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Research Article

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In vitro antioxidant potential study of triazole and thiadiazole analogues

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ABSTRACT

*The synthesis of 3-(2-aroylaryloxy)methyl-5-mercapto-4-phenyl-4H-1,2,4-triazole analogues (**6a**-e) and 2-(2-aroylaryloxy)methyl-5-N-phenylamino-1,3,4-thiadiazole (**7a-e**) were reported earlier. The antioxidant properties of these compounds **6a-e** and **7a-e** were evaluated by DPPH, nitric oxide and hydrogen peroxide radical scavenging methods. The investigation of antioxidant screening revealed that some of the tested compounds showed good to moderate antioxidant activity. Interestingly, the results show that the compounds containing electron releasing groups like methyl and methoxy exhibit good activities. In the above set of compounds **7e**, **6e** and **6d** have shown very good antioxidant activity.*

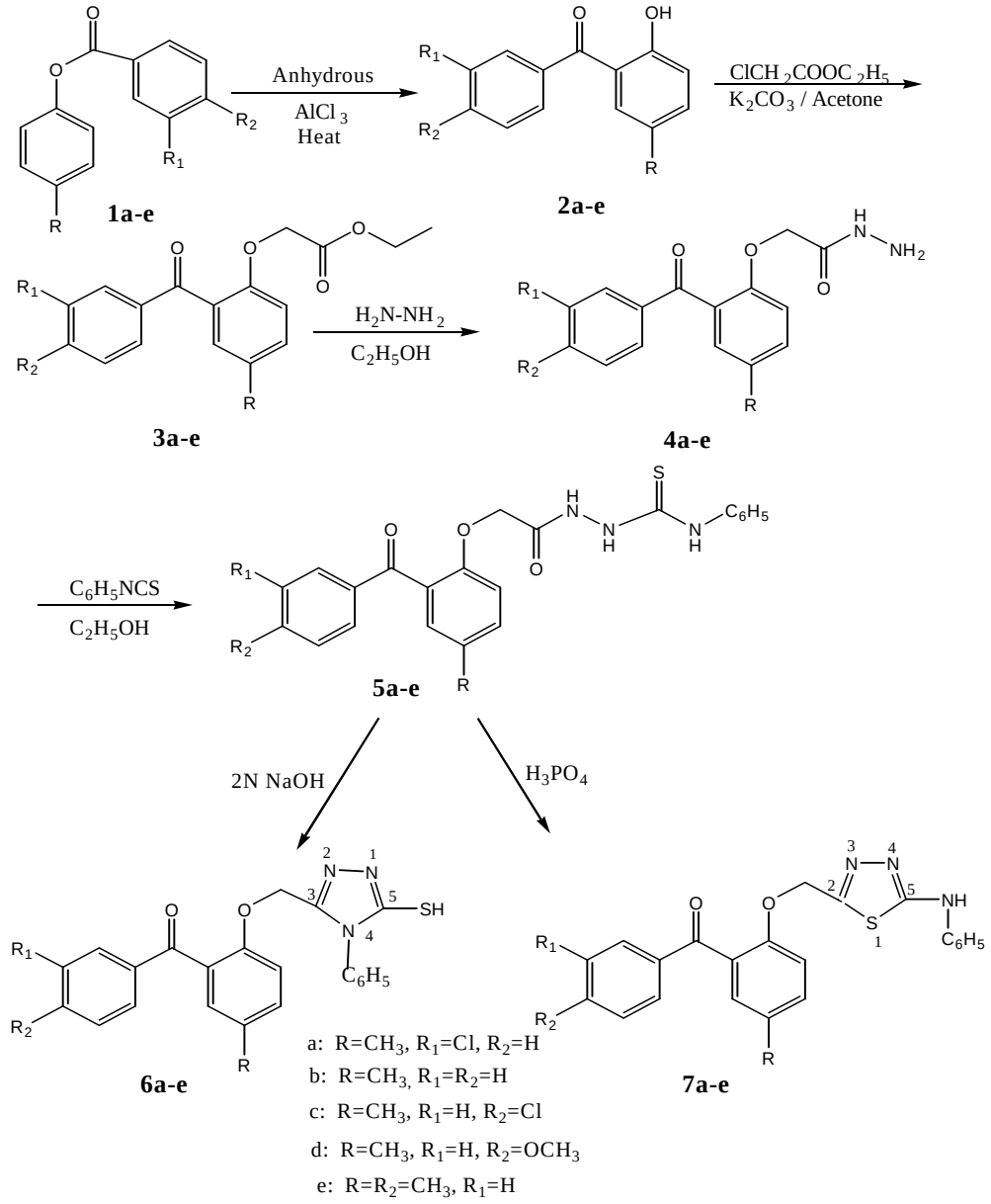
Keywords: Triazole, thiadiazole analogues, antioxidant activity.

INTRODUCTION

The imbalance between reactive oxygen species production and antioxidant defense leads to oxidative stress and impairs cell functions, immunity, promotes cell death and DNA damage, which can cause mutations and ultimately contribute towards the development of chronic diseases such as cancer [1,2**]**. Any compound, natural or synthetic with antioxidant properties might contribute towards the partial or total alleviation of this type of damage. The harmful effect of reactive oxygen species is neutralized by a broad class of protective agents termed antioxidants, which prevent oxidative damage by reacting with free radicals before any other molecules can become a target. Antioxidants are probably now regarded as the new generation superheroes to maintain the health [3]. There are enormous evidences revealing that enrichment of our body systems with antioxidant agents may correct the vitiated homeostasis and can prevent the onset as well as treat diseases caused and/or fostered due to free-radical mediated oxidative stress. Free radicals can damage tissues and have been implicated in the pathology of many diseases, such as cardiovascular diseases, cancer, neurodegenerative diseases, diabetes and ageing [4,5]. In recent years there is an increase in the areas related to newer developments in prevention of diseases specially the role of free radicals and antioxidants. So it will be pertinent to examine the possible role of 'free radicals' in disease and how 'antioxidants' play an important role in their prevention, especially the current status of the subject matter and future prospects. Recently, the search for antioxidant principles that lead to the identification of natural resources, isolation of active principles and further modification and refinement of active antioxidant molecules is being accelerated [6]. Additionally, there is an increased interest in the application of antioxidants to medical treatment as information is constantly gathered linking the development of human diseases to oxidative stress [7].

1,2,4-triazoles, 1,3,4-thiadiazole and their derivatives represent one of the most biologically active classes of compounds, which possess a wide spectrum of activities, their nucleus being associated with diverse pharmacological activities such as antibacterial [8], antifungal [9], anti-inflammatory [10], analgesic [11], antiviral [12], anti tumor [13], antitubercular [14], and anticonvulsant activities [15]. These biological activities prompted us to evaluate antioxidant activity of synthesized 1,2,4-triazoles and 1,3,4-thiadiazole analogues [16] by three methods: Scavenging of DPPH, nitric oxide and hydrogen peroxide radical activity.

EXPERIMENTAL SECTION



Scheme 1

All solvents and reagents were purchased from Sigma Aldrich Chemicals Pvt Ltd. Melting points were determined on an electrically heated VMP-III melting point apparatus. The FT-IR spectra were recorded using KBr discs and Nujol on FT-IR Jasco 4100 infrared spectrophotometer. ^{1}H NMR spectra were recorded using Bruker DRX 400 spectrometer at 400 MHz with TMS as an internal standard. Mass spectra were recorded on LC-MS/MS (API-4000)

mass spectrometer. Further elemental analysis of the compounds was performed on a Perkin Elmer 2400 elemental analyzer.

Experimental/Methodology

Chemistry

The strategy employed in the synthesis of desired compounds is as follows (scheme 1). Substituted phenyl benzoates (**1a-e**) on Fries rearrangement using anhydrous aluminum chloride gave corresponding substituted 2-hydroxybenzophenones (**2a-e**) in excellent yield [17]. Condensation of **2a-e** with ethyl chloroacetate in the presence of anhydrous potassium carbonate in dry acetone gave ethyl (2-aroylaryloxy)acetates (**3a-e**), which on treatment with 80% hydrazine hydrate in ethanol yielded the respective 2-(2 aroylaryloxy)acetohydrazides (**4a-e**). The compounds (**4a-e)** with phenyl isothiocyanate in dry ethanol gave 2-[2-(aroylaryloxy)acetyl]-N-phenylhydrazinecarbothioamides (**5a-e**), which on cyclization with 2N sodium hydroxide and anhydrous orthophosphoric acid gave 3-(2 aroylaryloxy)methyl-5-mercapto-4-phenyl-4*H*-1,2,4-triazoles (6a-e) and 2-(2-aroylaryloxy)methyl-5-N-phenylamino-1,3,4-thiadiazoles (**7a-e**) analogues respectively in excellent yield [16].

Biological assay

In vitro Antioxidant activity

DPPH free radical scavenging activity:

The free radical scavenging activities of synthesizes compounds were studied in *vitro* by their ability to bleach the stable radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) [18]. This assay has often been used to estimate the antiradical activity of antioxidants. Because of its odd electra, DPPH gives a strong absorption band at 517 nm in visible spectroscopy. Stock solution of the drug was diluted to different concentrations in the range of **25**, **50**, **75**, **100** µM in methanol. Methanol solution of the synthesized compounds (2ml) was added to methanol solution of DPPH (1ml). The samples were kept in the dark for 30 Min after which the optical density was measured at 517 nm and the percentage of scavenging activity was calculated. Ascorbic acid was used as standard.

Nitric Oxide (NO) Scavenging Activity:

The nitric oxide scavenging activity of the synthesized compounds was determined according to the method of Green *et al* [19]. Sodium nitroprusside (5 mM) in phosphate buffer pH 7.4 was incubated with 100 mM concentration of test compounds dissolved in a suitable solvent (dioxane/methanol) and tubes were incubated at 25 ^{o}C for 120 min. Control experiment was conducted with equal amount of solvent in an identical manner. At intervals, 0.5 ml of incubation solution was taken and diluted with 0.5 ml of Griess reagent (1% Sulfanilamide, 0.1% N-naphthylethylenediamine dihydrochloride and 2% *o*-phosphoric acid dissolved in distilled water). The absorbance of the chromophore formed during diazotization of nitrite with sulfanilamide and subsequent N-naphthylethylenediamine dihydrochloride was measured at 546 nm.

Hydrogen peroxide scavenging activity:

The H_2O_2 scavenging ability of the synthesized compounds was determined spectrophotometrically according to the method of Ruch. *et al* [20]. Briefly, a solution of hydrogen peroxide (2 mM) was prepared in 0.17 M phosphate buffer (pH 7.4). Various concentrations of the samples (in methanol) were added to the reaction mixture containing 2 mM hydrogen peroxide. After 10 min incubation at room temperature, the absorbance was measured against a blank at 230 nm.

The inhibition ratio (I %) of all the tested compounds was calculated according to the following equation: I % = (Ac-As) / Ac × 100, where Ac is the absorbance of the control and As is the absorbance of the sample. The radical scavenging activity was expressed as IC_{50} which was determined from the plot of percentage inhibition against concentration (µM/ml). All the tests and analyses were done in triplicate and the results were averaged.

RESULTS AND DISCUSSION

Antioxidant activity

All the compounds were screened for in vitro antioxidant activity by various scavenging methods like DPPH, nitric oxide and hydrogen peroxide radical activity. In vitro antioxidant activating of synthesized compounds are summarized in Tables (1, 2 & 3).

Table 1. In-vitro antioxidant activity of compounds 6a-e and 7a-e in DPPH method

Compound	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	56.25±1.84	62.39±1.09	65.64± 0.98	71.67± 1.69	22.31± 1.38
6b	69.99±1.07	72.58± 0.88	77.32± 1.33	82.48±1.06	19.15± 1.08
6c	65.34±1.45	69.33±1.22	74.62± 1.57	78.25±0.96	19.89± 1.14
6d	74.24± 0.20	78.12± 0.46	79.98± 0.47	84.53± 0.71	17.31± 0.61
6e	73.62± 0.37	76.57±0.61	79.46± 0.71	84.53± 0.87	17.99± 0.53
7a	60.41±1.18	65.76± 1.25	69.52±0.89	73.64± 0.96	21.36± 0.77
7b	69.11± 1.27	73.64± 1.42	75.44± 1.57	80.36±1.05	19.52± 1.11
7c	65.31±1.06	70.24± 1.41	74.22± 0.83	78.25± 1.64	20.23±1.08
7d	71.42±0.27	77.17±0.44	78.32± 0.66	82.58± 0.71	18.13± 0.7
7e	77.36± 0.29	80.31± 0.50	84.16± 0.62	86.35± 0.79	17.24± 0.29
Ascorbic acid	81.98±0.11	82.99±0.37	84.88±0.43	86.79±0.52	14.98±0.43
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ±SD.

Table 2. In- vitro antioxidant activity of compounds 6a-e and 7a-e in nitric oxide (NO) method.

Compound	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	61.46±1.53	62.25± 1.22	68.94± 1.32	73.63±0.72	20.99 ± 0.95
6b	72.64± 0.89	75.42± 1.08	78.36± 1.42	83.26± 1.28	18.22± 0.96
6c	69.63±0.91	71.84± 1.40	75.74± 0.96	81.73± 1.23	19.99± 1.07
6d	76.48 ± 0.27	80.71 ± 0.38	83.26 ± 0.58	86.65 ± 0.75	16.89 ± 0.57
6e	74.68 ± 0.27	79.38 ± 0.46	83.77 ±0.63	85.41 ± 0.80	17.97 ± 1.0
7a	63.84 ± 1.42	67.35 ± 1.57	71.72 ± 0.79	74.33 ±1.08	20.51± 0.98
7b	73.29 ± 0.86	75.47 ±0.95	79.64 ± 1.07	83.72 ± 1.42	18.63 ± 0.57
7c	64.74 ± 1.33	72.81 ± 1.25	77.82 ±1.05	79.26± 0.80	20.11 ± 1.13
7d	72.52±0.178	77.61 ± 0.36	80.33 ± 0.55	84.95 ±0.70	17.23 ± 1.16
7e	79.48 ± 0.29	84.81 ± 0.48	85.76 ± 0.62	87.44 ± 0.81	16.67 ± 0.78
Ascorbic acid	83.63±0.17	84.65±0.35	87.54±0.51	89. 97±0.68	13.97±0.54
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ±SD.

Table 3. In vitro antioxidant activity of compounds 6a-e and 7a-e in hydrogen peroxide (H_2O_2) method.

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	52.84 ± 1.17	55.31± 0.87	59.60± 0.98	64.31± 0.64	25.44 ± 1.05
6b	62.51± 0.87	64.72± 1.59	69.51± 1.08	72.60 ± 1.38	20.10± 1.24
6c	59.72±1.14	63.79± 1.18	66.66 ± 1.48	70.23± 1.56	21.99± 1.08
6d	64.33± 0.31	66.74± 0.47	69.51±0.66	73.49± 0.79	19.98± 1.27
6e	67.40± 1.13	70.66± 1.32	72.41± 0.61	75.45± 0.72	18.88± 0.61
7a	54.26± 0.90	57.13± 1.40	60.25 ± 0.79	65.64± 1.06	24.79± 0.43
7b	61.57±1.07	64.28± 1.28	68.35± 1.58	71.33± 0.84	21.10± 0.53
7c	69.34± 0.29	72.31 ± 0.46	75.22± 0.62	80.25± 0.83	19.68± 0.96
7d	65.55± 0.28	68.26± 0.65	70.24± 0.70	73.41± 0.81	20.20± 0.27
7e	69.50± 0.29	72.33±0.46	75.26± 0.62	80.72± 0.83	19.05± 0.96
Ascorbic acid	75.96±0.17	77.75±0.32	81.48±0.60	85.62±0.70	15.73±0.25
Blank	-	-	-	-	-

(-) Showed no scavenging activity. Values were the means of three replicates ±SD

The investigation of antioxidant screening revealed that some of the tested compounds showed good to moderate antioxidant activity. Particularly, compounds **7e**, **6d**, **6e**, **7d, 6b** and **7b** have shown the best antioxidant activity with IC_{50} values of 17.24, 17.31, 17.99, 18.13, 19.15 and 19.52 µg/ml respectively**,** and **6c, 7c, 6a** and **7a** showed moderate activity as compared to the standard, ascorbic acid by DPPH. Further nitric oxide activity methods have little different scavenging activity. Mainly, compounds **7e**, **6d**, **7d**, **6e, 6b** and **7b** have shown the best antioxidant activity with IC_{50} values of 16.67, 16.89, 17.23, 17.97, 18.22 and 18.63 µg/ml respectively**,** In hydrogen peroxide IC_{50} values of compounds **6e**, **7e**, **7c** and **6d** have showed good activities. In general, compounds **6e** and **7e** with methyl group and **6d** and **7d** with methoxy group at *para* position of benzoyl ring have shown highest activity compared to other analogues.

The strong antioxidant activity of these compounds indicated their potential application in the drug development, and also provided an effective approach for value-added application of triazole and thidiazole derivatives of benzophenone.

CONCLUSION

The present work describes the antioxidant activity of compounds **6a-e** and **7a-e** by DPPH, nitric oxide and hydrogen peroxide radical scavenging methods. The investigation of antioxidant screening revealed that some of the tested compounds showed good to moderate antioxidant activity. In general, compounds **6e** and **7e** with methyl group and **6d** and **7d** with methoxy group at *para* position of benzoyl ring have shown highest activity compared to other analogues.

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