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Scientific Assessment of Antioxidant Potential of Pyrimidine-2,4(*1H*, 3*H*)-diones

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ABSTRACT: The aim of present research work is to screen some pyrimidine-2,4-diones for their potential anti-oxidant activity to prevent the progress of free radical mediated disorders using different in-vitro models like nitric oxide(NO) scavenging activity and ferric reducing anti-oxidant power. Results revealed that pyrimidine-2,4-diones have significant anti-oxidant activity and OBP-05 is the most potent antioxidant as per the overall radical scavenging capabilities, but OBP-10 have specifically higher nitric oxide scavenging(IC_{50} 3.38 µg/ml) potential than that of OBP-05(IC_{50} 4.51 µg/ml).

KEYWORDS: Pyrimidine-2,4-diones; Antioxidant; Free Radical Scavenging; Nitric Oxide Scavenging.

INTRODUCTION

Heterocyclic compounds containing five or six membered ring with one or more nitrogen atoms are always of great importance in the pharmaceutical sector as having the bio-isosteric factor. Pyrimidine-2,4-dione is a 6-membered heterocyclic ring system having two nitrogen atoms at 1 & 3 position of the ring. In the 1967, a number of substituted pyrimidine-2,4-diones were synthesized and intensively studied as good reversible inhibitors of thymidine phosphorylase[1-3].

Nitric oxide is an important chemical mediator generated by endothelial cells, macrophages, neurons and is involved in the regulation of various physiological processes[4]. Excess concentration of nitric oxide is associated with several diseases[5,6]. Oxygen reacts with the excess nitric oxide to generate nitrite and peroxynitrite anions which acts as free radicals[7]. Nitric oxide can react rapidly in the intracellular environment to form nitrate, nitrite and s-nitrosothiols. These metabolites play a key role in mediating many xenotoxic effects such as DNA damage. Nitric oxide causes DNA damage via peroxynitrite.

Diazotization takes place between nitrite and sulphanilamide, this diazotized product is coupled with naphthylene diamine to form chromophore, which is reduced by antioxidant when measured at 546nm[8]. Nitric oxide was generated from sodium

nitroprusside and was measured by the Griess reagent. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide[9,10]. which interacts with oxygen to produce nitrite ions that can be estimated by the use of Griess Reagent. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[10,11].

MATERIALS & METHODS

Synthetic Pyrimidine-2,4-diones:

Some pyrimidine-2,4(*1H, 3H*)-diones were synthesized by our team and already explained with their antimicrobial activity[1, 2]. Same molecules were taken to screen their antioxidant potential.

Anti-oxidant activity

Anti-oxidant activity of newly synthesized pyrimidine-2,4-dione derivatives were find out by nitric oxide scavenging activity and ferric reducing anti-oxidant power in-vitro models.

Nitric oxide scavenging model

0.5 ml of Sodium nitropruside (10mM) was mixed with 0.5ml of different concentration of synthesized compounds dissolved in methanol and incubated at room temperature for 150 min. The same reaction mixture without the sample but with equivalent amount of phosphate buffer saline served as control. After, the incubation period, 0.5ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride) was added. The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546nm. While 0.5ml of Ascorbic acid (40µg/ml) was used as positive control. Percent inhibition was determined by comparing the results of the test and control samples[12].

Ferric reducing anti-oxidant power model

Different concentrations of synthesized compounds in 1ml of distilled water were mixed with phosphate buffer (2.5ml, 0.2M, pH 6.6) and potassium ferricyanide solution (2.5ml, 1%). The mixture was incubated at 50ºC for 20 min. Then, 2.5ml of trichloroacetic acid solution (10%) was added to mixture which was then centrifuged for 10 min. at 3000 rpm. The upper layer of solution (2.5ml) was mixed with 2.5ml distilled water and ferric chloride solution (0.5ml, 0.1%). The absorbance was measured at 700 nm against a control/blank using uv-spectrophotometer. Increased absorbance of the reaction mixture indicates increase in reducing power[13].

RESULTS & DISCUSSION

Procedure for the preparation of used pyrimidine-2,4(*1H, 3H*)-diones were detailed in our previous works[1,2] along their spectral parameters. All the synthesized pyrimidine-2,4-dione compounds were screened for their potential anti-oxidant activity by using in-vitro models. It includes nitric oxide scavenging model and ferric reducing anti-oxidant power model.

Table 1: Anti-oxidant activity of pyrimidine-2,4-dione derivatives by nitric oxide scavenging model

S. NO.	Compound Code	$IC_{50\%}$ in µg/ml
1.	OBP01	5.68
2.	OBP02	4.65
3.	OBP03	5.81
4.	OBP04	23.30
5.	OBP05	4.51
6.	OBP06	4.76
7.	OBP07	4.98
8.	OBP08	16.93
9.	OBP09	6.62
10.	OBP10	3.38

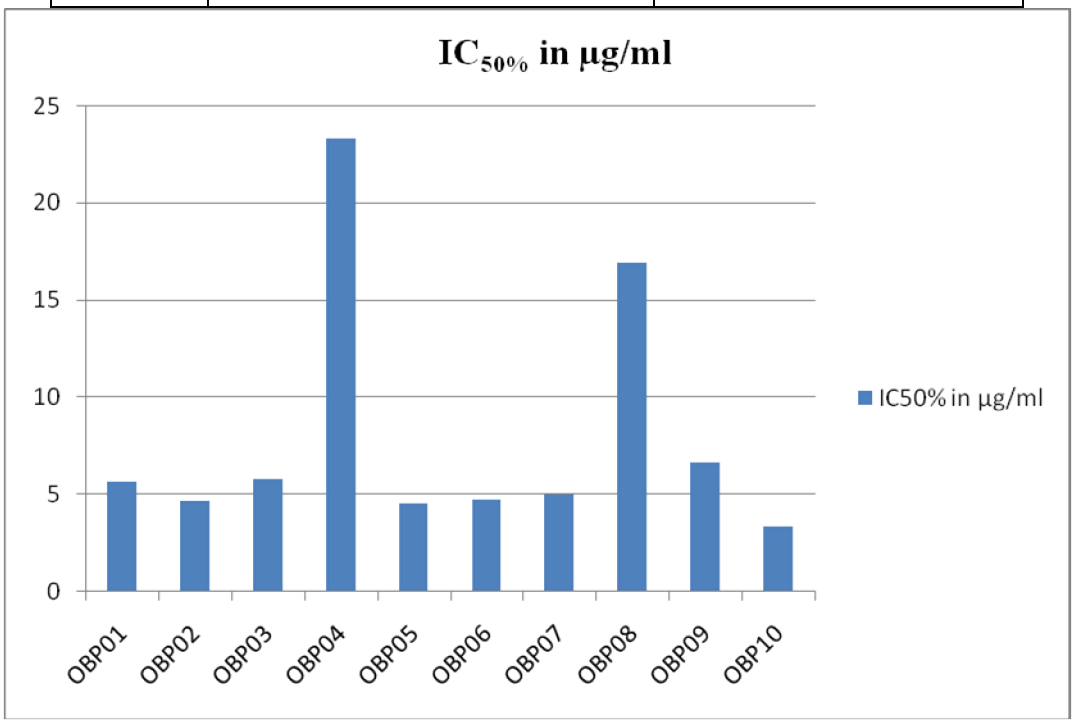

Fig. 1. Anti-oxidant activity by nitric oxide scavenging model

Table 2: Anti-oxidant activity of pyrimidine-2,4-dione derivatives by ferric reducing anti-oxidant power model

S. NO.	Compound Code	$IC_{50\%}$ in µg/ml
1.	OBP01	24.0
2.	OBP02	18.7
3.	OBP03	46.1
4.	OBP04	49.8
5.	OBP05	17.8
6.	OBP06	19.3
7.	OBP07	20.6
8.	OBP08	45.1
9.	OBP09	27.6
10.	OBP10	85.0

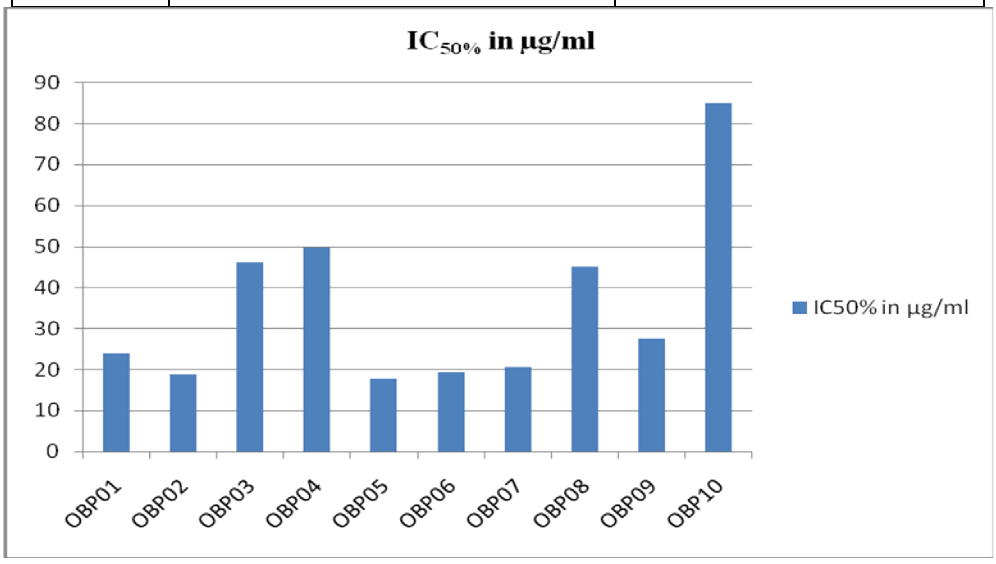

Fig. 2. Anti-oxidant activity by ferric reducing anti-oxidant power model

According to the results of nitric oxide scavenging activity(**Table 1, Figure 1**), all the ten pyrimidine-2,4-diones have IC_{50} value in the range of 3.38 – 23.30 µg/ml, out of which the most potent one is OBP-10. But astonishingly, OBP-10 hasn't had same capability to scavenge ferric ions. As according to the results of ferric antioxidant power model(**Table 2, Figure 2**), all molecules have IC_{50} in the range of 17.8 to 85 µg/ml, where OBP-05 is the most potent one having IC_{50} 17.8 µg/ml while the OBP-10(best nitric oxide

scavenger) have IC_{50} 85.0 µg/ml. This could certainly because of the different mechanism of action for both nitric oxide and ferric ions. Overall, OBP-05 which can scavenge both nitric oxide and ferric ions to a good extent, considered to be the most potent out of all. Further modifications on OBP-05 and mechanistic approach for analysis of its antioxidant potential may help to reach analogue of pyrimidine-2,4(*1H*,*3H*)diones to the bed side .

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