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Synthesis, Biological Evaluation and Molecular Modeling Study of 3,4-Disubstituted 5-Mercapto-1,2,4-triazoles

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Based on the outcome of computational docking to the active site of cytochrome P450 14α-demethylase (CYP51), diverse 3,4-disubstituted 5-mercapto-1,2,4-triazoles were prepared and screened for antioxidant and antifungal activities. The docking study of synthesized compounds showed promising binding affinity towards docked enzyme, sterol 14α-demethylase(CYP51) from trypanosome cruzi obtained from a RCSB protein data bank (PDB ID: 3KHM). The synthesized compounds were characterized by IR, ^{1}H NMR and Mass spectral data. Among the novel synthesized compounds **IV-6**, **IV-1** and **IV-2** showed maximum antifungal activity against *A. niger* and *C. albicans* organism when compared the standard fluconazole. For antioxidant activity, all the compounds showed moderate activity but compound **IV-6** and **IV-7** showed significant activity when compared to standard ascorbic acid.

Keywords: 1,2,4-Triazoles, Antioxidant activity, Antifungal activity, Molecular docking study.

INTRODUCTION

At the present scenario, antioxidants awaken researcher's attention in both medicinal plants and synthetic compounds. Antioxidant properties of diverse compounds were investigated by employing various *in vitro* systems, interaction of 2,2-diphenyl-1-picrylhydrazyl (DPPH) and scavenging of superoxide radical, microsomal NADPH-dependent inhibition of lipid peroxidation (LP), microsomal ethoxyresorufin O-deethylase (EROD) activity [1]. Antioxidants inhibit or delay oxidation, which appears to have a role in the prevention of many diseases [2]. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging measurements were used to determine antioxidant capacity with ascorbic acid as standard.

Contagious microbial diseases remain vital problems for humanity, because confrontation to an amount of antimicrobial agents among the variety of clinically important species of microorganisms [like methicillin-resistant *Staphylococcus aureus* (MRSA)] has turned into an important universal health problem [3-5]. Hence, there will constantly be a crucial need to find out new antimicrobial agents.

1,2,4-Triazole derivatives were found to posses a variety of pharmacological activities like anti-HIV [6,7], antimicrobial [8-10], antiviral [11], anti-inflammatory [12], antioxidant, *etc*. Among the azoles, especially triazole antifungal agents were used broadly and efficiently. For example, voriconazole, fluconazole and itraconazole, currently play an important role in the treatment of invasive fungal infection. These antifungal drugs act by inhibiting CYP51, a necessary enzyme in the biosynthesis of ergosterol, through a mechanism in which the heterocyclic nitrogen atom (N-4 of triazole) binds to the heme iron atom [13]. These efficient analogues are ideally suited for further alteration to obtain more efficacious antibacterial compounds.

Various biologically active compounds having therapeutic activities and diverse drawback lead to the scientist to prepare most biologically effective compounds with minimal side effects. In this paper, we have tried to report some novel synthesized 3,4-disubstituted 5-mercapto-1,2,4-triazoles and screened for their antioxidant activity as well as docking for antifungal activity.

EXPERIMENTAL

All the chemicals were of synthetic grade and commercially procured from SD Fine chemicals, Hyderabad. Melting points were determined in open capillary method and were uncorrected. Purity of the compound was checked on silica Gel TLC plates. IR spectra were recorded on FT-IR8400S, Fourier transform (SHIMADZU) infrared spectrophotometer

using KBr disc method. The proton magnetic resonance spectra (^{1}H NMR) were recorded on Perkin Elmer spectrophotometer-300 MHz in DMSO-d_6, chemical shifts are reported as parts per million (ppm) using TMS as an internal standard.

Preparation of methyl 2-(naphthalen-6-yloxy)acetate and acetohydrazide (I and II) [14]: Appropriate quantities of acid (0.1 mol) and methanol (50 mL) was introduced into a clean and dry round bottom flask and stirred well for 10 min to the above mixture, few drops of concentrated sulfuric acid were added and the reaction mixture was refluxed for 6 h. The reaction mixture was concentrated by distilling the excess ethanol under reduced pressure and treated with saturated solution of sodium bicarbonate. The ester formed in the reaction was used for the preparation of hydrazides directly. The appropriate ester (0.1 mol) was dissolved in 50 mL of ethanol in a clean dry round-bottomed flask and to this hydrazine hydrate (0.1 mol) was added. The reaction mixture was then refluxed for a period of 15 to 18 h. The excess ethanol was distilled off under reduced pressure. The resultant mixture was then poured into ice cold water and the obtained solid was filtered, recrystallized from ethanol.

2-(Naphthalen-6-yloxy)dithiocarbazinates (III) [14]: An appropriate quantity of acid hydrazide (0.01 mol) was taken in a clean and dry round bottomed flask. Carbon disulphide and alcoholic potassium hydroxide (1.5 mol) was introduced into the round bottomed flask containing acid hydrazide. The reaction mixture was refluxed for a period of 3-4 h. After cooling, the separated product was collected by filtration, washed with water and dried.

General procedure for the synthesis of 3,4-disubstituted 1,2,4-triazole (IV 1-7) [15]: The appropriate dithiocarbazinates salt (0.01mol), substituted amines (0.01 mol) and water (5 mL) was transferred in a clean and dry round bottomed flask and the reaction mixture was refluxed for 6 h till profuse H_2S gas evolves. The reaction mixture was further boiled for 0.5 h at the same temperature. After reflux the reaction mixture was cooled and the solid material was separated by filtration. The solid was washed with dil HCl (50 %, 25 mL) and cold water (3 × 25 mL) and dried. The resultant compounds (**IV 1-7**) were recrystallized by using ethanol as solvent.

4-(4-Fluorophenyl)-3-[(naphthalen-6-yloxy) methyl]-4H-1,2,4-triazole-5-thiol (IV 1): Yield 58 %, m.p. 116-117 °C; TLC solvent chloroform: ethanol (8:2), R_f value: 0.62. IR (KBr, ν_{max}, cm^{-1}): 3030.10 (aromatic-H), 2659.50 (SH), 1635.10 (C=N) 1145.36 (C-O-C). ^{1}H NMR δ (ppm): 3.328 (1H, -SH), 4.766 (2H-CH_2), 7.188-7.896 (11H, -Ar-H), Mass *m/z* 351.1.

4-(5-Mercapto-3-[(naphthalene-2-yloxymethyl)-4H-1,2,4-triazol-4-yl]-N-(5-methyl-1,3,4-oxadiazol-2-yl) benzenes sulfonamide (IV-2): Yield 62 %, m.p. 169-170 °C; TLC solvent chloroform: ethanol (8:2), R_f value: 0.53. IR (KBr, ν_{max}, cm^{-1}): 3333.42 (NH), 2837.26 (aliphatic-H), 3095.30 (aromatic-H), 2633.23 (SH), 1623.33 (C=N) 1110.09 (C-O-C). ^{1}H NMR δ (ppm): 4.765 (1H, -NH, 2° amine), 4.252 (1H, -SH), 6.087-7.877 (11H, -Ar-H), 4.765 (2H-CH_2), 2.289 (3H-CH_3).

6-Choloro-7-[(5-mercapto-3-((naphthalene-2-yloxy)-methyl)-4H-1,2,4-triazol-4-yl)sulphonyl]-3-4-dihydro-2H-benzo[e][thiadiazine-1,1-dioxide (IV-3): Yield 61 %, m.p. 220-221 °C; TLC solvent chloroform: ethanol (8:2), R_f value: 0.61. IR (KBr, ν_{max}, cm^{-1}): 3314.66 (NH), 2604.84 (SH), 3098.67 (aromatic-H) 1600.11 (C=N) 1122.27 (C-O-C). ^{1}H NMR δ (ppm): 4.289 (1H, -NH, 2° amine), 4.73 (1H, -NH, 2° amine) 3.325 (1H, -SH), 7.266-7.994 (9H, -Ar-H), 5.33 (2H-CH_2 aliphatic ring). 6.97 (2H-CH_2), Mass *m/z* 538.8.

N-(5-Mercapto-3-[(naphthalen-6-yloxy) methyl]-4H-1,2,4-triazol-4-yl)isonicotinamide (IV-4): Yield 60 %, m.p. 190-191 °C; TLC solvent chloroform: ethanol (8:2), R_f value: 0.54. IR (KBr, ν_{max}, cm^{-1}): 3305.94 (NH), 1667.50 (C=O), 2619.30 (SH), 3118.29 (aromatic-H) 1624.37 (C=N) 1118.54 (C-O-C). ^{1}H NMR δ (ppm): 10.08 (1H, -NH, 2° amine), 4.748 (2H-CH_2), 3.321 (1H, -SH), 7.721-8.713 (11H, -Ar-H),

4-(5-Mercapto-3-[(naphthalen-6-yloxy)methyl]-4H-1,2,4-triazol-4-yl)benzoic acid (IV-5): Yield 70 %, m.p. 171-172 °C; TLC solvent chloroform: ethanol (8:2), R_f value: 0.53. IR (KBr, ν_{max}, cm^{-1}): 3458.66 (OH), 1661.47 (C=O), 2659.99. (SH), 3030.10 (aromatic-H), 1604.10 (C=N) 1122.34 (C-O-C). ^{1}H NMR δ (ppm): 3.323 (1H, -SH), 4.765 (2H-CH_2), 7.242-7.877-(11H, -Ar-H), 10.298 (1H-OH).Mass *m/z* 391.

4-(Naphthalen-1-yl)-3-[(naphthalen-6-yloxy) methy])-4H-1,2,4-triazole-5-thiol (IV-6): Yield 68 %, m.p. 123-125 °C; TLC solvent chloroform: ethanol (8:2), R_f value: 0.56. IR (KBr, ν_{max}, cm^{-1}): 2933.82 (aliphatic-H), 3057.44 (aromatic-H) 2637.26 (SH), 1598.40 (C=N), 1112.24 (C-O-C). ^{1}H NMR δ (ppm): 4.781 (2H-CH_2), 3.312(1H, -SH), 6.778-8.069 (14H, -Ar-H), Mass *m/z* 383.1.

N-(N,N-dimethylcarbamimidoyl)-5-mercapto-3-[(naphthalene-2-yloxy)methyl]-4H-1,2,4-triazole-carboxaimidamide. (IV-7): Yield 61 %, m.p. 129-130 °C; TLC solvent chloroform:ethanol (8:2), R_f value: 0.60. IR (KBr, ν_{max}, cm^{-1}): 3320.84 (NH), 2904.84 (aliphatic-H), 3098.67 (aromatic-H). ^{1}H NMR δ (ppm): 5.513 (1H, -NH, 2° amine), 4.765 (2H, -NH, 2° amine), 3.347 (1H, -SH), 6.756 (2H-CH_2), 7.210-7.872 (7H, -Ar-H), 2.929 (6H-CH_3). Mass *m/z* 367.1.

Free radical scavenging activity: The percentage of antioxidant activity of each substance was assessed by DPPH free radical assay. The measurement of the DPPH radical scavenging activity was performed according to the methodology described by Braca *et al.* [16]. One milliliter of the sample (0.75, 1.5 and 3 μg/mL) was added to 3 mL of 0.1 mmol methanol solution of DPPH. When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The changes in colour (from deep violet to light yellow) were read at 517 nm was determined after 0.5 h of reaction using a UV-visible spectrophotometer and the percent inhibition activity was calculated.

Molecular docking studies [17]: In the present study, the X-ray crystal structure of the antifungal agent fluconazole bound to sterol 14α-demethylase (CYP51) from trypanosome cruzi obtained from a RCSB protein data bank (PDB ID: 3KHM). Resolution of protein structure with 464 amino acid residues was 2.85 Å. The protein was further processed by removing water and fluconazole. Docking simulation studies were carried out in Autodock vina. Polar and aromatic hydrogen's and gasteiger charges were added in the protein using MGLtools1.5.4 and the pdb file was subsequently

converted to pdbqt format. Pre-optimized compounds were also pre-processed similarly and converted to pdbqt format. All the torsion angles in the small-molecules were set free so as to perform flexible docking. Grid box of size 22 × 22 × 18 with 1 Å spacing was defined along x, y and z axis. The defined grid box was large enough to cover an active site of the protein. The analysis of binding free energy and interactions of ligands with residues at an active site was carried out by using Pymol and Discovery studio 3.5.

Antifungal activity

Standard nutrient agar medium: Meat extract was taken and made up the volume to 100 mL with water and to this were added weighed quantities of peptone, salt and agar. The contents were dissolved by heating and the mixture was filtered and the pH was adjusted to 7.5. The medium was sterilized by autoclaving at 121 °C for 15 min, cooled to 45 °C and then poured in 20 mL quantities to petri dishes. A loopful of an overnight broth culture was spread evenly over the whole part with a sterile cotton-wool swab [18].

The culture plates were dried in the incubator with the lid until its surface was free from visible moisture without further delay; the known concentration of the drug applied with adequate spacing to the surface of the culture plates with sterile fine pointed forceps and pressed gently to ensure full contact with the medium. It was then transferred to the incubator for 24 h at 37 °C. At the end of 24 h, the diameters of the zone of inhibition produced were measured.

RESULTS AND DISCUSSION

For the synthesis of the target compounds, the reaction sequences are outlined in **Scheme-I**. Herein, the aryloxy acetic acid was prepared by reacting β-naphthol with sodium hydroxide followed by addition of chloro acetic acid. At first the powdered β-naphthol treated with sodium hydroxide. In this exothermic reaction the solution mixture immediately converted to the sodium salt of β-naphthol, which followed by addition of freshly prepared chloro acetic acid produces aryloxy acetic acid. Further the prepared aryloxy acetic acid was treated with methanol and a few drops of H_2SO_4 to get the corresponding ester **(I)**, without separating the corresponding ester, it was further reacted with hydrazine hydrate to get the aryloxy acid hydrazides (**II**). And then aryloxy acid hydrazides were treated with carbon disulphide and alcoholic KOH to get the potassium salt dithiocarbazinates (**III**). The diverse 3,4-disubstituted 1,2,4-triazole (**IV 1-7**) were obtained by the ring closure reaction, between the potassium salt dithiocarbazinates and different drug moieties containing free amino group in their lead structure.

A series of 3,4-disubstituted 5-mercapto-1,2,4-triazoles were synthesized and their antifungal activities were screened for human pathogenic fungi. The compounds **IV-6**, **IV-1** and **IV-2** showed maximum antifungal activity against *A. niger* and *C. albicans* organism when compared the standard fluconazole (Table-1). The obtained results indicated that for antifungal activity of these novel triazole derivatives it is very helpful to introduce the aryl group to interact with a hydrophobic pocket and also generates π-π stacking interaction with TYR A103 by traizole ring (Fig. 1, Table-2). For antioxidant activity, all the compounds shows moderate activity but compound **IV-6** and **IV-7** showed significant activity when compared to standard ascorbic acid (Table-3).

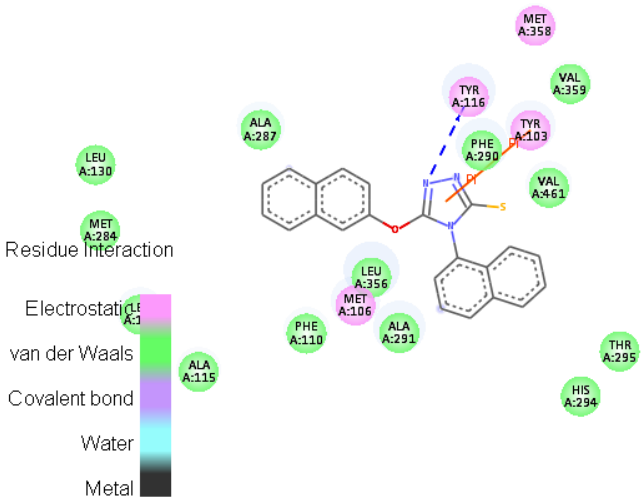
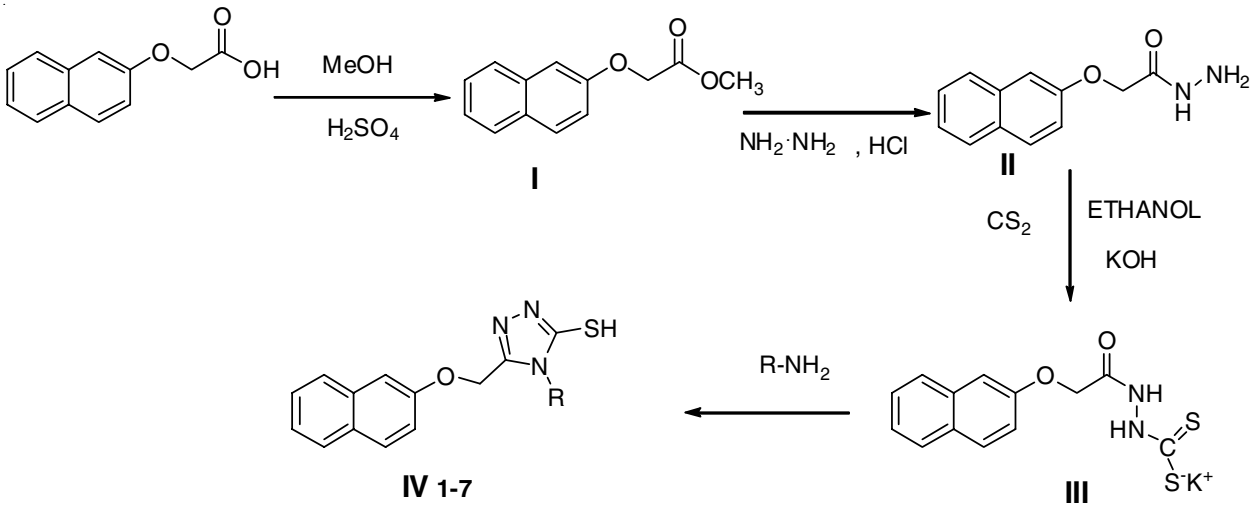
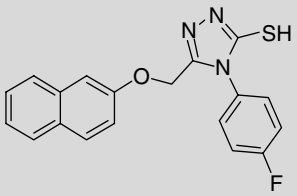
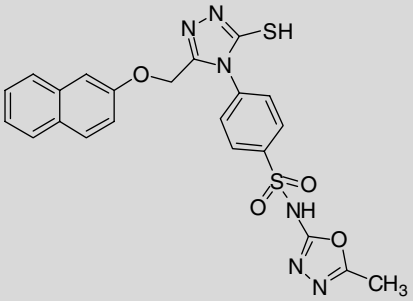
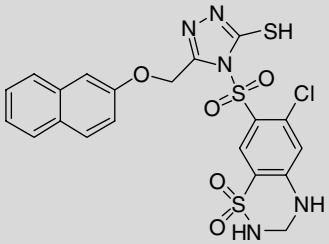
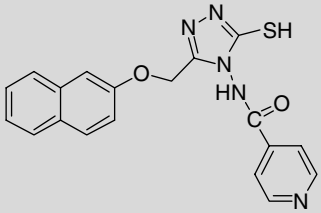
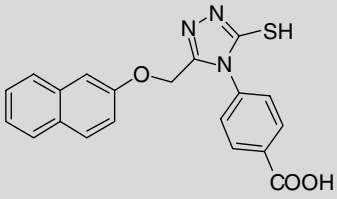
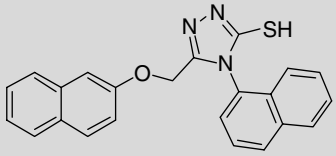
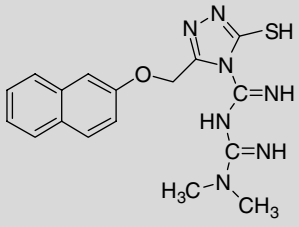


Fig. 1. Hydrophobic interaction (dotted lines) of Compound **IV-6** at the binding pocket of 14α-demethylase (CYP51) domain



Scheme-I: Synthesis of 3,4-disubstituted 1,2,4-triazole (**IV 1-7**)

TABLE-1
in vitro ANTIFUNGAL ACTIVITY OF SYNTHESIZED COMPOUNDS

Compound	Structure	*Inhibition zone diameter (mm)			
		A. nigier		*C. albicans*	
		50 µg	100 µg	50 µg	100 µg
IV-1		7 ± 0.03	11 ± 0.01	7 ± 0.05	12 ± 0.06
IV-2		7 ± 0.04	12 ± 0.03	8 ± 0.02	12 ± 0.04
IV-3		6 ± 0.01	10 ± 0.06	7 ± 0.02	10 ± 0.05
IV-4		7 ± 0.04	11 ± 0.03	8 ± 0.02	9 ± 0.04
IV-5		4 ± 0.01	7 ± 0.06	–	05 ± 0.05
IV-6		10 ± 0.02	17 ± 0.04	10 ± 0.05	18 ± 0.03
IV-7		–	4 ± 0.03	6 ± 0.02	9 ± 0.04
Fluconazole		9 ± 0.04	14 ± 0.03	8 ± 0.01	13 ± 0.01
DMF		–	–	–	–

– = denotes no activity

TABLE-2
PROTEIN LIGAND INTERACTIONS OF
3,4-DISUBSTITUTED 5-MERCAPTO-1,2,4-TRIAZOLES

Compound	Dock score
IV-1	-10.1
IV-2	-10.3
IV-3	-10.6
IV-4	-10.1
IV-5	-10.9
IV-6	-11.3
IV-7	-8.8
Fluconazole	-8.0

TABLE-3
DPPH RADICAL SCAVENGING EFFECT AND
% INHIBITION OF SYNTHESIZED COMPOUNDS

Compounds	Conc. (µg/mL)	Sample Absorbance	Control Absorbance	Scavenging effect (%)
IV-1	3.00	0.2925	1.1332	74.18
	1.50	0.3663	1.1332	67.67
	0.75	0.4784	1.1332	50.99
IV-2	3.00	0.2802	0.5247	46.59
	1.50	0.3963	0.5247	24.47
	0.75	0.4403	0.5247	16.08
IV-3	3.00	0.2336	0.5247	55.47
	1.50	0.2746	0.5247	47.66
	0.75	0.3792	0.5247	27.73
IV-4	3.00	0.2529	0.5247	51.80
	1.50	0.2620	0.5247	50.06
	0.75	0.2979	0.5247	43.22
IV-5	3.00	0.2131	0.6197	65.61
	1.50	0.2204	0.6197	64.43
	0.75	0.3104	0.6197	49.91
IV-6	3.00	0.1640	0.6197	73.53
	1.50	0.2221	0.6197	64.16
	0.75	0.3422	0.6197	44.77
IV-7	3.00	0.0746	0.6197	87.96
	1.50	0.1979	0.6197	74.88
	0.75	0.3104	0.6197	49.91
Ascorbic acid	3.00	0.0520	1.1332	95.41
	1.50	0.1692	1.1332	85.06
	0.75	0.3728	1.1332	67.10

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