Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2021, 13(4):01-07

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Synthesis and Evaluation of Antifungal and Anti-Oxidant Activities of Novel 1, 2, 4-Triazole Derivatives

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ABSTRACT

The key intermediate 4-amino-3-mercapto-1, 2, 4-triazoles (1) is prepared by reacting with methyl paraben carbohydrazide and CS2 to yield potassium dithiocarbazinate salt. The salt upon reaction with hydrazine hydrate will yield the compound(1).The title compounds 4-(6-phenyl-[1,2,4] triazole [3,4-b] [1,3,4] thiadiazole-3-yl)-phenol derivatives were prepared by reacting 4-amino-3-mercapto-1,2,4-triazole(1) with aromatic acids in the presence of phosphorous oxychloride medium. All the new compounds were evaluated for in-vitro antioxidant and antifungal activities. The new compounds were assigned on the basis of IR, NMR and mass spectral data.

Keywords: Ascomycota; Methyl paraben; Potassium dithiocarbazinate salt; Phosphorous oxychloride; Antioxidant activity; Antifungal activity

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INTRODUCTION

Nowadays research has been initiated on nitrogen containing heterocyclic compounds due to their synthetic and effective biological importance. The five membered heterocyclic compounds which includes three nitrogen as a hetero atoms is acquiring more interest in the present field of research and synthesis of new compounds. In the present work 1, 2, 4-triazole which is a five membered nitrogen containing heterocyclic compound has been taken for research work.

1,2,4-triazole and their fused heterocyclic derivatives represents an interesting class of compounds owing to their varied synthetic as well as biological activities.1,2,4-triazole showed wide range of biological activities such as antibacterial [1], antifungal [2], anti-inflammatory [3], anticancer [4], antiviral [5], antitubercular [6,7], anticonvulsant [8] etc. In view of all these facts the aim of present study was to synthesize 1, 2, 4-triazole derivatives with the hope that they may possess potent antioxidant and antifungal activities.

The increase in the concentration of reactive oxygen species can lead to oxidative stress which contributes to many diseases such as hypertension, neurological disorder, atherosclerosis, cancer, diabetes, quickening of age process etc. So the antioxidants are the agents which counteract these problems by binding with free radicals. The relative importance of antioxidants in oxidative stress related problem is a current area of research and development. So in

the present study various in-vitro methods are being used for the confirmation of antioxidant activity of synthesized compounds.

Antifungal agents are the drugs that are used to kill or prevent the growth of fungi or mycoses such as candidiasis, tinea and serious infections like cryptococcal meningitis etc. Unlike bacteria, both fungi and human are eukaryotes, have similar biological level that makes difficult to discover drug for fungal infection without affecting human cell, so because of this many antifungal drugs cause side effects. In order to overcome this there is a demand for the synthesis of new compounds with less side effects and potent antifungal activity [9].

MATERIALS AND METHODS

All the reagents and the solvents that are used in the reactions such as methyl paraben, carbon disulphide, hydrazine hydrate were purchase from Loba Chemie Pvt. Ltd mumbai and were used without further purifications. The melting point of the synthesized compound were recorded by open capillary tube method.IR spectra were recorded on Bruker spectrum using a thin film on KBr pellet technique and frequencies are expressed in cm-1. The H1NMR spectra were recorded on Bruker Avance II at 300 MHZ NMR spectrophotometer. All the spectra were obtained in DMSO solution. Mass Spectrum were recorded on Perkin Elmar Clarus 680 GC-MS spectrometer.

Synthesis of 4-Amino-3-Mercapto-1, 2, 4-Triazole (1)

Potassium dithiocarbazinate salt (0.01 mol) was dissolved in water (5 ml) and hydrazine hydrate (6 ml) added to this solution and refluxed for 6 hrs. The reaction mixture was cooled and poured into crushed ice (100 ml) and acidified with HCl (10%). The precipitated compound was filtered, washed with water and dried [10] (Table 1)**.**

Table 1: Physical data of the compound (1a-j)

Comp	Ar-COOH	Molecular formula	Molecular weight	M.P (ºC)	Yield (%)
1a	4-NH_2	C15H11N5OS	309	222-224	69
1b	CH=CH	C17H11N4OS	319	202-204	67
1c	2,4-$(Cl)^2$	C15H8Cl2N4OS	363	196-198	65
1d	5-Cl-2-OH	C16H9ClN5O2S	344	218-220	71
1e	4-OCH_3	C16H12N4O2S	324	227-229	75
1f	2,4-$(OH)^2$	C16H11N3O3S	325	186-188	73
1g	2-Br	C16H9BrN5O2S	373	247-249	79
1h	3,5-(NO_2)2-2-OH	C15H8N6O6S	400	234-236	74
1i	3-Cl	C15H9ClN4OS	328	258-260	71
1j	2-Cl-4-NO_2	C15H8ClN5O3S	373	270-272	83

Synthesis of 4-(6-Phenyl-[1, 2, 4] Triazole [3, 4-b] [1, 3, 4] Thiadiazole-3-yl)-Phenol Derivatives (1a-j)

4-amino-3-mercapto-1, 2, 4-triazole (0.01 mol) and aromatic acids (0.01 mol) was dissolved in phosphorous oxychloride (6-7 ml) and refluxed for 15 hrs. The reaction mixture was cooled to room temperature and poured into crushed ice (100 ml) and basified with sodium bicarbonate (10%). The precipitated compound was filtered washed with water and dried [11]. The physical data of the compounds (1a-j) is given in the Table 1 (Scheme 1). The

synthesized compounds (1a-j) were evaluated for in-vitro antioxidant activity using Nitric oxide inhibition method as well as by using DPPH radical scavenging method and these compounds (1a-j) were also evaluated for in-vitro antifungal activity using cup plate method. The physical data of the synthesized compounds (1a-j) are given in the (Tables 2 and 3).

Table 2: Data of Nitric oxide inhibition of the compounds (1a-j)

Percentage inhibition											
Conc (µg/ml)	**Std**	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**
10	56.74	7.11	14.69	15.64	11.37	22.75	8.53	0.47	4.74	3.79	15.64
20	98.72	15.64	27.49	23.7	12.32	35.07	9.48	5.69	5.69	11.37	21.33
30	99.43	20.85	32.23	30.81	14.22	36.97	10.43	9.00	8.06	17.54	22.27
40	99.57	32.23	45.5	31.75	30.33	39.81	32.7	13.74	15.17	21.8	27.01
50	99.86	47.73	45.97	32.23	58.77	42.81	44.55	56.87	33.65	36.02	55.45
IC50	26.92	42.74	40.88	73.02	38.36	61.86	44.95	46.23	75.59	64.09	80.13

Table 3: Data of DPPH radical scavenging activity of the compounds (1a-j)

Percentage inhibition											
Conc(µg/ml)	**Std**	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**1j**
10	65.2	0.43	17	28.14	36.43	32.57	30	21.57	-0.14	23.3	2.14
20	69.71	1.86	17.86	30	37.57	33.71	33.71	24.14	1.71	28.2	5.71
30	77.1	16.57	23.86	30.86	37.57	34.57	34.86	24.86	10.86	33.3	16.7
40	82.5	36.86	23.86	35.14	37.86	37	35.57	26.14	28.86	38.3	30.33
50	84.4	94.26	92.59	94.94	93.83	93.79	93.67	93.16	48.71	45.26	48.7
IC50	6.22	28.98	29.52	24.45	21.17	22.92	23.43	28.28	45.62	50.22	17.65

2-(3-(4-Hydroxyphenyl)-[1, 2, 4] Triazole [3,4-b][1, 3, 4] Thiadiazol-6-yl)-4,6-Dinitrophenol

1h: IR (KBr) (Vmax cm^{-1}): 3080 (C-H), C=N (1607), OH (3338) C=C (1519).

^1H NMR (MHz, $CDCl_3$) 6.86-8.87 (m, Ar-H, 6H), 5.35 (Ar-2 x OH); MS m/z: 390 [M^+]

4-(6-(2-Chloro-4 Nitrophenyl)-[1, 2, 4] Triazole [3, 4-b][1, 3, 4] Thiadiazol-3-yl)Phenol

Ij : IR (KBr) (Vmax cm^{-1}): 1605 (C=N), 2916 (C-H),3338 (OH), 1388 1520 (NO_2).

^1H NMR (MHz, $CDCl_3$) 6.86-8.41 (m, Ar-H, 7H) 5.35 (s, Ar-OH); MS m/z : 363[M^+]

Antioxidant Activity

Nitric oxide inhibition method: Nitric oxide generated from sodium nitropruside in aqueous solution at physiological pH interact with oxygen to produce nitrite ions, which were measured by the method of Garret. Sodium nitropruside (1 ml of 10 mM) is mixed with 1 ml of 1,2,4-triazole derivatives at different concentrations(10-50 μg/ml) in phosphate buffer(pH-7.4). The mixture is incubated at 25ºC for 150 minutes. After incubation, 1 ml of the incubated solution containing nitrate was pipetted out and mixed with 1 ml of Griess's reagent (1% sulphanilamide, 2% O-phosphoric acid and 0.1% Naphthyl ethylene diamine dihydrochloride). The reaction was allowed to stand for 30 minutes. Ascorbic acid is used as a standard drug for comparison purpose. Absorbance is read at 546 nm [12].

DPPH (2,2-diphenyl-1-picryle-hydrazyle) free radical scavenging activity: 0.2 Mm solution of DPPH in methanol was prepared and 100 μl of this solution was added to 1,2,4-triazole derivatives (10-50 μg/ml). The mixture was shaken vigorously and left to stand for 30 minutes and absorbance was measured at 517 nm. Ascorbic acid was used as reference standard and all the tests were performed in triplicate. The percentage of inhibition was calculated by comparing the absorbance value of control and test sample [13] (Table 4).

Table 4: Data of antifungal activity of the compounds (1a-j)

Percentage inhibition		
Compounds	***Aspergillus niger***	***Aspergillus fumigatus***
Std (Bavistin)	99.87 ± 0.02	99.87 ± 0.02
1a	65.49 ± 0.086	46.68 ± 0.061
1b	74.44 ± 0.139	55.51 ± 0.063
1c	56.74 ± 0.138	57.74 ± 0.071
1d	86.53 ± 0.075	52.26 ± 0.103
1e	52.11 ± 0.066	58.54 ± 0.058
1f	72.38 ± 0.085	43.41 ± 0.116
1g	61.21 ± 0.061	60.34 ± 4.066
1h	44.42 ± 0.113	52.24 ± 0.087
1i	68.79 ± 0.151	40.25 ± 0.15
1j	70.77 ± 0.384	54.52 ± 0.097

Antifungal Activity

The antifungal activity of the compounds were evaluated by using cup plate method against fungal organisms such as *Aspergillus niger* and *Aspergillus fumigatus***.** In this method cups were filled with test solution in petridish which was inoculated with the organisms. After the incubation the plates were observed for diameter of zone of inhibition was calculated which is directly proportional to sensitivity of the compounds against organism and compared with the standard drug bavistin [14].

RESULTS AND DISCUSSION

The main aim of this work was to synthesize substituted 1,2,4-triazoles and the intermediate compound which was performed by using reagents like methyl paraben carbohydrazide, carbon disulphide were reported and illustrated in the Scheme-01. The title compounds were also synthesized by using phosphorous oxychloride, 4-amino-3-mercapto-1,2,4-triazoles with corresponding aromatic acids as per the reported procedure. All the compounds were in conformity with the structural envisaged. The structures were proved on the basis of spectral data. All the synthesized compounds absorption band at 1550 cm-1, 1600 cm-1 indicated the presence of C=N, C=C in the molecules respectively. The 1H-NMR spectra of the compounds 1a-j, showed the appearance of multiplet in the region δ 6.86 to 8.87 integrating for the presence of aromatic protons. A sharp singlet is observed at δ 5.35 indicated the presence of OH group. In the mass spectrum of the compounds (1a-j) molecular ion peak observed at 365 m/z consistent with the molecular formula CHNO (Figure 1).

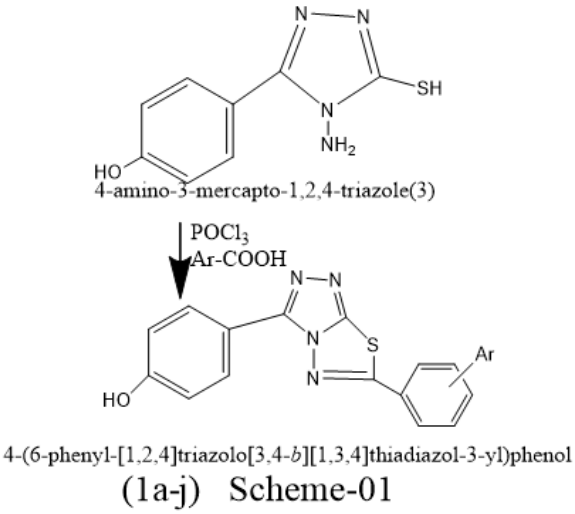

Figure 1: Scheme-01(1a-j)

Some of the tested compounds 1c, 1e, and 1h showed good activity in antioxidant and some of the tested compounds 1d, 1h also displayed moderate antifungal activity.

The synthesized 1, 2, 4-triazoles derivatives were evaluated for in-vitro antioxidant activity using DPPH radical scavenging method as well as Nitric oxide inhibition method. The synthesized compounds exhibited good antioxidant activity. The synthesized 1, 2, 4-triazole derivatives also evaluated for *in vitro* antifungal activities using two fungal strains by cup plate method. All the synthesized compounds were exhibited wide range of moderate antifungal activities.

CONCLUSION

The chemistry of 1, 2, 4-triazoles and their fused heterocyclic derivatives have wide range of synthetic as well as biological activities, so the present work require further structural modification to get better pharmacological activities.

The synthesis of 1, 2, 4-triazole derivatives by prescribed method resulted in the product with good yields. All the newly synthesized compounds were characterized spectral data by IR, ^{1}H-NMR and mass spectra.

ACKNOWLEDGEMENT

The author would like to thank NGSM institute of Pharmaceutical Sciences, Nitte Deemed to be university, Mangalore, Karnataka for providing all the necessary facilities for the work.

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