohexyl	458	$C_{22}H_{31}N_7S_2$	5.73
12	4e	n-butyl	406	$C_{18}H_{27}N_7S_2$	4.84
13	4f	p-methoxy phenyl	506	$C_{24}H_{23}N_7O_2S_2$	5.48
14	4g	2-fluoro phenyl	482	$C_{22}H_{17}F_2N_7S_2$	5.95

***In vitro* antifungal activity**

In vitro anti fungal activity of the synthesized compounds was evaluated by two fold serial dilution method[13]. Media used was Potato Dextrose Broth (PDB). Initially, the stock culture of *Aspergillus niger, Aspergillus flavus, Aspergillus terrius* and *Candida albicans* were revived by inoculating in broth media and grown at 37^{0}C for 48 hrs. The tubes of the above media PDB (5 ml) were prepared and each tube was added with compounds (10-500 µg) and inoculated with 100 µl of 48 hr old cultures. The control tubes with fluconazole and DMSO were also prepared. All the tubes were incubated at 37^{0}C for 48 h with constant shaking and the absorbance of biomass were measured 660 nm against autoclaved, uninoculated media as blank. The result of i*n vitro* anti fungal activity is expressed as Minimum Inhibitory concentration (MIC) and is given in **Table 2**.

Table 2: In vitro antifungal and cytotoxic activity of test compounds

Compound	Antifungal activity MIC in µg/ml				Cytotoxic Activity IC_{50} in µM
	A. niger	*A. flavus,*	*A. terrius*	*C. albicans*	A-549
3a	50	100	100	50	45.25 ± 3.87
3b	25	50	50	25	33.37 ± 4.55
3c	25	25	25	12.5	29.23 ± 3.36
3d	100	100	100	100	54.73 ± 2.98
3e	25	25	50	50	20.25 ± 2.92
3f	50	50	50	50	25.19 ± 2.53
3g	12.5	25	25	12.5	15.09 ± 2.68
4a	100	100	100	50	61.26 ± 3.23
4b	200	200	200	200	46.94 ± 2.49
4c	100	100	100	100	18.36 ± 4.53
4d	100	100	100	100	51.81 ± 4.74
4e	100	100	200	100	33.88 ± 4.45
4f	200	200	200	200	23.27 ± 1.35
4g	50	50	50	50	21.19 ± 4.16
Fluconazole	6.25	6.25	6.25	6.25	-
Doxorubicin	-	-	-	-	0.09 ± 0.01

The cytotoxicity of the compounds was evaluated *in vitro* against the human lung carcinoma cancer cell line (A-549). The cells were procured from National Centre for Cell Sciences, Pune, India, and were cultured in DMEM medium supplemented with 10%FBS, 1%L-glutamine and 50µg/ml gentamycin sulphate in a CO_2 incubator in a humidified atmosphere of 5%CO_2 and 95% air. Effect of test compounds on cell proliferation of cancer cells was tested using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay.[14-15] Cells were cultured in duplicates at a density of 1 × 10^5 cells/well. After 24 h, the test compounds were added at a concentration of 10, 20 and 50 µM and incubated for 48 h. Cells were harvested and incubated with MTT reagent (5 mg/ml, Sigma–Aldrich, USA). The resulting insoluble MTT formazan product was then dissolved in detergent containing 50%

N,N-dimethylformamide (Sigma–Aldrich, USA) and 10% of sodium dodecyl sulphate (Amresco, USA). Absorbance of the resulting colored solution was measured at 570 nm on a multiwell ELISA plate reader. Cells treated with DMSO grown in culture media were used as a vehicle control. The experiment was repeated three independent times and the percentage cytoxicity was calculated using the following formula.

% Cytotoxicity= (Control abs – Test abs) X 100/ Control abs.

The drug concentration that causes 50% cell growth inhibition after 48h of continuous exposure to the test compounds (IC_{50}) was determined by performing the regression analysis. The IC_{50} values of the test compounds are shown in **Table 2**.

RESULTS AND DISCUSSION

Chemistry

The synthesis of bis-triazoles and thiadiazoles encompassing pyrrole moiety was carried out in three steps in good yield as depicted in the scheme of the synthesis.

The di-carbohydrazide (**1**) was prepared by refluxing respective di-ester in presence of excess of hydrazine hydrate under inert atmosphere. The absence of characteristic ethoxy signals and appearance of CONH and NH_2 signals at 10.5 and 4.3ppm in the PMR, indicate the conversion of ester to hydrazide.

The above dicarbohydrazide was further treated with different aryl, cycloalkyl and alkyl isothiocyanates to obtain respective thiosemicarbazides (**2a-g**) in almost quantitative yield. These compounds when subjected to cyclization under different reaction conditions yielded bis 1,2,4-triazoles (**3a-g**) and 1,3,4-thiadiazoles (**4a-g**).

In PMR spectrum of the bis-1,2,4-triazole derivative **3a**, absence of the group signals corresponding to the CONH, CSNH and Ar-NH group of the respective thiosemicarbazide and appearance of a signal due SH at 12.5 ppm confirms the cyclization into mercapto triazoles. The high down field signal for the SH proton is due to the thione-thiol tautomerism of the 5-mercapto 1,2,4-triazoles system. The LCMS data showed M+ peak at 446 m/z.

The thiadiazole derivative **4a**, which was prepared by reacting **2a** with cold concentrated sulphuric acid exhibited a broad peak at 4.84ppm due to aryl amino proton while the group signals of thiosemicarbazide were absent. The MS data showed a stable base peak at 446 m/z units representing the molecular mass of the compound. The fragmentation was very minimum as the spectra were recorded in ESI mode. Similarly other compounds were characterized and found to be in agreement with the proposed structure.

Lipophilicity

The efficiency of an anti infective drug will depend in part on its ability to accumulate in microorganisms. Further, certain resistant strains of microorganism develop resistance by decreasing the accumulation of the drug substance in the cytoplasm of the microorganism. Lipophilicity (Log P) of the drugs hence, plays a vital role in the antimicrobial effect of the compounds. ClogP of the compounds was determined by the fragment based prediction software and found to be in the range of 3.61 to 5.61 for bis-triazoles; while that of bis-thiadiazole were found to be between 4.84 to 6.62.

The *in vitro* anti fungal activity was carried out against four different fungal species by two fold serial dilution method. From the MIC data presented in Table 2, it is clear that the triazole derivatives exhibited better antifungal activity than the thiadiazole derivatives. The lower MIC values suggested that the compounds **3c** and **3g** showed significant activity as compared to the standard drug.

The compounds were screened for their cytotoxicity against human lung carcinoma cells (A-549) by using standard MTT assay protocol[14-17]. The inhibition concentration (IC_{50}) defined as the concentration of the drug that causes 50% cell-growth inhibition after 48 h of continuing exposure to the test compounds and the mean of the results obtained from triplicate assays are shown in Table 2. The IC_{50} values were compared with that of anticancer antibiotic doxorubicin. From the evaluation of the data reported in Table 2, the following observations can be made. All the synthesized compounds were far less cytotoxic than the standard drug doxorubicin (IC_{50} value 0.09 ± 0.01μM) as evident from higher IC_{50} values. However, among the synthesized compounds 3c & 4c and 3f & 4f which contain electron releasing functional groups such as ethoxy and methoxy respectively exhibited moderate activity; however the fluoro substituted compounds 3g and 4g were found to be more potent cytotoxic compound. The compounds with cyclohexyl and n-butyl substitution exhibited poor cytotoxicity.

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Authors declare no conflict of interest.

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