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EVALUATION OF THE ANTIOXIDANT ACTIVITY OF FIRST (G1) AND SECOND GENERATION (G2) PPI DENDRIMERS FUNCTIONALIZED WITH 4-HYDROXYCOUMARIN

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SUMMARY
The work presented focuses on the synthesis and characterization of first- and second-generation dendrimers (G1) & (G2) functionalized with 4-hydroxycoumarin of antioxidant utilities. Today, it is demonstrated with irrefutable evidence that free radicals are the main culprits of aging and degenerative diseases such as cancer, cardiovascular disease, cataracts, immune system decline and brain dysfunction. This is why it is more than important to isolate and ⁄or synthesize new molecules in order to effectively fight against these diseases. The study and evaluation of synthetic antioxidants has become a popular and reliable object of research as it aims to develop and discover powerful new antioxidant molecules without risk to the body. The study of antioxidant activity carried out by the DPPH• method revealed that the synthesized dendrimers exhibit antioxidant activity. All the results obtained have been encouraging. The C2 compound, with a percentage of 56.87% catches our attention.

KEYWORDS: Dendrimers, 4-Hydroxycoumarin, Antioxidant, DPPH, PPI.

1. INTRODUCTION

Dendrimers are large tree molecules constructed by an iterative process from a molecule with at least three reactive sites.[1] They are often comparable to certain proteins ranging in size from 3 to 10 nm.[2] The three parts that make up the structure of a dendrimer are: the heart, the skeleton and the periphery. There are several types of dendrimers such as: PPI (PolyPropyleneImine), PAMAM (PolyAmidoAmine), metallodendrimers, PolyGlycols-co-Succinic Acid dendrimers, Poly(L-Lysine) dendrimers, polyglycerol dendrimers, Poly (2,2'-bis (HydroxyMethyl) Propionic acid and melanin dendrimers. They find their applications in several areas. In medicine, for example, they can be used as anticancer drugs, antimicrobials, antivirals and as transfection agents, gene therapy agents, drug carriers.[3]

This work aims to develop molecules based on PPI dendrimers functionalized with 4-hydroxycoumarin using the "one pot" synthesis method based on the Manich reaction 4-Hydroxycoumarin is a heterocyclic chemical compound with ketone function in the α position relative to oxygen and carrying a hydroxyl group on heterocycle carbon 4. It belongs to the coumarin family characterized by its multiple beneficial properties. The latter is classified as phenolic compounds which are natural antioxidants. Their structures are similar to those of flavonoids.[4] They are used as: anticancer, anti-inflammatory, antibacterial and antiviral. A free radical is an atom or molecule that carries on its peripheral electron layer one or more electrons not coupled to another electron of opposite spin giving it great instability and therefore great reactivity.[5,6] Their primary role, especially when produced in small quantities, is to protect the body from pathogens. Their importance is not limited only to this level, as they participate in the functioning of the body, the transduction of cellular signals, the immune defense against pathogens and also in the process of fertilization, maturation and cell movement.[7] They also play a significant role in blood pressure regulation and heart protection.[8] However, they become dangerous when they are produced in the body excessively. An overproduction of ROS and especially when it occurs in unwanted places can cause damage when they react with important cellular components such as: DNA lipids (peroxidation), proteins.[9,10] There are two modes of production of free radicals: endogenous production and

exogenous production. To cope with this scourge, the use of antioxidants is recommended. By definition, an antioxidant is a natural or synthetic molecule that can inhibit or slow down the oxidation of other molecules that occurs at different stages of the oxidation process.[11] In order to protect certain cellular components such as DNA, lipids and proteins, the body deploys a system of enzymatic antioxidants characterized by the presence of enzymatic antioxidants such as superoxides dismutases (SOD),[12,13] catalysis[14,15] and glutathione peroxidase (GPX) and non-enzymatic antioxidant systems.[16] These non-enzymatic antioxidant systems are divided into two categories such as endogenous antioxidants (molecules from biosynthesis) and exogenous antioxidants (vitamins, trace elements and synthetic antioxidants). Synthetic antioxidants are usually prepared in the laboratory and mainly from chemical components. Often their skeletons derive from existing natural antioxidants.[17] Among the best known we can mention: the BHT, the BHA, the TBHQ ..... Thanks to their stability and resistance to the temperatures reached during cooking or frying, they are much more interesting than natural antioxidants. Antioxidant potency is assessed using several free radical trapping methods such as DPPH, FRAP test, TRAP test, ABTS test and β-carotene.

2. MATERIALS AND METHODS

2.1. EQUIPMENT

The PPI G1-G5 dendrimers were purchased from SyMO-Chem B. V/University of Heindoven (Netherlands). The NMR spectra of the compounds were recorded at 400 MHZ for proton 1H and 75.5 MHZ for 13C on a BRUKER AM 400 WB high-field spectrometer from the Regional Center for Physical Measurements of the West (CRMPO) of the University of Rennes 1.

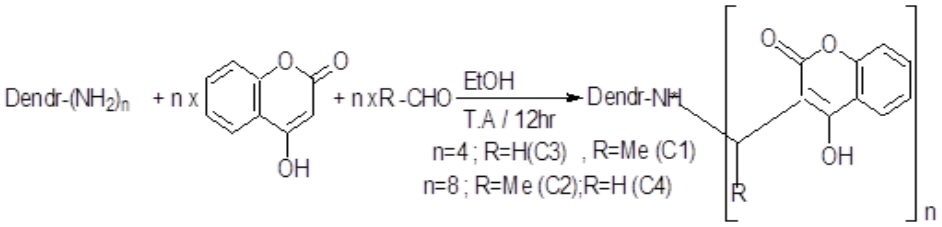
The evaluation of the antioxidant activity of the synthesized compounds was made using a UV spectrophotometer (SPECORD 200 PLUS) of control absorbance (0.3 mL of DPPH and 2.7 mL of methanol) at the Water, Energy, Environment and Industrial Processes Laboratory (LE3PI), at the Polytechnic School of Cheikh Anta Diop University of Dakar (Senegal).

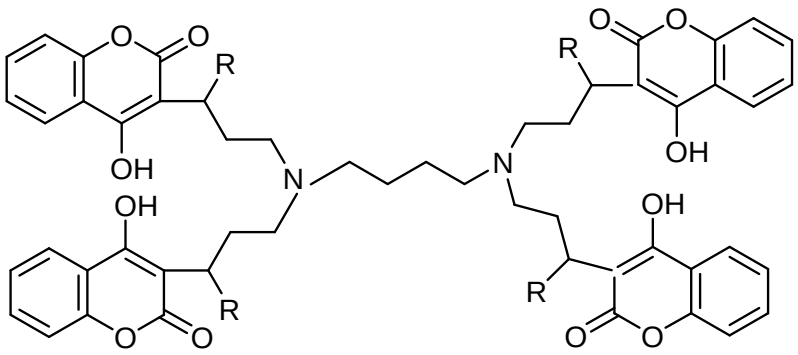
2.2. GENERAL PROCEDURE FOR SYNTHESIS

In a 150 ml Erlenmeyer protected from light, a solution of suitable Dendr-(NH2)4n in 10 mL of absolute etahnol is added to a suspension of 4-hydroxycoumarin in20 ml of absolute ethanol under magnetic stirring at room temperature. The gradual disappearance of the suspended solid observed is marked by the formation of a white solution. In order to ensure complete training, the solution is left stirred for 15 minutes. It is at this time that the aldehyde considered is added with the help of a syringe. The resulting reaction mixture is then left under magnetic stirring at room temperature for 12 hours away from light.

Thus, the precipitate formed is wrung out, washed with ethanol (2 times) and then with petroleum ether (2 times) before drying it in an oven thermostated at 45 ° C for 1 hour.

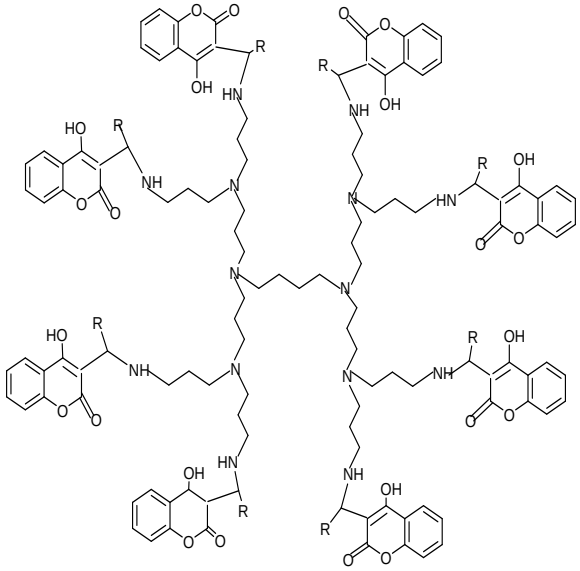
The compounds C1, C2, C3 and C4 have been prepared according to the diagram below:





R : Hydrogen for C3

R: Methyl for C1



R = H for C4
R = CH3 for C2

Characterization of compound C1: **IR (KBr) ν_{max}/cm^{-1}:** 3399 (ν_{O-H}); 3058(ν_{C-H}); 2954 (ν_{C-H}); 1678 ($\nu_{C=O}$); 1603 ($\nu_{C=C}$); 1524 (δ_{N-H}); 1278 (ν_{C-O}).

RMN ^{1}H (DMSO-d_6, 400 MHz): δ (ppm) ; 7,76 (d, *J* = 7,8 Hz, H^{10}-*Coum*, 4H) ; 7,73 (t, $^3J_{HH}$ = 7.5 Hz, H^{12}-*Coum*, 4H); 7,61 (d, *J* = 7,2 Hz, H^{13}-*Coum*, 4H) ; 7,36-7,39 (t, H^{11}-*Coum*, 4H) ; 5,53 (s, H^6, 4H) ; 2,90 (s, H^5, 8H) ; 2.40-2,00 (s [2,28 ppm (H^3, 8H) + 2,11 ppm (H^2, 4H)], ; 1,69 (s, H^4, 8H) ; 1,22 (s, H^1, 4H).

^{13}C RMN (DMSO-d_6, 400 MHz): δ (ppm) 165 (d, *J* = 88 Hz, C=O) ; 164,0 (d, *J* = 88 Hz, C-OH) ; 152,5 (t, C =); 131,8 (CH ,Coum) ; 123,9 (d, CH-*Coum*) ; 133,6 (CH-*coum* ; 131,3 (C^{IV}- ; 125,3 (CH-*coum*) ; 19,70 (CH_3) ; 111,2 (C^{IV}, C^3-*coum*) ; 57,7 (RCHN) ; 52,2 (t; $NCH_2CH_2CH_2CH_2N$) ; 50,5 (d, $NCH_2CH_2CH_2NH$) ; 44,7 (t, $NCH_2CH_2CH_2NH$) ; 22,4 (s, $NCH_2CH_2CH_2CH_2N$) ; 23,5 (s, $NCH_2CH_2CH_2NH$).

Characterization of compound C2: IR (KBr) ν_{max}/cm^{-1}: 3388 (ν_{O-H}); 2054 (ν_{C-H}); 2966 (ν_{C-H}); 1673 ($\nu_{C=O}$) ; 1607 ($\nu_{C=C}$) ; 1532 (δ_{N-H}) ; 1272 (ν_{C-O}).
RMN ^{1}H (DMSO-d_6, 400 MHz): δ (ppm) 7,87 (d, H^{13}-*Coum*, 8H); 7,45 (t, H^{15}-*Coum*, 8H); 7,21 (d, *J* = 8,4 Hz, H^{16}-*Coum*, 8H); 7,16 (t, H^{14}-*Coum*, 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^{19}, 24H) ; 1,23 (s, H^4 + H^1, 12H)

^{13}C RMN (DMSO-d_6, 400 MHz) : δ (ppm) 178,9 (d, *J* = 84 Hz, C=O) ; 170,0 (d, *J* = 88 Hz, C-OH) ; 138,4 (d, C^{IV}-coum) ; 133,6 (CH-*coum*) ; 131,3 (C^{IV}-*coum*) ; 130,9 (CH-*coum*) ; 128,2 (CH-*coum*) ; 127,7 (CH-*coum*) ; 127,6 (CH-*coum*) ; 125,3 (CH-*coum*) ; 125,0 (CH-*coum*) ; 111,2 (C^{10}-*coum*) ; 58,7 (RCHN); 19,70 (CH_3) ; 52,2 (t; $NCH_2CH_2CH_2CH_2N$) ; 50,5 (d, $NCH_2CH_2CH_2NH$) ; 44,7 (t, $NCH_2CH_2CH_2NH$) ; 22,4 (s, $NCH_2CH_2CH_2CH_2N$) ; 23,5 (s, $NCH_2CH_2CH_2NH$).

Characterization of compound C3: IR (KBr) ν_{max}/cm^{-1}: 3400 (ν_{O-H}); 3068 (ν_{C-H}); 2964 (ν_{C-H}) ; 1633 ($\nu_{C=O}$) ; 1600 ($\nu_{C=C}$) ; 1569 (δ_{N-H}) ; 1279 (ν_{C-O}).

RMN ^{1}H (DMSO-d_6, 400 MHz): δ (ppm) 7,91 (d, *J* = 7,8 Hz, H^{10}-*Coum*, 4H) ; 7,88 (t, $^3J_{HH}$ = 7.6 Hz, H^{12}-*Coum*, 4H) ; 7,52 (d, *J* = 8,4 Hz, H^{13}-*Coumt*, 4H) ; 7,30 (t, H^{11}-*Coum* 4H) ; 5,55 (s, H^6, 8H) ; 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 RMN (DMSO-d_6, 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}-*coum*) ; 133,6 (CH-*coum*) ; 131,3 (C^{IV}-*coum*) ; 130,9 (CH-*coum*) ; 128,2 (CH-*Ph*) ; 127,7 (CH-*Ph*) ; 127,6 (CH-*Ph*) ; 125,3 (CH-*coum*) ; 125,0 (CH-*coum*) ; 111,2 (C^{IV}, C^3-*coum*) ; 58,7 (PhCHN) ; 52,2 (t; $NCH_2CH_2CH_2CH_2N$) ; 50,5 (d, $NCH_2CH_2CH_2NH$) ; 44,7 (t, $NCH_2CH_2CH_2NH$) ; 22,4 (s, $NCH_2CH_2CH_2CH_2N$) ; 23,5 (s, $NCH_2CH_2CH_2NH$).

Characterization of compound C4: IR (KBr) ν_{max}/cm^{-1}: 3369 (ν_{O-H}) ; 3068(ν_{C-H}) ; 2881 (ν_{C-H}) ; 1679 ($\nu_{C=O}$) ; 1633 ($\nu_{C=C}$) ; 1568 (δ_{N-H}) ; 1279 (ν_{C-O}).

RMN ^{1}H (DMSO-d_6, 400 MHz): δ (ppm) 7,85 (d, H^{13}-*Coum*, 8H); 7,46 (t, H^{15}-*Coum*, 8H); 7,22 (d, *J* = 8,4 Hz, H^{16}-*Coum*, 8H); 7,16 (t, H^{14}-*Coum*, 8H); 4,56 (s, H^9, 16H); 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)

^{13}C RMN (DMSO-d_6, 400 MHz): δ (ppm) 178,9 (d, *J* = 84 Hz, C=O); 170,0 (d, *J* = 88 Hz, C-OH); 150,4 ($C_{=}$); 138,3 (d, C^{IV}-*coum*) ; 133,6 (CH-*coum*) ; 131,3 (C^{IV}-*coum*) ; 130,9 (CH-*coum*) ; 28,2 (RCH_2) ; 125,3 (CH-*coum*) ; 125,0 (CH-*coum*) ; 111,2 (C^{IV}, C^3-*coum*) ; 52,2 (t; $NCH_2CH_2CH_2CH_2N$) ; 50,5 (d, $NCH_2CH_2CH_2NH$) ; 44,7 (t, $NCH_2CH_2CH_2NH$) ; 22,4 (s, $NCH_2CH_2CH_2CH_2N$) ; 23,5 (s, $NCH_2CH_2CH_2NH$).

2.3. EVALUATION OF ANTIOXIDANT ACTIVITY

2.3.1. EXPERIMENTAL PROTOCOL

The antioxidant activity of the compounds was evaluated by following the trapping kinetics of the DPPH radical (1,1-diphenyl-2-picryl-hydrazyl). Tests were performed with 2,2-diphenyl-1-pycrilhydrazyl (DPPH) following the method reported by Oliveira et al. (2014) with some adjustments. A mass of 0.10 g of extract was diluted in a volume of 25 mL of methanol to obtain a concentration solution 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, then incubated in the dark and 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) is determined at this wavelength. The anti-radical activity is expressed as a percentage of reduced DPPH according to the following equation:

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

2.3.2. RESULTS AND DISCUSSION

2.3.2.1. RESULTS

The results of the actual tests are presented in Table 1 as a percentage of DPPH trapping and reproduced on a diagram for comparison.

Table 1: Percentage of DPPH reduced of C1, C2, C3 and C4 compounds.

Echantillons	C1	C2	C3	C4
AAR (%)	36,98	56,872	27,939	5,425

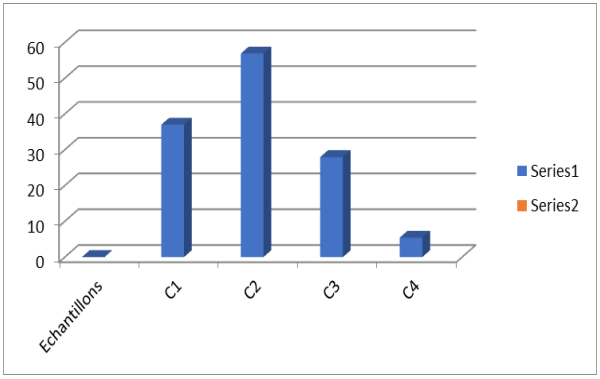


Diagram of percentages of reduced DPPH of C1, C2, C3 and C4 compounds

2.3.2.2. DISCUSSION

To obtain all the products, the synthesis method developed by Baramée et al. 2006, has fundamentally inspired us. However, the thermal conditions underwent a change in the context of our syntheses, as revealed by Neves et al. Thus, the syntheses were carried out at room temperature and away from light due to the degradation of the products after heating to 40 ° C. The order of introduction of the reagents is as follows: 4-hydroxycoumarin, then the dendrimer and finally the aldehyde. The products obtained have been isolated after precipitation, their purification is done by a simple washing with ethanol and then with petroleum ether. Yields of the order of 80% have been obtained. The products were then subjected to chemical characterizations: 1H NMR, 13C NMR {1H}, IR without any further purification operation. The results obtained from the DPPH free radical absorbance measurement test allowed us to trace the histogram of the reduced DPPH percentages of the four synthesized products. In the light of these results, it can be seen that all synthesized dendrimers have a free radical scavenging power and that the values of the reduced DPPH percentages are very different.

Comparing the percentages of reduced DPPH showed us that PC4 <PC3< PC1< PC2. All four products are synthesized on the basis of coumarin. Their only differences are in the generation and the presence or absence of a methyl group (CH3). C4 being the compound that has the lowest antioxidant activity is a second-generation dendrimer (G2) and without methyl group. It is followed by the compound C3 which is a first-generation dendrimer (G1) and with an absence of methyl group. This proves that when the synthesized dendrimer does not have a methyl group, the generation does not influence antioxidant activity. In third place comes the first generation C1 compound (G1) and having four methyl groups. And finally comes the C2 compound which has exhibited the most important antioxidant activity, it is a second-generation dendrimer (G2) with 8 methyl groups. This remark confirms that the antioxidant activity of our products depends in part on the methyl group and the generation if the latter is methylated. 3,3',5,5'-tetra-t-butyl-diphenyl-4,4'-diol has shown a protective effect against free radical attacks and a protective effect of neuronal cells.[18,19] This synthetic product has in its structure twelve methyl groups. So it is very likely that its significant antioxidant activity is on the one hand attributable to hydroxyl groups, the conjugation of π bonds in the two benzene nuclei, but also to the presence of methyl groups. Regarding flavonoids, their antioxidant power can be improved by some structural factors such as: the number of AVAILABLE OH groups, 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.[20] The effect of steric clutter is felt when there are no methyl groups in the structure of the dendrimer. This is confirmed by comparing on the one hand the activities of C4 (5.42%) and C3 (27.93%) on the other hand those of C1 compounds (36.98%) and C2 (56.87%). Our results also show us that the activity of synthesized dendrimers does not depend on the number of hydroxyl groups (OH) contained in the molecule. As proof, the compound C1 has the same number of groups OH as C3 while their antioxidant activities are very different. This remark is more than valid in view of the results of the C1 and C3 compounds which each have four OH groups with an antioxidant activity much higher than that of the C4 compound which has eight OH groups.

4. 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 contribute significantly to the prevention of diseases. This is why, in the pharmaceutical industry, the development of new methodologies for the synthesis and preparation of molecules for therapeutic use are set as objectives and have become concerns among researchers. It is in this context that our team worked on the synthesis of new molecules based on PPI dendrimers functionalized by 4–hydroxycoumarin for antioxidant purposes.

The actual structures of the prepared molecules are consistent with those expected. They were confirmed by the usual characterization techniques: 1H NMR, 13C NMR and dept, infrared (IR) and mass spectrometry.

The study of antioxidant activity carried out by the DPPH• method revealed that synthesized dendrimers exhibit antioxidant activity.

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