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Comparative evaluation of antimicrobial and antioxidant efficacy of 7-methyl -2-(phenoxymethyl)-5H-[1,3,4]thiadiazolo [3,2-a] pyrimidin-5-one analogues- An *in-vitro* study

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ABSTRACT

Infectious diseases represent a critical issue for health and the major cause of morbidity and mortality worldwide. The widespread use of antibacterial and antifungal drugs resulted in resistance to drug therapy against bacterial and fungal infections which led to serious health hazards. The resistance of the wide spectrum antibacterial and antifungal agents has initiated discovery and modification of the new antibacterial and antifungal drugs. Besides, the desire of mankind to remain youthful and healthy is answered through antioxidants. The key role played by antioxidants is in achieving optimum health and longevity as free radicals play an important role in the inflammatory process. The much hype about antioxidants among people is attributed to their ability to conflict deadly diseases effectively. Hence efforts are going on continuously to discover more efficient antioxidant drugs to accomplish our health goals. By observing the potentiality of the synthetic compounds towards antimicrobial and antioxidant activities, comparative evaluation of antimicrobial and antioxidant efficacy has been done for the synthesized series of 7-methyl-2-(phenoxymethyl)-5H-[1,3,4]thiadiazolo[3,2-a]pyrimidin-5-one analogues ***6a-t****.*

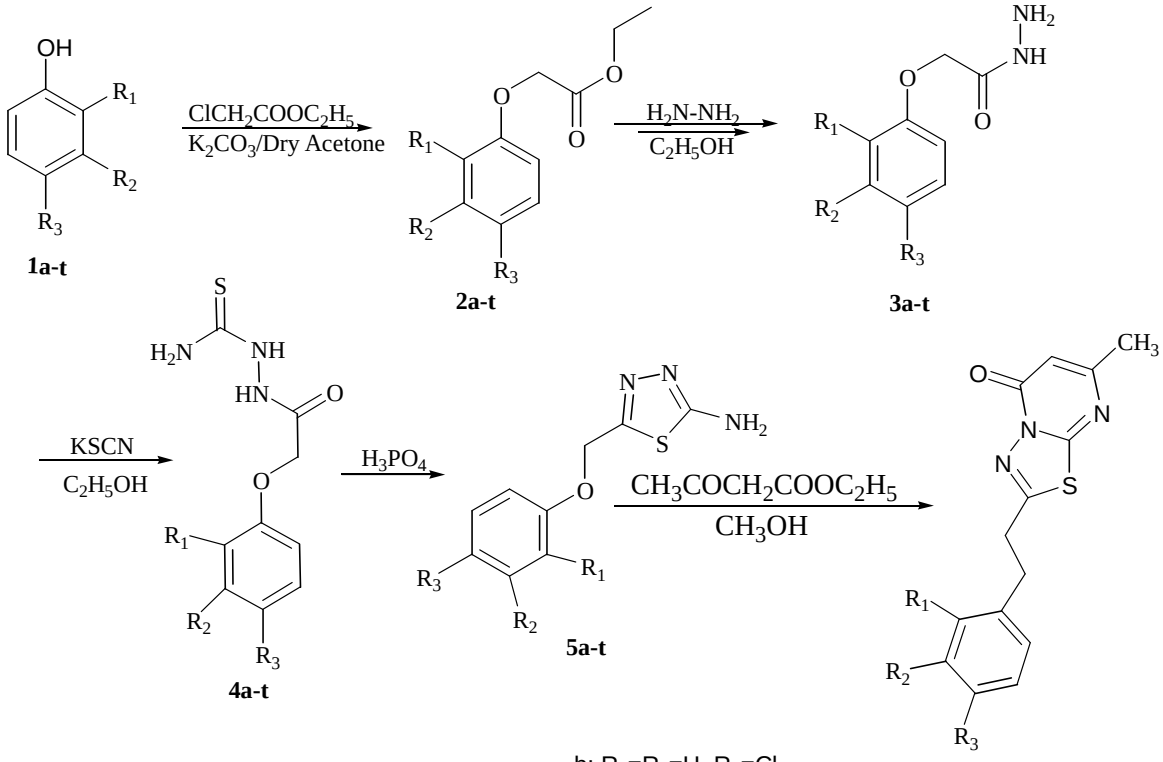
Keywords: pyrimidinone, thiadiazole, antioxidant, antimicrobial.

INTRODUCTION

In the fast growing world, the causes of microbial infections are many. Despite significant progress in human medicine, infectious diseases caused by microorganisms are still a serious threat to public health. The impact is even greater in developing countries due to unavailability of medicine in all the locations, the practice of self-medication and the emergence of microorganism drug resistance. Two possible ways to avoid getting pruned is, either to enhance our immunity or to decrease their resistance towards drugs. We, the chemists prefer the latter. As microorganisms have an ability of genetic modification for drug resistance, especially bacteria [1,2]. Pharmacologists are carrying an unstoppable research for better antibiotics to resist them. Though the development of antimicrobial agents is random, the clinical value is limited as the exposure of toxicity is high [3]. Hence chemists have followed a trend of inclusion of heterocyclic core in the antibiotics. The main aim is to offer efficient drugs for the sufferers by a new approach of synthesizing effective antibiotic.

Oxidative stress, defined as "an imbalance between oxidants and antioxidants in favour of the oxidants, potentially leading to damage", is associated with higher risks of many ailments including diabetes mellitus, hypertension, obesity, dyslipidemia and inflammation. Oxidative stress is induced by reactive oxygen species (ROS) such as superoxide anions ($^{\cdot}OOH$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\cdot}OH$). ROS are "generated as byproducts of aerobic respiration and metabolism" and modulated by antioxidant enzymes and non-enzymatic scavengers [4,5]. The antioxidants that scavenge reactive free radicals may be of great value in preventing the onset

and propagation of oxidative diseases like autoimmune diseases, cardiovascular diseases, cancer, diabetes, ageing and neurovascular diseases [6]. Hence antioxidants are now probably known to be "True scavengers". Highly reactive free radicals react with lipids, proteins and DNA, evoking irreversible damages in their bio-molecular structure [7,8]. Scavenging activity can be studied by 1,1-diphenylpicrylhydrazyl (DPPH), Nitric oxide (NO), hydrogen peroxide (H_2O_2) and lipid peroxidation (LPO) methods. Superoxide anion radicals, hydrogen peroxide and hydroxyl radicals, produced by activation of phagocytes, are considered to be involved in inflammation and tissue destruction.



a: R_1=R_2=H, R_3=OCH_3
b: R_1=R_2=H, R_3=Cl
c: R_1=Br, R_2=R_3=H
d: R_1= CH_3, R_2=R_3=H
e: R_1=Cl, R_2=R_3=H
f:: R_1=H, R_2=CH_3, R_3=Cl
g: R_1=R_3=H, R_2=CH_3
h: R_1=R_2=H, R_3=Br
i: R_1=R_2=H, R_3=F
j: R_1=NO_2, R_2= R_3=H
k: R_1=R_2=H, R_3=NO_2
l: R_1=R_3=CH_3, R_2=H
m: R_1=F, R_2=R_3=H
n: R_1=R_3=H, R_2=NO_2
o: R_1=R_2=H, R_3=CH_3
p: R_1=R_3=H, R_2=Cl
q: R_1=R_3=H, R_2=Br
r: R_1=R_3=H, R_2=F
s: R_1=R_2=R_3=H,
t: R_1=R_2=CH_3, R_3=H

SCHEME - 1

Pyrimidinone and their substituted derivatives have been showing certain biological activities including antimicrobial, anti-inflammatory and antioxidant properties [9]. In recent years, pyrimidones have been shown activity against various bacterial and fungal strains such as Aspergillus niger, Penicillium, Candida albican, Streptomyces, Bacillus subtilis, Streptococcus lactis, Escherichia coli and Pseudomonas [10]. Antimicrobial agents act selectively on vital microbial functions with minimal effects or without affecting host functions. Different antimicrobial agents act in different ways. The understanding of these mechanisms as well as the chemical nature of the antimicrobial agents is crucial in the understanding of the ways how resistance against them develops. However, the mechanism of action of antimicrobial agents can be categorized further based on the structure of the bacteria or the function that is affected by the agents. These generally include the following: Inhibition of the cell wall synthesis, Inhibition of ribosome function, Inhibition of nucleic acid synthesis, Inhibition of folate metabolism, Inhibition of cell membrane function [11]. Observing the above facts and based on the need for the development of efficient and safe antimicrobial and antioxidant agents. Further, in continuation of our research work on antimicrobial and antioxidant agents, we have focused on the evaluation of antimicrobial and antioxidant activities for previously reported 7-methyl-2-(phenoxymethyl)-5H-[1,3,4]thiadiazolo[3,2-a] pyrimidin-5-one analogues **6a-t** [12].

MATERIALS AND METHODS

Chemistry

Synthesis of the title compounds **6a-t** was clearly discussed earlier. Briefly, the synthetic route of the title compounds is outlined in Scheme 1. Substituted phenols **1a-t** on reaction with ethyl chloroacetate affords substituted ethyl phenoxy acetates **2a-t** in excellent yield. This on treatment with hydrazine hydrate yields corresponding phenoxy acetohydrazides **3a-t**. Condensation of **3a-t** with potassium isothiocyanate in dry ethanol resulted in the formation of substituted phenoxy acetyl-N-hydrazine carbothioamides **4a-t**. Intramolecular cyclization of **4a-t** with anhydrous orthophosphoric acid afforded 5-phenoxy methyl-2-amino-1,3,4-thiadiazoles **5a-t**. Finally, compounds **5a-t** on condensation with ethylacetoacetate in the presence of methanol afforded the title compounds **6a-t**.

Biology

Antimicrobial activity

Streptomycin was used as positive controls against bacteria. Ketoconazole (Himedia, Mumbai) were used as positive controls against fungi.

Tested microbes

The following gram positive bacteria were used for the experiments; staphylococcus aureus (MTCC 7443), Staphylococcus aureus (MRSA) (MTCC 84), Enterobacter aerogenes (MTCC 111), Micrococcus luteus (MTCC 1538). The gram negative bacteria included Klebsiella pneumonia (MTCC 109), Salmonella typhimurium (MTCC 2488), Salmonella paratyphi–B (MTCC 733), Proteus vulgaris(MTCC 321). In addition, fungi Candida albicans (MTCC 227), Botyritis cinerea (MTCC 2880), Candida krusei (MTCC 231), Malassesia pachydermatis, were also used for the experiments. All cultures were obtained from the Department of Microbiology, Manasagangotri, Mysore.

Preparation of Inoculums

Bacterial inoculums were prepared by growing cells in Mueller Hinton Broth (MHA) (Himedia) for 24 h at 37^{o}C. These cell suspensions were diluted with sterile MHB to provide initial cell counts of about 104 CFU/ml. The filamentous fungi were grown on sabouraud dextrose agar (SDA) slants at 28^{o}C for 10 days and the spores were collected using sterile doubled distilled water and homogenized [13,14].

Disc diffusion assay

Antibacterial activity was carried out using a disc diffusion method [15]. Petri plates were prepared with 20 ml of sterile Mueller Hinton Agar (MHA) (Himedia, Mumbai). The test cultures were swabbed on the top of the solidified media and allowed to dry for 10 mins. The tests were conducted at 1000 µg/disc. The loaded discs were placed on the surface of the medium and left for 30 min at room temperature for compound diffusion. Negative control was prepared using respective solvent. Streptomycin (10µg/disc) was used as positive control. The plates were incubated for 24 h at 37^{o}C for bacteria and 48 h at 27^{o}C for fungi. A zone of inhibition was recorded in millimeters and experiment was repeated twice (Table 1 and 2).

Minimum inhibitory concentration (mic)

Minimum inhibitory concentration studies of synthesized compounds were performed according to the standard reference method for bacteria [16] and filamentous fungi [17]. Required concentrations (1000 µg/ml, 500µg/ml, 250 µg/ml, 125 µg/ml, 62.5 µg/ml, 31.25 µg/ml and 15.62 µg/ml) of the compounds were dissolved in DMSO (2%), and diluted to give serial twofold dilutions that were added to each medium in 96 well plates. An inoculum of 100 ml from each well was inoculated. The anti-fungal agent's ketoconazole, fluconazole for fungi and streptomycin, ciprofloxacin for bacteria were included in the assays as positive controls. For fungi, the plates were incubated for 48-72 h at 28^{o}C and for bacteria the plates were incubated for 24 h at 37^{o}C. The MIC for fungi was defined as the lowest extract concentration, showing no visible fungal growth after incubation time. 5 ml of tested broth was placed on the sterile MHA plates for bacteria and incubated at respective temperatures. The MIC for bacteria was determined at the lowest concentration of the compounds inhibiting the visual growth of the test cultures on the agar plate (Table 3 and 4).

Antioxidant activity

Compounds (**6a-t**) were also tested for in vitro antioxidant property by DPPH, nitric NO, H_2O_2 and LPO methods (Table 5.6.7 and 8).

DPPH radical scavenging activity

In the DPPH radical scavenging activity the hydrogen atom or electron donating ability of the compounds was measured from the bleaching of the purple colored methanol solution of DPPH [18,19]. The spectrophotometric

assay uses the stable radical DPPH as a reagent. 1 ml of various concentrations of the test compounds (25, 50, 75, 100 and 100 mg/ml) in methanol was added to 4ml of 0.004% (w/v) methanol solution of DPPH. After a 30 min incubation period at room temperature, the absorbance was read against blank at 517 nm. The percent of inhibition (I%) of free radical production from DPPH was calculated by the following equation

% of scavenging = [(Acontrol – Asample) / Ablank] X 100 equation-1

Where Acontrol is the absorbance of the control reaction (containing all reagents except the test compound) and Asample is the absorbance of the test compound. Tests were carried at in triplicate [20].

Nitric oxide (NO) scavenging activity

NO scavenging activity was measured by slightly modified methods of Green et al [21-23]. NO were generated from sodium nitroprusside. 1 ml of sodium nitroprusside (10 mM) and 1.5 ml of phosphate buffer saline (0.2 M, pH 7.4) were added to different concentrations (25, 50, 75 and 100 mg/ml) of the test compounds and incubated for 150 min at 25^0C and 1 ml of the reaction mixture was treated with 1 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromatophore was measured at 546 nm. Nitric oxide scavenging activity was calculated using equation-1.

Hydrogen peroxide (H_2O_2) scavenging activity

The H_2O_2 scavenging activity of the test compound was determined according to the method of Ruch et al [24, 25]. A solution of H_2O_2 (40 mM) was prepared in phosphate buffer (pH 7.4). 25, 50, 75 and 100 mg/ml concentrations of the test compounds in 3.4 ml phosphate buffer were added to H_2O_2 solution (0.6 ml, 40 mM). The absorbance value of the reaction mixture was recorded at 230 nm. The percentage of scavenging activity of H_2O_2 was calculated using equation-1.

Lipid peroxidation (LPO) scavenging activity

In LPO inhibitory activity egg lecithin (3 mg/ml phosphate buffer, pH 7.4) was sonicated in an ultrasonic sonicator for 10 min to ensure proper liposome formation. Test samples (100 µλ) of different concentrations (25, 50, 75, 100 µg/ml) were added to the liposome mixture (1 ml); the control was without test sample. LPO was induced by adding ferric chloride (10, 400 mM) and L-ascorbic acid (10µλ ml, 200 mM). After incubation for 1 h at 37^oC the reaction was stopped by adding hydrochloric acid (2 ml, 0.25 N) containing trichloroacetic acid (150 mg/ml), thiobarbituric acid (3.75 mg/ml) and butylated hydroxy anisole (0.50 mg/ml). The reaction mixture was subsequently boiled for 15 min, cooled, centrifuged at 1000 rpm for 15 min and the absorbance of the supernatant was measured at 532 nm and compared with that of ascorbic acid [26,27].

For all the above antioxidant methods, experiments were done in triplicate and the average is taken, the % inhibition at different concentration was calculated by the following formula % Inhibition = $(1\text{-}v_t/v_c)$ x 100, where, Vt= mean absorption of test compound, Vc = mean absorption of control. The IC-50 value was derived from the % inhibition at different concentration.

RESULTS AND DISCUSSION

The antimicrobial activities of synthesized compounds were screened against eight bacteria and four fungi using in vitro disc diffusion method. The results revealed that most of the synthesized compounds exhibited antimicrobial activities against Staphylococcus aureus, Staphylococcus aureus (MRSA), Enterobacter aerogenes, Micrococcus luteus, Klebsiella pneumoniae, Proteus vulgaris, Salmonella typhimurium, Salmonella paratyphi-B, Candida albicans, Candida krusei, Botyritis cinerea and Malassesia pachydermatis organisms. The results revealed that most of the compounds showed moderate to excellent activities against the tested microorganisms. Among all the synthesized compounds **6m** and **6r** showed highest antibacterial activity compared with the standard drug. The compound **6m** having fluoro group at ortho position of phenyl ring showed good antibacterial activity against E. aerogenes, S. aureus (MRSA), S. aureus, P. vulgaris and K. pneumonia. While compound **6r** having fluoro group at meta position showed good antibacterial activity against E. aerogenes, S. aureus, M. luteus, P. vulgaris and S. typhimurium. Interestingly, the compounds **6b, 6h, 6i, 6j 6m, 6q** and **6r** with electron withdrawing groups showed good activity against some of the bacteria strain. Whereas, compounds **6a, 6d, 6g, 6l** and **6o** with electron releasing groups showed moderate activity and the results are summarized in Table **1.**

With respect to antifungal activity, compounds **6e** with a chloro group at ortho position, **6m** with the fluoro group at ortho position and **6r** with the fluoro group at the meta position exhibited maximum antifungal activity against some of the fungal strain. In contrast, compounds **6a** and **6g** with methoxy at para and methyl at the meta position respectively exhibited the least antifungal activity. Hence, a statement can be made that electron donating group

diminish antifungal activity, whereas electron withdrawing group enhance it. The results are summarized in Table 2**.** The MIC values of active compounds **6b, 6c, 6e, 6h, 6i, 6k, 6m, 6p, 6q** and **6r** against bacteria and fungi are given in Table 3 and 4 respectively. Significant MIC values were observed against Gram-positive and Gram-negative bacteria. The compound **6r** having fluoro group at the meta position shown the maximum MIC activity against S. aureus, E. aerogens and S. typhimurium. The compound **6m** with the fluoro group at the ortho the position shown higher MIC activity against S. aureus compared to the standard Streptomycin and Ciprofloxacin. Further, the compound **6r** shown the maximum MIC activity against C. albicans and B. cinerea fungal strains compared to the standard Fluconazole and Ketoconazole.

Table 1. In-vitro antibacterial activity of compounds 6a-t

Compounds	Zone of inhibition in mm							
	Gram positive bacteria				Gram negative bacteria			
	E. aerogenes	*S. aureus (MRSA)*		*M.*	*P.*	*K.*	*S. typhimurium*	*S.*
6a	9	14	15	13	12	9	12	15
6b	17	13	22	20	19	16	23	11
6c	9	8	11	12	9	11	10	8
6d	9	9	10	11	8	9	11	8
6e	12	9	10	12	9	12	10	8
6f	12	11	9	11	9	12	9	8
6g	9	9	11	10	13	12	11	9
6h	19	14	22	17	18	15	24	9
6i	18	17	23	21	19	16	23	11
6j	15	12	21	13	10	21	16	22
6k	14	13	17	16	10	15	17	11
6l	11	9	13	12	9	11	10	8
6m	20	11	22	21	17	15	24	13
6n	11	8	11	13	8	11	10	7
6o	12	17	18	19	15	19	9	12
6p	15	12	16	14	12	10	15	11
6q	14	18	17	21	15	17	22	10
6r	25	20	22	17	17	21	13	18
6s	14	17	9	14	15	10	12	18
6t	7	13	8	11	15	12	9	13
Streptomycin	26	21	24	23	19	23	25	25

Table 2. In-vitro antifungal activity of compounds 6a-t

Compounds	Zone of inhibition in mm			
	C. albicans	*C. Krusei*	*B. cinerea*	*M. pachydermatis*
6a	8	11	11	8
6b	10	14	8	12
6c	12	16	10	13
6d	11	15	11	12
6e	10	13	22	13
6f	12	13	10	15
6g	9	8	10	8
6h	11	15	12	18
6i	12	13	10	17
6j	12	15	11	13
6k	9	15	20	12
6l	13	12	8	7
6m	11	15	18	21
6n	8	13	12	8
6o	10	12	14	8
6p	9	11	16	10
6q	9	16	15	14
6r	14	16	21	17
6s	7	13	18	9
6t	9	13	10	8
Ketoconazole	14	18	23	24

Table 3. MIC (mg/ml) of compounds against tested bacteria

Compounds	Minimum inhibitory concentration (µg/ ml)							
	Gram positive bacteria				Gram negative bacteria			
	S. aureus	*S. aureus (MRSA)*	*E. aerogens*	*M. luteus*	*K. pneumonia*	*P. vulgaris*	*S. typhimurium*	*S. Paratyphi-B*
6b	31.25	62.5	62.5	15.62	125	250	500	62.5
6c	62.5	31.25	15.62	15.62	125	125	62.5	500
6e	62.5	31.25	15.62	15.62	62.5	62.5	125	500
6h	62.5	125	15.62	62.5	125	550	31.25	500
6i	250	62.5	31.25	31.25	62.5	250	31.25	250
6k	500	250	31.25	250	250	250	125	250
6m	125	15.62	31.25	62.5	125	31.25	62.5	125
6p	500	250	250	31.25	250	62.5	125	250
6q	125	250	31.25	250	250	500	125	250
6r	15.62	250	15.62	31.25	15.62	15.62	15.62	125
Streptomycin	6.25	>100	25	6.25	6.25	Ni	30	6.25
Ciprofloxacin	<0.78	>100	>100	<0.78	<0.78	6.25	>100	<0.78

Table 4. MIC (mg/ml) of compounds against tested fungi

Compounds	Minimum inhibitory concentration (µg/ ml)			
	C. albicans	*C. krusei*	*B. cinerea*	*M. pachydermatis*
6b	500	31.5	62.5	250
6c	31.5	62.5	125	500
6e	31.5	62.5	250	500
6h	62.5	250	500	31.5
6i	31.5	62.5	250	125
6k	62.5	250	500	125
6m	125	31.5	62.5	250
6p	62.5	125	250	500
6q	125	31.5	250	125
6r	15.62	62.5	15.62	31.5
Fluconazole	>100	12.5	ni	12.5
Ketoconazole	25	15	25	15

ni = no inhibition.

Table 5. In-vitro antioxidant activity of compounds 6a-t in DPPH method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	73.78 ± 0.21	76.96 ± 0.41	80.88 ± 0.50	82.96 ± 0.68	15.01 ± 0.23
6b	51.97 ± 1.73	57.75 ± 1.10	60.92 ± 0.97	64.99 ± 1.58	22.68 ± 1.47
6c	52.85 ± 1.19	55.75 ± 0.89	59.35 ± 0.99	64.31 ± 0.69	24.65 ± 1.08
6d	70.68 ± 0.30	73.78 ± 0.51	75.86 ± 0.60	80.88 ± 0.80	16.24 ± 0.47
6e	51.55 ± 0.81	54.66 ± 1.32	57.74 ± 0.72	62.75 ± 1.01	21.45 ± 0.36
6f	61.85 ± 1.38	65.86 ± 1.14	70.83 ± 1.50	74.69 ± 0.88	17.73 ± 1.08
6g	66.89 ± 1.07	68.69 ± 0.74	73.78 ± 1.26	78.79 ± 1.00	16.23 ± 1.01
6h	51.76 ± 1.18	54.68 ± 0.89	58.47 ± 0.98	63.65 ± 0.67	24.99 ± 1.07
6i	63.85 ± 1.05	68.91 ± 1.40	72.89 ± 0.84	76.88 ± 1.64	19.55 ± 1.08
6j	48.99 ± 0.67	52.95 ± 1.28	56.93 ± 0.58	61.89 ± 0.88	26.33 ± 1.17
6k	48.99 ± 0.67	52.97 ± 1.28	56.91 ± 0.58	62.88 ± 0.88	26.77 ± 1.14
6l	70. 84 ± 0.12	74.77 ± 0.37	76.92 ± 0.40	80.59 ± 0.61	15.15 ± 0.53
6m	60.55 ± 1.08	63.75 ± 1.29	67.85 ± 1.58	70.55 ± 0.87	20.35 ± 0.55
6n	47.34 ± 0.95	49.48 ± 1.25	52.76 ± 1.21	55.64 ± 1.31	27.58 ± 0.72
6o	60.95 ± 0.60	64.84 ± 1.27	69.32 ± 1.24	73.85 ± 1.30	17.56 ± 1.17
6p	54.72 ± 1.19	58.65 ± 0.89	62.85 ± 1.49	66.65 ± 0.87	22.83 ± 0.69
6q	54.83 ± 0.93	56.88 ± 1.43	60.34 ± 0.82	65.74 ± 1.15	25.34 ± 0.47
6r	56.79 ± 1.10	61.68 ± 1.17	65.86 ± 0.80	70.11 ± 0.87	18.49 ± 0.70
6s	67. 96 ± 1.27	71.99 ± 1.41	73.98 ± 1.58	78.94 ± 1.06	18.22 ± 1.11
6t	67.89 ± 0.20	72.96 ± 0.37	74.91 ± 0.60	78.72 ± 0.60	16.01 ± 0.50
Ascorbic acid	80.79 ± 0.07	81.77 ± 0.32	83.88 ± 0.37	85.78 ± 0.44	14.33 ± 0.40
Blank	-	-	-	-	-

Table 6: The in vitro antioxidant activity of compounds 6a-t in nitric oxide (NO) method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	75.96 ± 0.21	80.85 ± 0.40	81.88 ± 0.52	83.93 ± 0.71	13.89 ± 0.70
6b	68.48 ± 0.93	70.54 ± 1.39	74.53 ± 0.98	79.25 ± 1.26	19.57 ± 1.09
6c	60.86 ± 1.56	63.57 ± 1.22	67.85± 1.32	72.68 ± 0.73	20.36 ± 0.97
6d	70.21 ± 0.77	71.94 ± 0.87	75.85 ± 1.01	80.52 ± 1.30	15.63 ± 0.50
6e	60.46 ± 1.55	62.78 ± 1.21	66.86 ± 1.31	71.83 ± 0.72	20.21 ± 0.96
6f	58.90 ± 1.30	64.98 ± 1.56	68.92 ± 0.70	71.95 ± 1.04	16.97 ± 0.82
6g	68.32 ± 0.80	71.23 ± 1.01	74.84 ± 1.32	78.75 ± 1.20	15.36 ± 0.88
6h	62.78 ± 1.42	66.89 ± 1.59	70.58 ± 0.79	73.68 ± 1.12	20.69 ± 0.99
6i	63.84 ± 1.34	71.79 ± 1.26	76.84 ± 1.07	78.48 ± 0.79	18.89 ± 1.14
6j	51.99 ± 1.36	54.98 ± 1.24	58.95 ± 0.73	62.86 ± 1.08	21.52 ± 1.06
6k	62.86 ±1.59	63.68 ± 1.28	69.86 ± 1.39	72.68 ± 0.77	21.78 ± 0.99
6l	72.93 ± 0.20	76.87 ± 0.30	80.11 ± 0.50	82.86 ± 0.66	14.35 ± 0.50
6m	67.75 ± 0.92	69.91 ± 1.38	73.65 ± 0.97	78.77 ± 1.25	19.13 ± 1.08
6n	52.78 ± 1.37	55.89 ± 1.25	59.68 ± 0.74	63.68 ± 1.09	21.99 ± 1.07
6o	68.67 ± 0.10	73.95 ± 0.26	78.89 ± 0.45	80.85 ± 0.61	15.67 ± 0.83
6p	61.86 ± 1.41	65.99 ± 1.58	70.22 ± 0.78	72.96 ± 1.11	20.22 ± 0.98
6q	61.98 ± 1.58	62.88 ± 1.27	68.94 ± 1.38	71.77 ± 0.76	20.99 ± 0.98
6r	62.95 ± 1.33	70.92 ± 1.25	75.94 ± 1.06	77.88 ± 0.78	18.12 ± 1.13
6s	61.75 ± 1.10	66.79 ± 1.50	71.99 ± 1.30	76.88 ± 0.62	17.24 ± 1.18
6t	70.79 ± 0.20	75.94 ± 0.37	79.86 ± 0.58	81.99 ± 0.70	15.33 ± 0.80
Ascorbic acid	82.89 ± 0.14	83.87 ± 0.31	86.89 ± 0.46	88. 96 ± 0.74	13.33 ± 0.50
Blank	-	-	-	-	-

Table 7 The in vitro antioxidant activity of compounds 6a-t in hydrogen peroxide (H_2O_2) method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	61.99±1.01	64.98± 1.20	66.84 ± 0.50	70.15 ± 0.60	15.88 ± 0.48
6b	53.87±1.18	57.83 ± 0.98	61.97 ± 1.48	65.84 ± 0.86	22.21 ± 0.68
6c	51.99 ± 1.18	54.88 ± 0.88	58.96 ± 0.98	63.75 ± 0.68	24.33 ± 1.07
6d	61.75 ± 0.20	64.78 ± 0.57	66.95 ± 0.60	69.74 ± 0.70	18.11 ± 0.21
6e	54.72±1.19	58.65 ± 0.89	62.85 ± 1.49	66.65 ± 0.87	22.83 ± 0.69
6f	59.23 ± 0.81	60.98 ± 1.51	65.74 ± 1.01	69.25 ± 1.31	19.13 ± 1.17
6g	59.86 ±1.27	62.14 ± 1.12	66.79 ± 1.01	70.95 ± 1.50	17.83 ± 0.70
6h	52.85 ± 1.19	55.73 ± 0.89	59.31 ± 0.99	64.33 ± 0.69	24.67 ± 1.08
6i	59.92 ± 1.07	62.79 ± 1.28	66.93 ± 1.57	69.91 ± 0.86	20.22 ± 0.54
6j	49.55 ± 0.68	53.9 5± 1.29	57.671 ± 0.59	63.92 ± 0.89	25.77 ± 1.15
6k	49.94 ± 0.68	53.88 ± 1.29	57.77 ± 0.59	62.76 ± 0.89	26.97 ± 1.18
6l	64.85 ± 0.20	67.13 ± 0.40	70.85 ± 0.51	75.78 ± 0.74	16.01 ± 0.90
6m	50.81 ± 0.80	53.95 ± 1.31	56.82 ± 0.71	61.99 ± 1.01	21.17 ± 0.35
6n	46.88 ± 0.94	48.95 ± 1.24	51.99 ± 1.21	54.84 ± 1.31	27.28 ± 0.72
6o	60.11 ± 0.81	61.33 ± 1.51	66.21 ± 1.01	70.01 ± 1.31	19.01 ± 1.17
6p	50.12 ± 1.18	53.05 ± 0.89	57.18 ± 0.98	62.26 ± 0.67	24.13 ± 1.07
6q	55.39 ± 0.94	57.89 ± 1.44	60.99 ± 0.83	65.77 ± 1.16	25.13 ± 0.48
6r	55.87 ± 1. 04	60.13 ± 1.11	62.86 ± 1.40	66.57 ± 1.47	20.05 ± 1.01
6s	62.96 ± 0.31	64.98 ± 0.48	67.94 ± 0.66	71.93 ± 0.78	19.33 ± 1.27
6t	63.86 ± 1.07	66.79 ± 1.21	68.69 ± 0.51	71.82 ± 0.61	16.15 ± 0.51
Ascorbic acid	75.31±0.15	77.11±0.27	81.01 ± 0.58	85.05 ± 0.64	15.12 ± 0.21
Blank	-	-	-	-	-

Free radicals play a very important role in the pathogenesis of various human diseases and aging. In food products free radicals also cause damage, resulting in diminish taste and shelf life. Antioxidants are therefore protecting against free radicals and save health. In this context, the aim of our study was to explore the most potent antioxidant and examine the factors that give a picture and establish the antioxidant activity with frequent comparison to various pyrimidone tagged phenyl thiadiazoles. Consequently, the synthesized compounds screened for antioxidant activity through different *in vitro* models such as DPPH, NO, H_2O_2 and LPO free radical scavenging activity. Observing the results indicated that, few of the tested compounds are significant in their antioxidant properties. Particularly compounds **6a** and **6l** having methoxy and methyl group at the para position respectively, were most efficient among the series with its IC_{50} value almost comparable with the standard drug ascorbic acid in all the above methods.

The DPPH radical scavenging assay is widely used to investigate the radical scavenging activities of several natural as well as synthetic compounds. The scavenging activity has been studied in the process of hydrogen atom transfer to the stable free radical DPPH to compare the activity of compounds under investigation with that of the widely known antioxidant parameter. The free radical scavenging activity of DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The reduction

capability of DPPH radicals was determined by decreased absorbance at 517 nm, which is induced by antioxidants. The compounds **6a** and **6l** shown IC_{50} values of 15.01, and 15.15 µg/ml respectively, compared to the standard ascorbic acid 14.33 µg/ml.

NO has also been involved in a variety of biological functions, including neurotransmission, vascular homeostasis, antimicrobial, and antitumor activities. Despite the possible beneficial effects of NO, its contribution to oxidative damage is also reported. This is due to the fact that NO can react with superoxide to form the peroxynitrite anion, which is a potential oxidant that can decompose to produce OH and NO. In NO scavenging assay the compounds **6a** and **6l** exhibited IC_{50} values of 13.89 and 14.35µg/ml respectively, compared to the standard ascorbic acid 13.33 µg/ml.
The damaging action of hydroxyl radicals is the strongest among free radicals. In biochemical systems, superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently generate extremely reactive hydroxyl radicals in the presence of certain transition metal ions. Further, hydroxyl radical has the capacity to cause DNA strand breakage and leads to mutations. The compounds **6a** and **6l** displayed highest hydroxyl radical scavenging activity with IC_{50} values of 15.88 and 16.01 µg/ml respectively, compared to the standard ascorbic acid 15.12 µg/ml.

LPO refers to the oxidative degradation of lipids. It is the process in which free radicals steal electrons from the lipids in cell membranes, resulting in cell damage. Remarkably the same compounds **6a** and **6l** revealed highest LPO activity with IC_{50} values of 16.21 and 16.25 µg/ml respectively, compared to the standard ascorbic acid 15.16 µg/ml. In summary, the compounds having electron donating group such as methyl and methoxy are activating antioxidant property with good results comparable with IC_{50} of the standard. At the same time, compound with electron withdrawing group like halo and nitro exhibited less antioxidant activity. The results are summarized in Table 5-8.

Table 8 The in vitro antioxidant activity of compounds 6a-t in lipid peroxidation method

Compounds	Concentration (µg/ml)				
	25	50	75	100	IC_{50}
6a	70.11 ± 0.85	72.75 ± 1.04	75.96 ± 1.38	80.84 ± 1.24	16.21 ± 0.92
6b	60.84 ± 1.52	61.95 ± 1.21	67.99 ± 1.31	72.85 ± 0.71	20.31 ± 0.94
6c	52.82 ± 0.87	55.72 ± 1.37	58.73 ± 0.76	63.64 ± 1.03	22.75 ± 0.40
6d	66.99 ± 0.87	68.92 ± 1.36	72.98 ± 0.92	77.99 ± 1.18	17.33 ± 1.02
6e	62.49 ± 1.53	63.22 ± 1.22	69.98 ± 1.32	74.67 ± 0.72	21.25 ± 0.95
6f	58.37 ± 1.26	61. 37 ± 1.13	67.58 ± 1.03	68.67 ± 1.51	18.11 ± 0.71
6g	61.78 ± 1.14	66.37 ± 1.52	71.937 ± 1.36	76.56 ± 0.68	17.11 ± 1.12
6h	45.79 ± 0.56	50.83 ± 1.20	53.99 ± 0.52	59.87 ± 0.78	23.26 ± 1.01
6i	55.72 ± 1.09	61.73 ± 1.18	66.75 ± 0.85	70.48 ± 0.92	19.68 ± 0.73
6j	43.15 ± 0.85	44.45 ± 1.11	47.78 ± 1.21	50.88 ± 1.33	25.20 ± 0.58
6k	64.94 ± 1.64	67.95 ± 1.30	71.96 ± 1.40	76.89 ± 0.73	25.22 ± 1.18
6l	70.91 ± 0.33	73.88 ± 0.57	76.94 ± 0.67	81.91 ± 0.83	16.25 ± 0.50
6m	50.92 ± 0.86	53.86 ± 1.36	56.95 ± 0.75	61.91 ± 1.02	20.11 ± 0.40
6n	43.76 ± 0.85	44.77 ± 1.11	47.91 ± 1.21	50.96 ± 1.33	25.32 ± 0.58
6o	61.93 ± 1.14	66.88 ± 1.52	71.94 ± 1.36	76.84 ± 0.68	17.35 ± 1.12
6p	52.82 ± 0.87	55.72 ± 1.37	58.73 ± 0.76	63.64 ± 1.03	22.75 ± 0.40
6q	46.30 ± 0.57	51.23 ± 1.20	54.92 ± 0.53	60.32 ± 0.79	23.73 ± 1.01
6r	54.99 ± 1.08	60.89 ± 1.17	65.88 ± 0.84	69.77 ± 0.91	19.23 ± 0.72
6s	59.85 ± 1.27	62. 85 ± 1.14	66.65 ± 1.04	69.79 ± 1.52	18.50 ± 0.71
6t	75.78 ± 0.28	80.01 ± 0.37	82.84 ± 0.57	85.92 ± 0.74	16.78 ± 0.56
Ascorbic acid	77.94 ± 0.18	78.78 ± 0.28	82.94 ± 0.62	86.93 ± 0.68	15.16 ± 0.26

CONCLUSION

In conclusion, our study shows a strong evidence for the antimicrobial and antioxidant activities of pyrimidinone analogues. It is interesting and significant to note from the data that thiadiazole substituted pyrimidinone compounds exhibit antimicrobial and antioxidant activity more relevant to the reference compound against the strains**.**

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