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Research Article

ANTIOXIDANT ACTIVITY EVALUATION OF 2-AMINO-6-ARYL-4-(FURAN-2YL) PYRIMIDINES

***Udhayavani S.**
Department of Chemistry, Adhiparasakthi Engineering College, Melmaruvathur
**Author for Correspondence*

ABSTRACT
2-amino-6-aryl-4-(furan-2yl) pyrimidines compounds are found to be an efficient scavengers of free radicals such as DPPH free radical, $ABTS^{\cdot+}$,$^{\cdot}OH$, $O_2^{\cdot-}$, and nitric oxide in dose dependence manner. The various antioxidant activities of the compound were compared to the standard ascorbic acid.

Keywords: *DPPH Radical Scavenging Assay, ABTS Radical Cation Decolorization Assay, Superoxide Anion Scavenging Assay, Hydroxyl Radical Scavenging Assay and Nitric Oxide Radical Inhibition Assay*

INTRODUCTION
Free radical formation is associated with the normal natural metabolism of aerobic cells. The oxygen consumption inherent in cell growth leads to the generation of a series of oxygen free radicals. The interaction of these species with lipidic molecules produces new radicals: hydroperoxides and different peroxides (Aust and Sringen, 1952; Pyror *et al*., 1982). This group of radicals (superoxide, hydroxyl, and lipoid peroxides) may interact with biological systems in a cytotoxic manner. Free radicals and their uncontrolled productions, in fact, are responsible for several pathological processes, such as certain tumours (prostate and colon cancers) and coronary disease (Halliwell, 1997; El-Rayyes and Ramadan, 1987). Dietary antioxidants, including polyphenolic compounds, vitamin E and C, and carotenoids, are believed to be the effective nutrients in the prevention of these oxidative stress related diseases.
Reactive oxygen species are cytotoxic due to the intermediate formed from univalent reduction of molecular oxygen, including the superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), and the hydroxyl radical ($OH^{\cdot}$). These oxygen intermediates differ significantly in their interactions and can cause extensive cellular damage such as nucleic acid strand scission (Adelman *et al*., 1988), modification of polypeptides, lipid peroxidation, etc. (Pryor and Porter, 1990). The screening of the compounds, which scavenge the reactive oxygen species, could lead to promising radioprotectors. Most of the antioxidants used in therapy are derived from natural sources. About 28% of the drugs approved by the FDA between 1981 and 2002 are either natural products or chemicals derived from them (Weiss and Landauer, 2003; Clardy and Walsh, 2004). Therefore, exploration of chemicals as radioprotectors is a promising drug development strategy.
This study is devoted to the synthesis and comparative investigation of the antioxidant activity of a series of new 2-amino-6-aryl-4-furan-2ylpyrimidines. These compounds have lipophilc aryl ends and can be easily converted into water soluble by halides.

MATERIALS AND METHODS
Experimental
Redical-scavenging Activity
DPPH-Redical-scavenging Activity
The radical scavenging activity of compounds against $DPPH^{\cdot}$ was determined by spectrophotometrically by the method of Brand Williams *et al*., (1995).
DPPH is a stable free radical and accepts an electron, or hydrogen radical to become a stable diamagnetic molecule. DPPH reacts with an antioxidant compound that can donate hydrogen and gets reduced. The change in color (from deep violet to light yellow) was measured. The intensity of the yellow colour depends on the amount and nature of radical scavenger present.

The reaction mixture in a total volume of 3mL contained 1mL of DPPH, various concentrations of compounds (1, 2, 3, 4, 5, 6,7,8,9 & 10µg) and made up to 3mL with water. The tubes were incubated for 10 min at 37°C. A blue colour chromospheres was formed, the absorbance of which was measured at 517nm. Ascorbic acid was used as standards for comparison.

Note: DDPH **2, 2-Diphenyl-1-picryl hydrazyl**

ABTS-Redical Cationdecolorization Assay

The generation of the ABTS radical cation forms the basis of one of the spectrophotometric methods that has been applied for the measurement of the total antioxidant activity of solutions of pure substances (Welfended and Willson, 1982). The improved technique for the generation of ABTS described here involves the direct production of the green ABTS chromospheres through the reaction between ABTS and potassium persulphate. Addition of compounds and other antioxidants compete with ABTS diminishes the color formation.

ABTS was dissolved in water at a concentration of 7mM. The stock solution was mixed with 2.45mM potassium persulphate (final concentration). The mixture was allowed to stand in the dark at room temperature for 12-16hrs before use for incomplete oxidation of ABTS. The radical was stable in this form for more than two days when stored in the dark at room temperature. The incubation mixture in a total volume of 5mL contained 0.54mL of ABTS, 0.5 mL of phosphate buffer and varying concentrations of compounds (1, 2, 3,4,5,6,7,8,9 & 10µg). The blank contained water in place of sunphenon. The absorbance was read in spectrophotometer at 734nm and compared with standards ascorbic acid at same concentrations.

Note: ABTS **2, 2'-Azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)**

Superoxide Anion Scavenging Assay

Superoxide anion scavenging activity of sunphenon was determined by the method of Nishimiki *et al.,* (1972) with modifications. The assay was based on the oxidation of NADH by phenazinemethosulphate (PMS) to liberate PMS_{red}. PMS_{red} converted oxidized nitrobluetetrazolium (NBT_{oxi}) to the reduced form NBT_{red}, which formed a violet colour complex. The colour formation indicated the generation of superoxide anion, which was measured spectrophotometrically at 560nm. A decrease in the formation of colour after addition of the antioxidant was a measured of its superoxide radical scavenging activity.

To 1mL of NBT, 1mL of NADH solution and carrying volumes of compounds (1, 2, 3, 4, 5, 6,7,8,9 & 10µg) were added and mixed well. The reaction was started by the addition of 100µL of PMS. The reaction mixture was incubated at 30°C for 15 min. the absorbance was measured at 560nm. Incubation with water in plate of compounds was used as blank. Ascorbic acid was used as standards for comparison.

Hydroxyl Radical Scavenging Assay

The hydroxyl radical scavenging activity of sunphenon was determined by the method of Halliwell *et al.,* (1987). In this assay, hydroxyl radicals are produced by the reduction of H_2O_2 by the transition metal (iron) in the presence of ascorbic acid. The generation of hydroxyl radical is detected by its ability to degrade deoxyribose to form products, which on heating with TBA forms a pink colour chromate. Addition of sunphenon competes with deoxyribose for hydroxyl radicals and diminishes the colour formation.

The incubation mixture in a total volume of 1mL contained 0.1mL of buffer, varying volumes of compounds (1, 2, 3, 4, 5, 6,7,8,9 & 10µg), 0.2mL of ferric chloride, 0.1mL of ascorbic acid, 0.1mL of EDTA, 0.1mL of hydrogen peroxide and 0.2mL of 2-deoxyribose. The contents were mixed thoroughly and incubated at room temperature for 60 min. then added, 1mL of TBA and 1mL of TCA.

All the tubes were kept in a boiling water bath for 30 min. the absorbance of the supernatant was read in a spectrophotometer at 535 nm with reagent blank containing water in place of extract. The efficiency of compounds was compared with various concentrations (1, 2, 3, 4, 5, 6,7,8,9 & 10µg) of standards ascorbic acid.

Nitric Oxide Radical Inhibition Assay

The nitric oxide radical inhibition activity of sunphenon was measured by the method of Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously produces nitric oxide which interacts with oxygen to produce nitrite ions. This can by estimated using Griess reagent.

The reaction mixture (3mL) containing sodium nitroprusside (2mL), PBS (0.5mL) and compounds or standard solution (0.5mL) was incubated at 25° C for 15 min. after incubation, 0.5mL of the reaction mixture containing nitrite was pipetted and mixed with 1 mL of sulfanilic acid reagent and allowed to stand for 5 min for completing diazotization. Then 1mL of naphthalene diamine hydrochloride was added, mixed and allowed to stand for 30 min at 25° C. a pink colour chromospheres was formed, the absorbance of which was measured at 540nm.

RESULTS AND DISCUSSION

IC_{50} *Values for the Different Antioxidant Activity Assay*

Table 1: Comparison of the IC_{50} in µg/mL

Radicals	Ascorbate µg/mL	1 µg/mL	2 µg/mL	3 µg/mL	4 µg/mL	5 µg/mL	6 µg/mL
DPPH	4.94	5.59	3.24	3.52	4.47	4.77	3.40
ABTS	5.32	5.87	3.48	3.74	4.72	5.02	3.64
Superoxide	5.35	5.41	3.10	3.39	4.33	4.62	3.27
Hydroxyl	4.23	5.22	2.94	3.24	4.16	4.15	3.11
Nitricoxide	4.50	5.47	3.16	3.44	4.38	4.38	3.32

- 2-Amino-6-phenyl-4-(furan-2yl)pyrimidine (**1**)
- 2-Amino-6-(4-chlorophenyl)-4-(furan-2yl)pyrimidine (**2**)
- 2-Amino-6-(3-bromophenyl)-4-(furan-2yl)pyrimidine (**3**)
- 2-Amino-6-(4-methoxyphenyl)-4-(furan-2yl)pyrimidine (**4**)
- 2-Amino-6-[4-(dimethylamino)phenyl]-4-(furan-2yl)pyrimidine (**5**)
- 2-Amino-6-[3,4-(dimethoxy)phenyl]-4-(furan-2yl)pyrimidine (**6)**

All the compounds are found to be an efficient scavengers of free radicals such as DPPH free radical, $ABTS^{\cdot+}$, $^{\cdot}OH$, $O_2^{\cdot-}$, and nitric oxide in dose dependence manner. The various antioxidant activities of the compound were compared to the standard ascorbic acid. Compounds 1-6 showed more antioxidant properties whereas compound 1 exhibited low antioxidant property, when compared to standard ascorbic acid. The scavenging potential was in the order of 2>3>6>4>5>AA>1.

Conclusion

All the compounds are found to be an efficient scavengers of free radicals such as DPPH free radical, $ABTS^{\cdot+}$, $^{\cdot}OH$, $O2^{\cdot-}$, and nitric oxide in dose dependence manner. The various antioxidant activities of the compound were compared to the standard ascorbic acid.

The scavenging potential was in the order of 2>3>6>4>5> ascorbic acid >1.

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