Antioxidant Activity of Diphenylpropionamide

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Summary

In the present study we report the synthesis, antioxidant and antiproliferative activity of synthetic diphenylpropionamide derivatives. Synthesis of compounds was obtained by direct condensation of 2,2-(or 3,3)-diphenylpropionic acid and appropriate amine using 1-propylphoshonic acid cyclic anhydride (PPAA) as catalyst.

Structures of these compounds were elucidated by ^{1}H-NMR method and their melting points were measured. Antioxidant activity of these compounds was tested by using ABTS method.

Keywords*:* Diphenylpropionamide derivatives, antioxidant activity.

Introduction

Amides are very abundant in the plant, being extracted from the dry fruits. Various biological activities have been attributed to amides and their derivatives including pharmacological roles, prevention and treatment of tissue damage, the involving in inflammatory sites, the treatment of psoriasis, ulcerative colitis etc[1]. The easy access to plant material, the abundance of natural product as well the easy of extraction made amide a useful starting material for the preparation of potentially bioactive compounds[2].

Antioxidant activity (AOA) is a very important parameter used to characterize different plant materials. This activity is related with compounds capable of protecting a biological system against the potential harmful effect of oxidative processes. These reactions cause excessive oxidation, involving reactive oxygen and nitrogen species (RONS) [3,4]. Excess free radicals can result from a variety of condition such as tissue damage, overexposure to environmental factors, a lack of antioxidants, or destruction of free radical scavengers [5]. Then, antioxidants are essential to preserve the biological system from free radicals damage to biological molecules.

Antioxidants have received increased attention in the last years by nutritionist and medical researchers for their potential activities in the prevention of several degenerative diseases such as cancer and cardiovascular disorder as well as aging [6-8].

There are a great number of antioxidant compounds of natural sources and some of these are used in clinical trials [9-12]. In the recent years, several works have been published on structure-activities analysis on compounds with antioxidant activities. In the work of Rasulev [13] on flavonoids, it was shown that quantum-chemical descriptor are very important in the description of their activities.

Instead there are few reports on antioxidant activity of synthetic compounds. [14-16]. Therefore the synthesis of new active derivates with potential application, prepared with simple chemical procedure will be of increasing interest.

In the present study we report the chemical synthesis of some compounds structurally related to diphenylpropionamide derivatives, their biological activity.

Experimental Section

Reagents and apparatus. Analytical grade, methanol, dichloromethane, diethylether, ethanol were obtained from Carlo Erba (Italy). Methanol and dichloromethane (HPLC grade) from Merck (Darmstadt, Germany) were used. 2,2'-Azino-bis-(3 - ethylbenzothiazoline - 6 - sulfonic acid) (ABTS) as the crystallized diammonium salt was purchased from Fluka; 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox) was purchased from Aldrich and potassium persulfate ($K_2S_2O_8$) were purchased from Sigma Chemical Co. (Italy).

Spectrophotometric measurements were recorded at controlled room temperature (25 °C) with a Varian DMS 90 UV-VIS spectrophotometer.

Flash column chromatography was carried out using silica gel 60 (0.040-0.063 mm, Merck). Melting points were determined on a Gallenkamp hot stage apparatus and are uncorrected. ^{1}H-NMR spectra were recorded on a Bruker ARX 300 MHz spectrometer.

General procedure for preparation of compounds *1-11*. Triethylamine (4 eq) was added to a solution of PPAA (2 eq), acid (1 eq), and amine (1.2 eq) in dichloromethane (5 mL), and resulting reaction mixture was stirred overnight at room temperature. Solvent was removed under reduced pressure, and residue

was purified by flash chromatography (silica gel) using *n*-hexane/ AcOEt (1:1) as eluent.

Antioxidant activity assay by ABTS method.

The antioxidant activity of compounds was carried out in triplicate according to ABTS method as described by Miller et al. (*26*) and Miller and Rice-Evans (*27*) and expressed as percentage of the absorbance of the uninhibited radical cation solution and quantified in terms of T.E.A.C.

$ABTS^{\cdot+}$ radical cation is obtained by reacting ABTS with potassium persulfate;. Before the use the mixture is diluted in ethanol at a ratio 1:100 to give an absorbance at λ=734 nm of 0.70 $\pm$ 0.02. TROLOX is used as standard at a concentration of 1 mg/ml and aliquots of Trolox (0,5µl, 1µl, 2µl, 3µl, 5µl and10µl) are added to 1 ml of ethanolic $ABTS^{\cdot+}$ to have a standard curve to which all data are referred. All compounds are dissolved in dichloromethane at a concentration of 20 mg/ml and 5 µl are added to ethanolic $ABTS^{\cdot+}$ to measure absorbance after 1 min.

Antioxidant activity is expressed as percentage of the absorbance of the uninhibited radical solution according to the equation:

$$\% \text{ inhibition}_{(\lambda=734 \text{ nm})} = (1- Abs_c/Abs_0) \times 100$$

where Abs_c is the absorbance of uninhibited radical solution and Abs_0 is the absorbance measured 1 min after addition of compound to assay. The antioxidant activity of samples is expressed as T.E.A.C. (Trolox Equivalent Antioxidant Capacity - µM) [28].

Antioxidant activity assay by in vitro method.

Measurement of reactive oxygen species (ROS). The formation of ROS was evaluated by means of the probe 2',7'-dichlorofluorescein (DCF) according to

the method described by S.L. Hempel et al.[29] and J.P. Crow[30] . Briefly, J774.A1 cells were seeded at a density of 5×10^3 cells/well into 96-well plates and allowed to grow for 48 h. After cell adhesion, compounds 3, 4 and 8 (0.01, 0.1 and 1 μM) were added to the culture medium 6 h before and always simultaneously to dichlorofluorescein-diacetate. 2',7'-Dichlorofluorescein-diacetate (H_2DCF-DA, Sigma) was then added directly to the growth medium at a final concentration of 5 μM and the cells incubated for 1 h at 37°C. H_2DCF-DA is a non-fluorescent permeant molecule which diffuses passively into cells; the acetates are then cleaved by intracellular esterases to form H_2DCF which is thereby trapped within the cell. In the presence of intracellular ROS, H_2DCF is rapidly oxidized to the highly fluorescent DCF. Therefore, cells were washed twice with phosphate-buffered saline (PBS), placed in fresh medium and treated with H_2O_2 3 mM for 30 min. After treatment, cells were washed twice with PBS and the plates placed in a fluorescent microplate reader (LS 55 Luminescence Spectrometer; Perkin Elmer, Beaconsfield, Bucks, UK). Fluorescence was monitored using an excitation wavelength of 490 nm and an emission wavelength of 520 nm. Results were expressed as Relative Fluorescence Units (RFU).

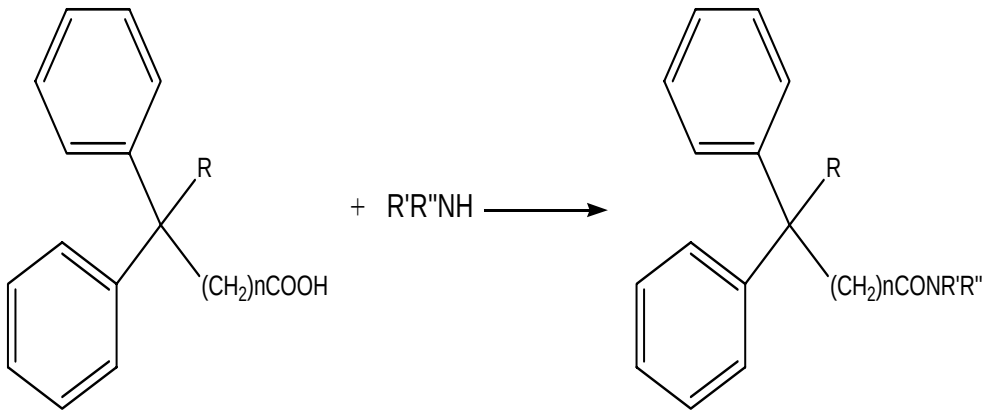
Brine Shrimps Test. The brine shrimps (*Artemia salina*) assay was performed in triplicate with appropriate amounts of samples dissolved in DMSO (1% final volume) to reach final concentrations of 1, 10 and 100 ppm, using 10 freshly hatched larvae suspended in 5 ml of artificial sea water [34]. Briefly, for each dose tested, surviving shrimp were counted after 24 h, and the data analyzed by the Finney program [35], which affords LD_{50} values with 95% confidence intervals.

Results and discussion

As shown in Scheme 1, amides **1-11** were obtained by direct condensation of 2,2-(or 3,3)-diphenylpropionic acid and appropriate amine using 1-propylphoshonic acid cyclic anhydride (PPAA) as catalyst [17].

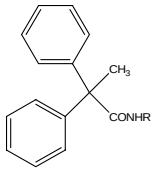
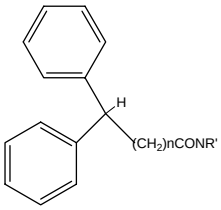
In Table 1 are reported the structures of compounds tested as well as their antioxidant activity and biological activity in brine shrimp test.

The maximum antioxidant activity was exhibited by compounds **3** and **8** (78.19% and 71.4% respectively), having a morpholinic ring as substitute R''. Also compounds **4** and **9**, with a methoxyphenyl-methyl as substitute R'', showed a moderate antioxidant activity (41.8% and 33.93% respectively).



Scheme 1. Reagents and conditions: PPAA, TEA, dichloromethane, RT overnight

Table I. **(a) Percentage of the absorbance of the uninhibited radical cation solution obtained by using ABTS method. (b) Antioxidant activity of samples expressed as T.E.A.C (µM). (c) Biological activity in brine shrimps assay of samples. In square parenthesis are shown the values of 95% confidence intervals; n.a. no activity, (d) n:1.**

Cmp	R	(a) % inhibition ABTS +	(b) T.E A.C (µM)	(c) LD_{50} (ppm) [95% confidence intervals]
				
1	4-methoxy-phenyl	3.4	0.8	4.94 [11.04/1.73]
2	3,4-dimethoxy-benzyl	3.06	0.7	9.45 [16.25/5.43]
3	4-morphiln-phenyl	78.19	20.3	9.66 [17.51/5.27]
4	4-methoxy-benzyl	41.8	10.8	5.9941 [10.7/3.16]
5	4-methoxy-phenyl-ethyl-	0.67	0.17	3.4918 [5.77/2.05]
				
6[(d)]	4-methoxy-phenyl	n.a.	n.a.	14.34 [54.34/4.8]
7	3,4-dimethoxy-benzyl	7.63	1.98	11.97 [28.93/5.22]
8	4-morphiln-phenyl	71.4	18.6	n.a.
9	3,5-dimethoxy-benzyl	33.93	8.8	25.13 [123.08/9.36]
10	4-fluoro-N,N-diethylbenzylamine	4.17	1.08	1.38 [2.19/0.75]
11	4-methoxy-phenyl-ethyl-	n.a.	n.a.	14.45 [66.38/4.35]

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