AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

In-Vitro Studies of Antioxidant and Membrane Stabilization Activity of 2-Substituted 4, 5-Diphenyl Imidazole Derivatives

G.P.Gigi Sam*, A. Jerad Suresh, Ajithadas Aruna, V. Niraimathi

1. Department of Pharmaceutical Chemistry, Madras Medical College, Chennai-3

ABSTRACT

A novel series of 2-substituted 4,5-diphenyl imidazoles were synthesized and investigated for their antioxidant activity and membrane stabilization activity. 2,2-diphenyl-1-picrylhydrazyl(DPPH) radical assay was carried out to evaluate the antioxidant potential of the extract. The antioxidant activity of the synthesized compounds increased in a concentration dependent manner. In DPPH radical scavenging assay the IC50 value of the compounds ranged from 40 to 200µg/ml. The membrane stabilization activity of the compounds was evaluated using human red blood cells (HRBC) membrane stabilization method. The concentration of 88.88 to 444.44µg/ml showed a dose dependent inhibition of haemolysis of erythrocytes induced by hypotonic solution.

Key words: DPPH, antioxidant, membrane stabilization

*Corresponding Author Email: gigipharma@gmail.com

Received 18 March 2012, Accepted 14 June 2012

Please cite this article in press as: Sam GPG *et al.*, In-vitro studies of antioxidant and membrane stabilization activity of 2-substituted 4, 5-diphenyl imidazole derivatives. American Journal of PharmTech Research 2012.

INTRODUCTION

The term 'membrane stabilizer' was used by Shanes (1958) to designate chemical agents which depress excitability in nerves and muscle without causing obvious changes in the resting membrane potential. To the extent examined, these agents also protect erythrocytes against hypotonic hemolysis and stabilize lysosomes and other cell organelles under in vitro conditions.

MATERIALS AND METHODS:

Study of anti-inflammatory effect by membrane stabilizing property[1,2,3]

Preparation of erythrocyte suspension:

Blood was collected from median cubital vein of healthy volunteers. The blood was mixed with isosaline and centrifuged at 3000 rpm. The packed cells were further washed with isosaline and the sedimented erythrocytes were collected. A suspension of 2%v/v in isosaline was prepared.

Procedure:

The assay mixture consisted hyposaline (2mL), sodium phosphate buffer at pH 7.4 (1mL), varying volumes of drugs (2mg/mL) (0.0-1.0mL) and erythrocyte suspension (0.5mL) were made up with isosaline to give a total assay volume of 4.5mL.The control was prepared as above except the drug was omitted.

The reaction mixtures were incubated at 56^0C for 30 minutes. The tubes were cooled under running water followed by centrifugation at 5000rpm. The supernatant were collected followed by reading the absorbance of the released hemoglobin at 560nm.

The percentage membrane stability was estimated using the expression:

% Membrane Stability = C-T/C*100

Study of antioxidant effect by measurement of DPPH free radical scavenging activity

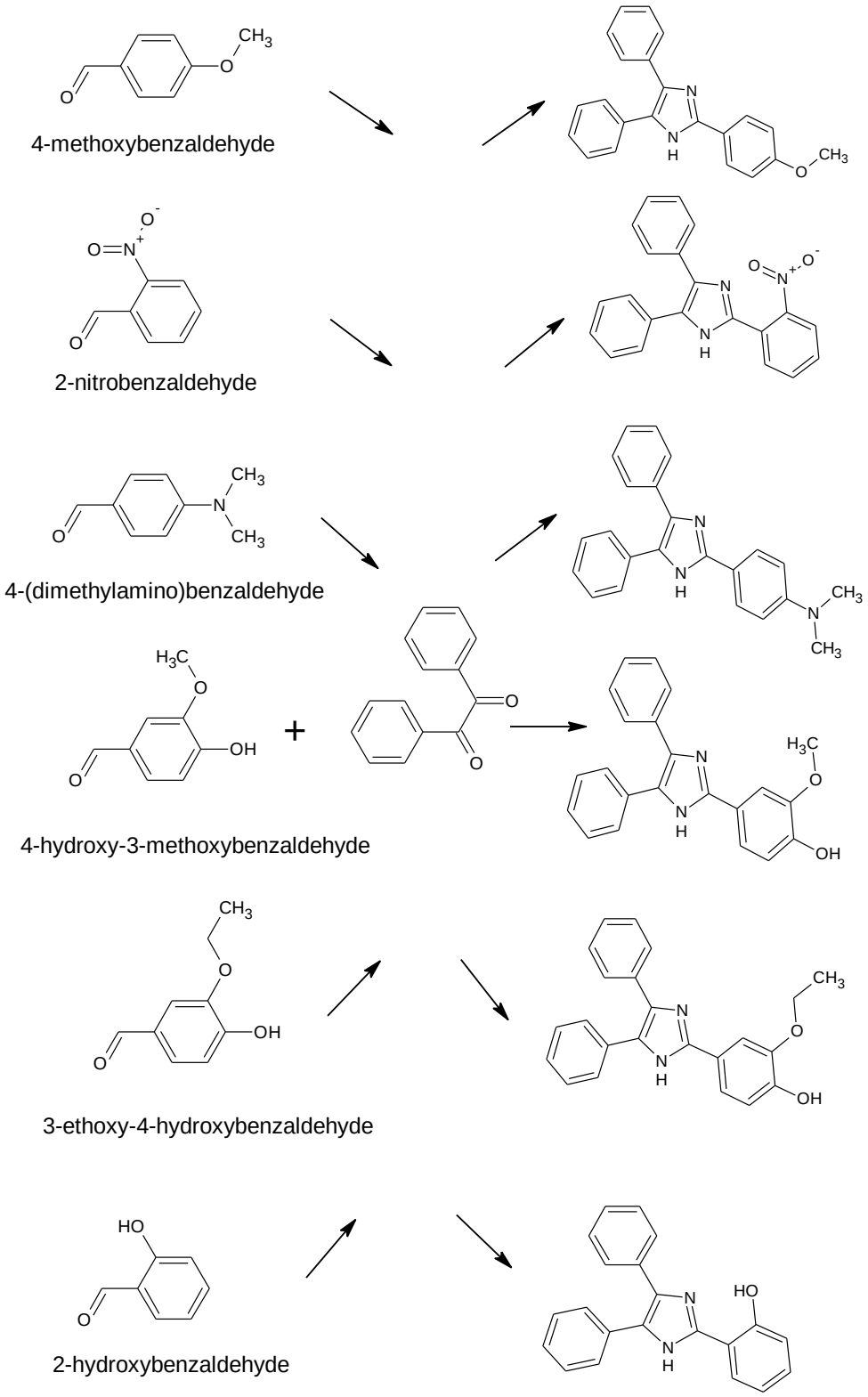
Preparation of 10^{-4} M DPPH:

3.9g of 2, 2-diphenyl-1-picrylhydrazyl (DPPH) was accurately weighed and dissolved in ethanol to prepare 10^{-4} M solution of DPPH in ethanol.

Procedure:

To 2mL of 10^{-4}M solution of DPPH in ethanol, 1mL of various concentration of drug was added. The total assay volume was made up to 5mL with ethanol. The mixture was allowed to stand in water bath at 30^0C for 30 minutes after which the absorbance was measured at 517nm against a DPPH control containing 1mL of ethanol in the place of the drug.

SYNTHETIC SCHEME



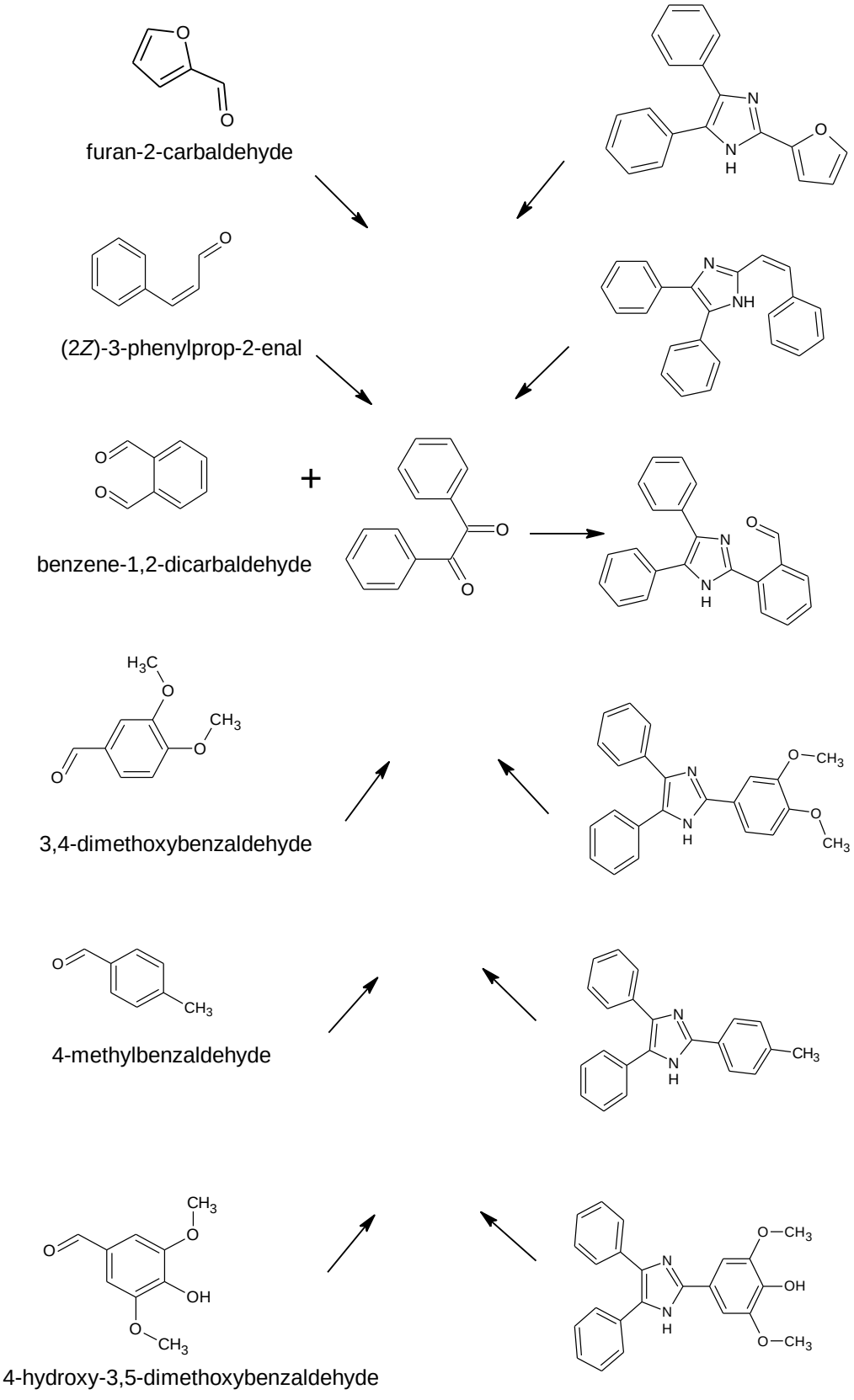

furan-2-carbaldehyde
(2Z)-3-phenylprop-2-enal
benzene-1,2-dicarbaldehyde
3,4-dimethoxybenzaldehyde
4-methylbenzaldehyde
4-hydroxy-3,5-dimethoxybenzaldehyde

Table-1 Membrane Stabilizing Effect

Conc. µg/mL	% Membrane Stabilizing Effect												
	Std (Diclofenac Potassium)	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
88.88	48.71	46.69	33.49	30.66	48.5	45.2	41.98	38.20	42.45	42.20	41.98	37.40	27.35
177.77	56.62	48.58	42.45	32.54	50.4	47.60	43.86	42	48.92	45.98	44.33	45.62	33.01
266.66	65.98	52.35	48.11	36.32	53.7	52.01	47.16	49.52	52.05	51.05	50.11	54.40	36.32
355.55	73.22	56.60	55.66	39.62	59.4	55.32	53.30	56.60	56.78	59.35	53.35	57.08	46.22
444.44	78.71	58.01	57.07	43.39	64.6	59.05	55.66	61.32	58.96	62.82	58.01	57.98	54.71
IC_{50}	96.61	195.74	305.59	635.27	151.87	220.5	305.50	276.23	226.66	229.54	276.26	258.28	403.77

Table-2 Antioxidant activity

Conc. µg/mL	% Free radical scavenging effect												
	Std (Ascorbic acid)	G1	G2	G3	G4	G5	G6	G7	G8	G9	G10	G11	G12
40	47.07	32.38	26.80	31.70	38	30.34	38.23	38.91	34.96	27.61	26.39	30.20	31.15
80	52.10	37.00	33.74	37.82	43.8	36.46	40.54	45.71	42.44	31.83	28.43	31.83	36.19
120	58.23	43.80	43.67	40	45.31	45.17	45.17	48.16	50.74	35.23	33.46	33.74	41.90
160	65.17	50.47	50.47	44.76	50.47	50.47	50.47	52.65	55.23	40.81	40	39.18	45.44
200	69.93	55.64	53.19	49.38	58.36	55.83	56.32	57.27	57.27	50.06	45.30	47.07	51.83
IC_{50}	62.16	160.95	155.99	207.64	143.73	160.54	153.43	133.37	133.04	215.70	243.78	252.35	188.74

G1 2-(4-methoxyphenyl)-4,5-diphenyl-1*H*-imidazole, **G2** 2-(2-nitrophenyl)-4,5-diphenyl -1*H*-imidazole, **G3** 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-*N*,*N*-dimethylaniline, **G4** 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-2-ethoxyphenol, **G5** 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-2-ethoxyphenol, **G6** 2-(4,5-diphenyl-1*H*-imidazol-2-yl)phenol, **G7** 2-(furan-2-yl)-4,5-diphenyl-1*H*-imidazole, **G8** 4,5-iphenyl-2-[(*Z*)-2-phenylethenyl]-1*H*-imidazole, **G9** 2-(4,5-diphenyl-1*H*-imidazol-2-yl)benzaldehyde, **G10** 2-(4-methylphenyl)-4,5-diphenyl-1*H*-imidazole, **G11** 2-(3,4-dimethoxyphenyl)-4,5-diphenyl-1*H*-imidazole, **G12** 4-(4,5-diphenyl-1*H*-imidazol-2-yl)-2,6-dimethoxyphenol.

Percentage scavenging activity was calculated using the expression:

% Scavenging Activity= C-T/C*100

IC_{50} values for DPPH free radical scavenging activity was determined by linear regression analysis.

RESULTS AND DISCUSSION:

The results of the membrane stabilizing activities of the synthesized compounds are presented in **Table-1**. The results showed that the synthesized compounds are good on human erythrocyte adequately protecting it against heat and hypotonic induced lysis. The activity was compared to that of the standard anti-inflammatory drug (Diclofenac potassium). The synthesized compounds were allowed to react with a stable radical (DPPH) in ethanol. The radical scavenging activities of the sample at different concentrations were determined from the reduction in absorbance at 517nm due to scavenging of stable DPPH free radicals.

$$DPPH + AH \longrightarrow DPPH\text{-}H + A$$

The scavenging effects of the drugs on DPPH radical are shown in **Table-2**. All the synthesized compounds exhibited good antioxidant activity.

REFERENCES:

1. Varadarasou Mouttaya Mounnissamy, Subramanian Kavimani, Vaithilingam Balu, Sabarimuthu Darlin Quine. Evaluation of anti-inflammatory and Membrane stabilizing properties of ethanol extract of Cansjera rheedii. J Gmelin(Opiliaceae). Iranian J Pharmacolo Therapeutics. 2007; 6:235-237.
2. Omale James, Okafor Polycarp Nnacheta, Hassan Sanusi Wara, Umar Rabiu Aliyu. *In-vitro* and *In-vivo* studies on the antioxidative activities, membrane stabilization and cytotoxicity of water spinach (Ipomoea aquatic Forsk) from ibaji ponds, Nigeria. Int J PharmTech Res 2009; 1(3): 474-482.
3. Cassidy SL, Henry JA, Hafizi S. Antagonist studies using the erythrocyte osmotic fragility membrane stabilization assay. Toxic. *In-vitro* 1989; 3:1-5.
4. Brand Williams W, Cuvelier ME, Berset C. Use of a free radical method to evaluate antioxidant activity. Lebensm Wiss Technol 1995; 28: 25-30.
5. Tsimogiannis DI, Oreopoulou V. Free radical scavenging and antioxidant activity of 5, 7, 3V, 4V-hydroxy-substituted flavonoids. Innovative Food Science and Emerging Technologies. 2004; 5:523– 528.