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ORIGINAL ARTICLE

A comparative study of the antioxidant activities of three series of first-and second-generation PPI dendrimers (G1 and G2) functionalized by 2-hydroxy-P-naphthoquinone

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ABSTRACT

The work presented focuses on the synthesis of molecules capable of blocking the deleterious effects of oxidative stress. Oxidative stress is caused by an imbalance between the pro-oxidant and antioxidant systems, with the former benefiting. It is implicated in numerous pathologies, either as a cause or a consequence. Oxidative stress is involvedinchronicprocessessuchasatherosclerosisanddiabetes. Thework presented in this article focuses firstly on the synthesis of a new class of first- and second-generation PPI (Polypropylene Imine) dendrimers (G1 and G2) functionalized with 2-hydroxy-p-naphthoquinone, and secondly onthe assessment of their antioxidantactivity.

A study of antioxidant activity using the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method revealed that the dendrimers synthesized have antioxidant activity. All the results obtained were interesting.

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Keywords: Dendrimers, 2-hydroxy-p-naphthoquinone, Antioxidant, DPPH, PPI

INTRODUCTION

Oxidative stress is caused by a high and continuous production of reactive oxygen species (ROS) combined with an antioxidant response by the body [1]. It damages biomolecules such as lipids, proteins and nucleic acids. Oxidative stress is the cause of a number of pathologies, including asthma, cancer, cardiovascular disease, diabetes, inflammatory diseases, liver disease and degenerative diseases.[2]

Under normal circumstances, the body establishes a balance known as the antioxidan-prooxidant balance by regulating the adaptive defense system.[3] . To protect itself, the body sets up an antioxidant defense system. Antioxidants are substances that reduce or inhibit oxidative stress [4] .

As a result, many researchers have turned their attention to antioxidants to find solutions to the problems caused by oxidative stress. Natural antioxidants play an important role in this research. Molecules are isolated and characterized from plants. Among the compounds studied, polyphenols (flavonoids, tannins, flavones, coumarins, etc.) play an important role. [5 6 7 8 9]

Research has been carried out into the synthesis of new antioxidants that are effective against diseases linked to free radicals. Synthetic molecules include a new α-aminophosphonic acid

Obtained by Madi Djelloul (2023)[10] . The biosynthesis and evaluation of the antioxidant activity of CuO nanoparticles was studied by Gharnout Ghizlane (2023)[11] .

In this context, the work presented in this article involves a comparative study of the antioxidantactivitiesofthreeseriesoffirst-andsecond-generationPPI dendrimers(G1and G2) functionalizedwith 2- hydroxy-p-naphthoquinone. Thedendrimersweresynthesized using the 'one pot' method of the modified Manich reaction.

Generally speaking, dendrimers refer to a family of polymers with a tree-like, three-dimensional, perfectly structured, monodisperse architecture. [12] and hyperbranched[13] .

These are original, innovative molecules whose chemistry has been booming for years. Dendrimers have been evaluated for various biomedical applications, in particular as contrast agents in medical imaging or as drugs.[14] .

Dendrimers have antiviral, antibacterial and antitumor properties. These molecules can be used to develop new organic/inorganic materials with controlled structures [13] but also to modify, on a nanometric scale, the surface of existing materials[15] .

The Lawsonused in thesynthesis of dendrimers is a derivative of para-naphthoquinone (p-NQ) substituted in position 2 bya hydroxyl group(OH). It is a compoundinthe quinone family. Individually, it has biological activity, particularly antifungal activity. It can be found in the branches and leaves of Lawsonia inertie and Lawsonia alba.

Themolecularstructures of naphthoquinonesgivethem redoxproperties, makingthem active in a number of oxidative biological processes. They are found in various plant families, confirming the antioxidant power of naphthoquinones.

They have been used in traditional medicine by indigenous Amerindian populations for the treatment of a number of diseases, such as cancer.[16 17] .

Based on reports from the study carried out by Dr Gonçalves de Lima in Recife (Pernambuco, Brazil)[18 19] and his team on the microbicidal activity of naphthoquinones, other biological activities were found. Today, naphthoquinones are used in a number of areas,

Particularly in medicine.

In this article, the antioxidant activity of the molecules synthesized was assessed using DPPH (1,1-diphenyl-2-picryl-hydrazyl) as the reducing agent.

MATERIALS AND METHODS

Hardware

PPI G1-G5 dendrimers were purchased from SyMO-Chem B. V/University of Heindoven (The Netherlands). NMR spectra of the compounds were recorded at 400 MHZ for the proton[1] H and at 75.5 MHZ for[13] C on a BRUKER AM 400 WB high-field spectrometer at the Western Regional Centre for Physical Measurements (CRMPO) at the University of Rennes 1. The antioxidant activity of the synthesized compounds was assessed using a control absorbance UV spectrophotometer (SPECORD 200 PLUS) (0.3 mL DPPH and 2.7 mL methanol) at the Electrochemistry and Membrane Processes Laboratory (LEPM), Higher Polytechnic School of Cheikh Anta Diop University of Dakar (Senegal).

General procedure of the synthesis

In a 150 mL Erlenmeyer flask protected from light, a solution of the appropriate Dendr-(NH)2n in 10 mL of absolute ethanol was added to a suspension of 4-hydroxynaphthoquinone in 20 mL of absolute ethanol with magnetic stirring at room temperature. The gradual disappearance of the suspended solid observed was marked by the formation of a white solution. To ensure complete formation, the solution was left to stir for 15 minutes. Thealdehydeinquestionwasthenaddedusinga syringe(acetaldehyde for compounds 1a and 2 and benzaldehyde for compound 1b).

The resulting reaction mixture is then left under magnetic stirring at room temperature for 12 hours in the dark.

The precipitate formed was wrung out, washed with ethanol (2 times) and then with petroleum ether (2 times) before being dried in a thermostatic oven at 45°C for 1 hour.

Compounds 1a, 1b, and 2 were prepared according to the reaction scheme below:

-CHARACTERISATION OF COMPOUND 1A

- IR (KBr) /cm$_{\nu max}$-1 : 3426 (γO-H) ; 3066 (γC-H) ; 2954 (γC-H) ; 2818 (γC-H) ; 1680 (γC=O) ; 1590 (γC=C) ; 1536 (δN-H) ; 1278 ( ). γC-O

- NMR ^{1}H (DMSO-d6 , 400 MHz):δ(ppm) 16.80 (br. s, OH); 8.95 (s, NH); 7.90 (d, J^{3}HH

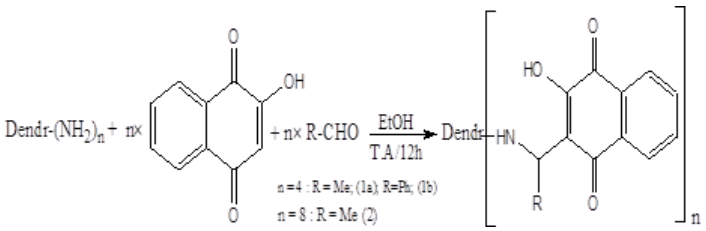

Figure 1 1: General equation for obtaining compounds 1a, 1b and 2

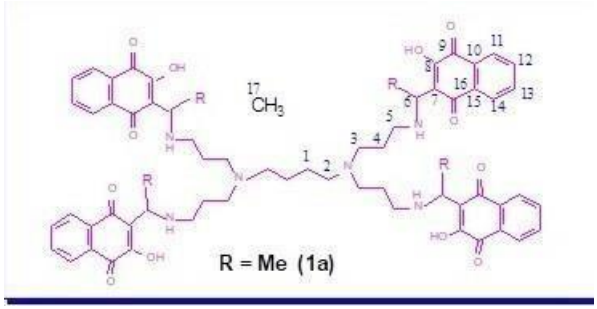

Figure 2 Structure of compound 1a

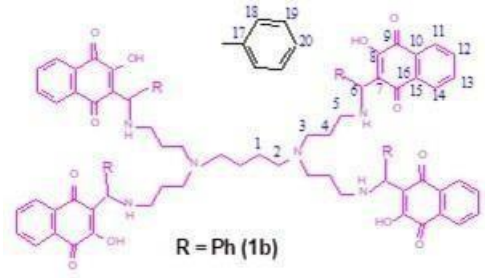

Figure 3 Structure of compound 1b

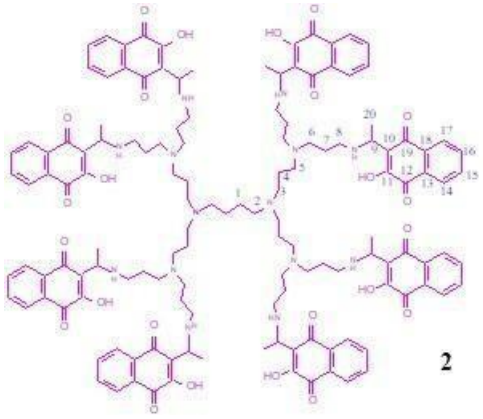

Figure 4 Structure of compound 2

= 6.8 Hz, H^{11} -Napht, 4H); 7.83 (d, J^3HH = 6.4 Hz, H^{14} -Napht, 4H); 7.68 (t, J^3HH = 7.6 Hz, H^{12} -Napht, 4H); 7.56 (t, J^3HH = 7.2 Hz, H^{13} -Napht, 4H); 4.56 (q, J^3HH = 4.4 Hz, H^6 , 4H);2.81 (s, H^5 , 8H); 2.40-1.95 (s [2.23 ppm (H^3 , 8H) + 2.14 ppm (H^2 , 4H)], 12H); 1.61 (s, H^4 , 8H); 1.43 (d, J = 4.4 Hz, H^{17} , 12H); 1.22 (s, H^1 , 4H).

- **NMR^{13} C (DMSO-d6 , 75.5 MHz): δ(ppm)** 184.7 (d, *J* = 28 Hz, C=O); 178.9 (d, *J* = 20 Hz, C=O); 170.2 (t, *J* = 64 Hz, C-OH); 134.6 (d; C^{IV} -Napht); 133.7 (CH-Napht); 131.3 (C^{IV} -Napht) ; 130.8 (CH-Napht); 125.4 (CH-Napht); 125.1 (CH-Napht); 111.6 (C^7); 52.5 (s, C^2); 51.3 (s, C^6) 50.7 (s, C^3); 43.6 (d, C^5); 23.6 (C^1); 23.0 (C^4); 17.42 (s; C^{17}).

- **Mass (ESI/ CH3 OH-CH3 Cl-95:5).** m/z theoretical [found (uncertainty)] **[M-4H+3Na]$^-$ (C H $N_{6468612}$ O Na3**) 1181.45938 [1181.4594 (0 ppm)].

-CHARACTERISATION OF COMPOUND 1B

- **IR (KBr) /cm$^{\upsilon}$ max-1** : 3424 (γO-H) ; 3060 (γC-H) ; 2950 (γC-H) ; 1677 (γC-H) ; 1679 (γC=O) ; 1591 (γC=C) ; 1522 (δN-H) ; 1276 ( ). γC-O

- **NMR^1 H (DMSO-d6 , 400 MHz):δ (ppm)** 16.50 (s, OH) ; 9.60 (s, NH) ; 7.88 (d, J^3_{HH} = 8 Hz, H^{11} -Napht, 4H) ; 7.81 (d, J^3HH = 7.6 Hz, H^{14} -*Napht*, 4H); 7.66 (t, J^3HH = 7.2 Hz, H^{12} -*Napht*, 4H); 7.50-7.60 (m, H^{13} -*Napht* + *H-Ph*, 12H); 7.21-7.33 (m, *H-Ph*, 12H); 5.55 (s, H^6 , 4H); 2.91 (s, H^5 , 8H); 2.40-2.00 (s [2.28 ppm (H^3 , 8H) + 2.11 ppm (H^2 , 4H)],12H); 1.69 (s, H^4 , 8H); 1.22 (s, H^1 , 4H).

- ^{13} **C NMR (DMSO-d6 , 400 MHz):δ (ppm)** 184.5 (d, *J* = 88 Hz, C=O); 178.9 (d, *J* = 84

Hz, C=O); 170.0 (d, *J* = 88 Hz, C-OH); 138.4 (C^{IV} -Ph); 134.3 (d, C^{IV} -*Napht*); 133.6 (CH-*Napht*); 131.3 (C^{IV} -*Napht*); 130.9 (CH-*Napht*); 128.2 (*CH-Ph*); 127.7 (*CH-Ph*) ; 127.6 (*CH-Ph*); 125.3 (CH-*Napht*); 125.0 (*CH-Napht*); 111.2 (C^{IV} , C^3 -*Napht*); 58.7 (PhCHN);52.2 (t; NCH2 CH2 CH2 CH2 N); 50.5 (d, NCH2 CH2 CH2 NH); 44.7 (t, NCH2 CH2 CH2 NH); 22.4 (s, NCH2 CH2 CH2 CH2 N); 23.5 (s, NCH2 CH2 CH2 NH).

• **Mass (ESI/CH3 OH-CH3 Cl-95:5).** m/z theoretical [found (uncertainty)] **[M-H]⁻ (C H $N_{8479612}$)** O1363.57615 [1363.5758 (0 ppm)] ; **[M-2H+Na]⁻ (C H $N_{8478612}$ ONa)** 1385.55809 [1385.5564 (1 ppm)] ; **[M-3H+2Na]⁻ (C H $N_{8477612}$ ONa2)** 1407.54004 [1407.5389 (1 ppm)]); **[M-4H+3Na]⁻ (C H $N_{8476612}$ ONa3)** 1429.52198 [1429.5200 (1 ppm)].

-CHARACTERISATION OF COMPOUND 2

- **IR (KBr) /cmγmax-1** : 3412 (γO-H) ; 3066 (γC-H) ; 2954 (γC-H) ; 2823 (γC-H) ; 1679 (γC=O) ; 1591 (γC=C) ; 1522 (δN-H) ; 1277 ( ). γC-O

- **NMR^1 H (DMSO-d6 , 400 MHz):** δ **(ppm)** 16.85 (s, OH); 8.97 (s, NH); 7.88 (s, H^{14} - *Napht*, 8H); 7.81 (s, H^{17} -*Napht*, 8H); 7.64 (d, J^3HH = 8 Hz, H^{15} -*Napht*, 8H); 7.53 (s, H^{16}-*Napht*, 8H) ; 4.56 (s, H^9 , 8H); 2.79 (s, H^8 , 16H); 2.26 (s, H^6 + H^5 + H^3 + H^2 , 36H); 1.62 (H^7 , 16H); 1.43 (s, H^{20} , 24H); 1.23 (s, H^4 + H^1 , 12H).

- **NMR^{13} C (DMSO-d6 , 400 MHz):** δ**(ppm)** 184.7 (s, C=O) ; 179.1 (s, C=O) ; 169.9 (t, *J*= 64 Hz, C-OH) ; 134.4 (d, C^{IV} -*Napht*) ; 133.6 (d, *CH-Napht*) ; 131.6 (*CH-Napht*) ; 131.2(C^{IV} -*Napht*) ; 130.7 (*CH-Napht*) ; 125.3 (*CH-Napht*); 125.0 (*CH-Napht*); 111.6 (s, C^{10} - *Napht*); 51.3 (s, C^9); 50.5 (s, NCH2 CH2 CH2 NH); 43.8 (d, NCH2 CH2 CH2 NH); 23.2 (s; NCH2 CH2 CH2 NH); 17.47 (s, C).^{20}

• **Mass (ESI/CH3 OH-CH3 Cl-80 : 20).** m/z theoretical (found (uncertainty)) **[M-H]⁻ (C H $N_{1361591424}$)** O2372.16572 (2372.1561 (4 ppm)).

ASSESSMENT OF ANTIOXIDANT ACTIVITY

2.3.1. **Principle**

The principle of this method is to use the violet-colored DPPH as a free radical scavenger. In the presence of an antioxidant (A-H), DPPH turns yellow. As the change in color is proportional to the antioxidant power of a substance, it can be monitored by UV-visible spectrometry by measuring the decrease in absorbance at 517 nm caused by the antioxidant.

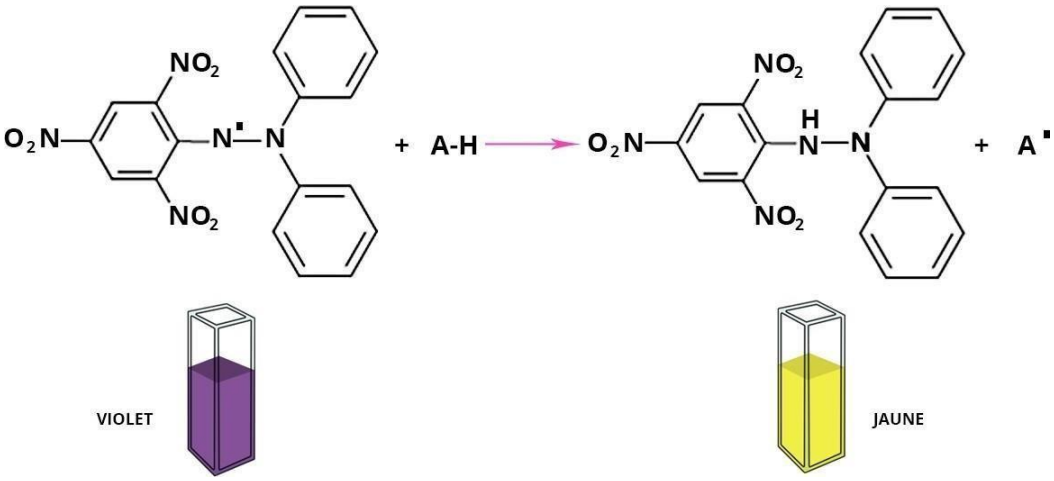

Figure 5 Modification of DPPH- during electronic transfer

EXPERIMENTAL PROTOCOL

The antioxidant activity of the compounds was assessed by monitoring thescavenging kinetics of the DPPH (1,1-diphenyl-2-picryl-hydrazyl) radical. Tests were carried out with 2,2- diphenyl-1-pycrilhydrazyl (DPPH) using the method reported by Oliveira et al., [20] with some adjustments. A mass of 0.10 g of compounds (1a, 1b and 2) was dissolved in 25 mL of

Methanol to obtain a solution with a concentration of 4000 μg. mL^{-1}. Thus, 2.7 mL of DPPH (40 μg. mL^{-1}) prepared in methanol was introduced into a test tube containing 0.3 mL of the solution. The mixture was stirred for five (5) minutes and then incubated in the dark at room temperature for 30 minutes. After this incubation period, the absorbance was read at 517 nm against a blank (0.3 mL of the solution and 2.7 mL of methanol) using a UV spectrophotometer (SPECORD 200 PLUS). The absorbance of the control (0.3 mL DPPH and 2.7 mL methanol) was determined at this wavelength. The free radical scavenging activity (FSA) is expressed as a percentage of DPPH reduced using the following equation:

$$AAR\ (\%) = 100\ x\ \left(\frac{Absorbance_{Contrôle} - Absorbance_{échantillon}}{Absorbance_{Contrôle}}\right)$$

RESULTS

The results of the real-life tests are presented in Table 1 as a percentage of DPPH trapping and plotted on a diagram for comparison.

Table 1: Percentage of DPPH reduced by compounds 1a, 1b

Samples	1a	1b	2
AAR (%)	23,237	2,441	5,244

DISCUSSION

The products were obtained using the synthesis method developed by Baramee et al., in 2006. However, the thermal conditions were modified for our synthesis, as revealed by Neves et al. [20] .

Syntheses were carried out at room temperature and protected from light due to the degradation of the products after heating above 40°C. The order of introduction of the reagentswasasfollows: 2-hydroxy-p-naphthoquinone, thenthe dendrimer andfinallythe aldehyde.

The products obtained were isolated after precipitation, and purified by washing with ethanol and then petroleum ether. Yields of around 80% were obtained. The products were then

Subjected to chemical characterization: NMR[1] H, NMR[13] C {[1] H}, IR without any further purification operations.

The results obtained from the DPPH free radical absorbance test gave us the percentages of DPPH reduced by the three synthesized products listed in Table 1. In the light of these results, it can be seen that all the dendrimers synthesized have free radical scavenging capacity and that the values for the percentages of DPPH reduced are very different.

A comparison of the percentages of DPPH reduced shows that P 1b<P2< P1a. All three products are synthesized on the basis of naphthoquinone. The only differences are in the generation or in the alkyl radical. 1b, the compound with the lowest antioxidant activity, is a first-generation dendrimer (G1) bearing a phenyl group as a radical. It is followed by compound 2, which is a second-generation dendrimer (G2) with eight methyl groups. This proves that the antioxidant character of the synthesized dendrimers is enhanced more by the methyl groups than by the phenyl groups. However, in the case of compound 2, the antioxidant activity seems to be attenuated by steric hindrance. This observation confirms that the antioxidant activity of the products depends in part on the methyl group and the environment of the dendrimer. For example, 3,3',5,5'-tetra-t-butyl-diphenyl-4,4'-diol showed a protective effect against free radical attack and a protective effect on neuronal cells [21] . This synthetic product has twelve methyl groups in its structure. It is therefore highly likely that its significant antioxidant activity is due in part to the hydroxyl groups, the conjugation of the π in the two benzene rings, but also to the presence of methyl groups. Regarding flavonoids, their antioxidant power can be improved by a few structural factors such as: the number of OH groups available, the C2-C3 double bond and a single OH in the 4' position, a catechol function on the B ring, the presence of C4'-OH and methylation, which has

variable effects[21] . The effect of steric hindrance is felt in dendrimer 2. This is confirmed by comparing the activities of 1a (23.237%) and 2 (5.244%) with those of compounds 1a (23.237%), which is a first-generation dendrimer with four methyl groups, and 1b (2.441%), which has four phenyl groups and is of the same generation as 1a. Our results also show that the activity of the dendrimers synthesized does not depend on the number of hydroxyl (OH) groups in the molecule. For example, compound 1a has the same number of OH groups as 1b, even though their antioxidant activities are very different. This observation is more than valid in view of the results for compound 1a, which has four OH groups and a much higher antioxidant activity than compound 2, which has eight OH groups.

CONCLUSION

This work is devoted to the synthesis and evaluation of the antioxidant activity of new molecules, by developing conjugated PPI dendrimers, easily accessible by a ”one pot” synthesis based on the Mannich reaction. Antioxidants make a significant contribution to disease prevention. This is why, in the pharmaceutical industry, the development of new methodologies for the synthesis and preparation of molecules for therapeutic use has been set as an objective and has become a preoccupation for researchers. It is in this context that three new molecules based on first (G1) and second (G2) generation PPI dendrimers functionalized by 2-hydroxy-p- naphthoquinone have been synthesized for antioxidant purposes.

L The actual structures of the molecules prepared are in line with those expected. They were confirmed by the usual characterization techniques: H NMR[1], C NMR[13] and dept, infrared (IR) and massspectrometry.

Assessment of antioxidant activity using the DPPH method• showed that the dendrimers synthesized (1a, 1b and 2) have very significant antioxidant power.

DECLARATIONS:

Authors' contributions:

Contributor Role	Degree of Contribution		
	Lead	Equal	Supporting
Conceptualization		TN	EGD
Data curation			
Formal analysis	CG	EGD	TN
Funding acquisition		EGD	TN
Investigation		AKD	TN, EGD
Methodology		AKD, CG	TN, EGD
Project administration	AF	MK, MLN	
Resources			
Software		AF; MK	MLN
Supervision		AKD	MD; EGD
Validation		AKD; EGD	MD
Visualization			
Writing-original draft		TN	EGD

Writing-review & editing	TN	EGD

Conflict of interest: None

Ethical Approvals: The research was conducted by ethical standards and guidelines, and any necessary approvals from institutional review boards or ethical committees were obtained.

Funding Resources: None

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