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Synthesis and antimycotic activity of new unsymmetrical substituted zinc phthalocyanines

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Abstract—The synthesis of new unsymmetrical substituted zinc phthalocyanines derivatives has been described; moreover the photodynamic activity of some compounds tested against *Candida albicans* has been reported.

1. Introduction

The importance of phthalocyanine derivatives is rapidly growing in many fields.[1–4] One of these is the photodynamic therapy (PDT).[5] A number of substituted phthalocyanines are already known as effective photosensitizers but the interest in this field and the need for new molecules with improved characteristics is always high owing to the large variety of therapeutic applications.

While the synthesis of symmetrically substituted phthalocyanines has been largely investigated,[6–11] only minor attention has been devoted to the synthesis of unsymmetrical substituted phthalocyanines, owing to the problems associated with the low reaction yields and the mixtures of differently substituted products which render their isolation a difficult step.[8]

As part of our current work we were interested to synthesize phthalocyanines comprising of three identical (A) and one different (B) isoindole subunit presenting a single functional group (A_3B type) potentially useful as PDT photosensitizers. The presence of a single reactive functional group might be also particularly interesting for the perspective possibility of a covalent binding of the phthalocyanine moiety to a suitable carrier molecule.[8–12]

Usually the strategies to synthesize unsymmetrical substituted phthalocyanine are: (a) statistical condensation of two differently substituted precursors; (b) the sub-phthalocyanine approach and, (c) the polymeric support method. As previously reported,[13] each strategy is affected by a number of disadvantages; therefore we decided to synthesize the described compounds, according to the statistical approach that is the most useful method for the synthesis of A_3B structures according to our experiences.

2. Results and discussion

The synthesis of substituted phthalocyanines is related to the preparation of the corresponding phthalonitrile precursors. Initially, with the aim at obtaining octasubstituted phthalocyanines endowed with light absorption maxima above 700 nm, we synthesized the 4,5-bis(phenylsulfanyl)phthalonitrile (**2**) as isoindole subunit A, starting from 4,5-dichlorophthalonitrile (**1**) and PhSH in 90% yield by modification (acetone, K_2CO_3, rt, 18 h) of a previously described synthesis.[14] The isoindole component B used, was the 4-[4-(hydroxymethyl)phenoxy]-5-(phenylsulfanyl)-phthalonitrile (**5**) obtained from 4-chloro-5-[4-(hydroxymethyl)phenoxy]phthalonitrile (**4**) which in turn was obtained from **1** and 4-hydroxybenzylic alcohol (**3**) (Scheme 1).

The derivative **5**, was obtained in moderate yield (40%) by treating **4** with thiophenol. In this case the reaction must be carefully controlled because thiophenol is also able to remove the aryloxy moiety giving the undesired **2**.

The cyclotetramerization of phthalonitriles **2** and **5** was performed in refluxing pentanol with diazabycicloundecene (DBU), according to conditions previously reported[14] for

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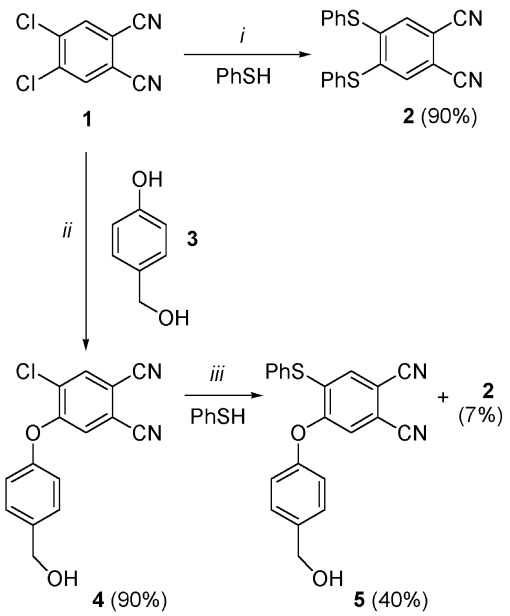


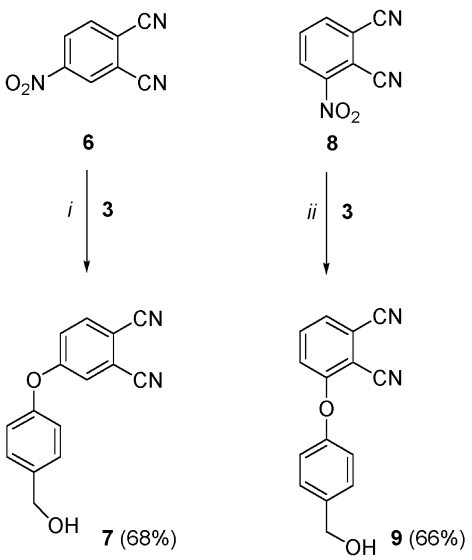
Scheme 1. (i) Acetone, K_2CO_3, rt, 18 h. (ii) Acetone, K_2CO_3, △, 24 h. (iii) Acetone, K_2CO_3, Et_3N, rt, 24 h.

the cyclotetramerization of compound **2** and in the presence of zinc acetate. The reaction was performed using a 9:1 ratio between dinitriles **2** and **5**; according to a published report[13] supporting this ratio to promote the A_3B structure instead of A_2B_2 or AB_3 species. Actually, the crude product of reaction revealed, by ESI-MS analysis, (Table 1, entry 1) a mixture of structures A_4, A_3B, A_2B_2. Unfortunately, this crude mixture was not useful as was impossible to accomplish any isolation of the components from this complex mixture.

To obtain a more manageable mixture of products we progressively attempted at reducing the complexity of isoindoline subunit B by structural variation of the reacting dinitriles (see Table 1). We then allowed the cyclotetramerization of dinitrile **2** with dinitrile **4**, of the dinitrile **2** with 4-[4-(hydroxymethyl)phenoxy]phthalo nitrile (**7**) in turn obtained from 4-nitrophthalonitrile (**6**) (Scheme 2) and, finally, we attempted at the cyclotetramerization of the dinitrile **2** with the 4-nitroderivative **6** or the dichloroderivative **1**.

Table 1. ESI-MS molecular peaks and UV data in the crude of reaction between **2** and the phthalonitriles **5**, **4**, **7**, **6**, **1**

B	Mixture components	Observed molecular peaks *m/z*	Mixture's λ_{max} (nm)
5	A_4	1443.9	709
	A_3B	1457.8	
	A_2B_2	1471.8	
4	A_4	1444.0	707
	A_3B	1384.0	
	A_2B_2	1323.9	
7	A_4	1443.9	709
	A_3B	1349.9	
	A_2B_2	1254.8	
6	A_4	1443.9	710
	A_3B	1272.7	
	A_2B_2	1101.6	
1	A_4	1443.9	706
	A_3B	1295.8	
	A_2B_2	1148.6	

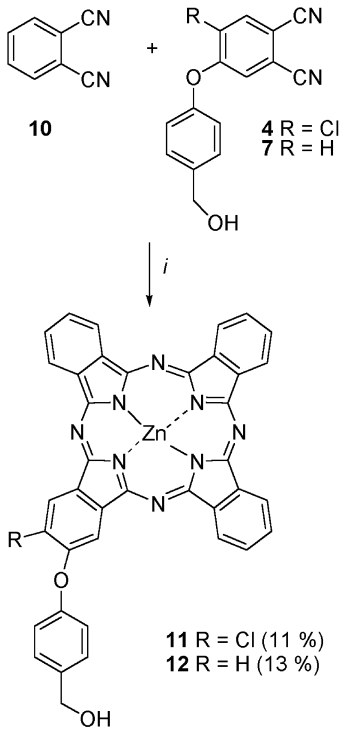


Scheme 2. (i) DMSO, K_2CO_3, rt, 2 d. (ii) DMSO, K_2CO_3, rt, 22 h.

Unfortunately also in all these cases the reactions products resulted in a very complex mixture from which we were unable to separate the components.

On the basis of these results we decided to drastically change the precursor of the isoindoline subunit A, from 4,5-bis(phenylsulfanyl)phthalonitrile (**2**) to the structurally simpler 1,2-dicyanobenzene (**10**).

By allowing the reaction of **10** with derivative **4** in the same reaction conditions (Scheme 3) previously used for dinitriles **2** and **4**, we obtained a mixture of zinc phthalocyanines (identified by ESI-MS spectrum) that can be resolved (see Section 3) by atmospheric pressure silica



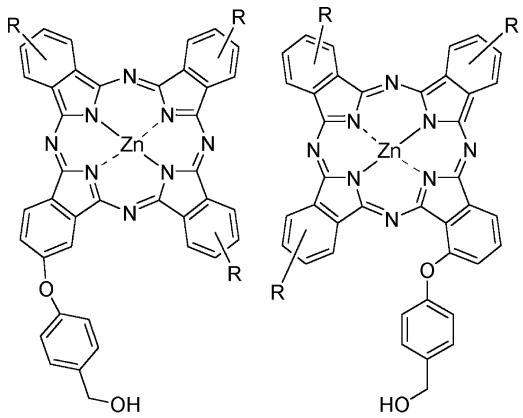
Scheme 3. (i) *n*-Pentanol, $Zn(OCOCH_3)_2$, DBU, △, 48 h.

gel chromatography. We separated zinc phthalocyanine (A_4, 45%); zinc 2-chloro-3-[4-(hydroxymethyl)phenoxy]-phthalocyanine (A_3B, **11**, 9%), and a very little amount of product which was assigned to a structurally related A_2B_2 species by ESI-MS spectra.

As part of this study, we also attempted (results not shown) to vary the reaction conditions to improve the reaction yield and/or the A_3B amount in the reaction products. To this purpose the solvent was changed (neat DBU or DBU in *n*-butanol), the concentration of the reagents was increased and the refluxing time was varied with no improvements. In fact, the only way to improve the yield of A_3B was found by changing the ratio of the dinitriles precursors of the isoindoline subunits A/B to 3:1.

The above reported reactant ratio was then adopted for the following experiments. From reaction between dinitriles **10** and **7** we isolated the desired compound **12** (A_3B, Scheme 3) and a mixture of A_2B_2 phthalocyanines (structures not shown, indicated as **13** in Section 3). Similarly, from the reaction between **10** and **9** we isolated the desired compound **14** (A_3B, 15%, Scheme 4) and a mixture of A_2B_2 phthalocyanines (structures not shown, indicated as **15** in Section 3, 5%).

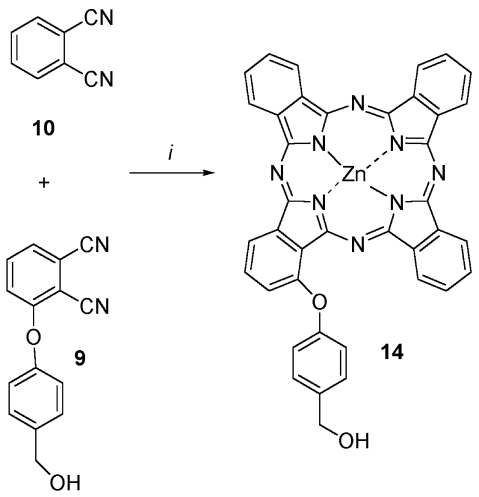
Finally, to evaluate the influence of the 4-(hydroxymethyl)-phenoxy substituent in the phthalocyanine core on both λ_{max} and antimicrobial activity, we synthesized the phthalocyanines corresponding to B_4 structures and prepared from 4-[4-(hydroxymethyl)phenoxy]phthalonitrile (**7**) or from 3-[4-(hydroxymethyl)phenoxy]phthalonitrile (**9**), as mixture of constitutional isomers corresponding to **16** (31%) or **17** (43%), respectively.



16 R = $OC_6H_4CH_2OHp$ **17** R = $OC_6H_4CH_2OHp$

2.1. Antimicrobial activity

Phthalocyanines **11**–**17** were all tested for their photoinhibitory activity against *Candida albicans* as a model to assess the microorganism inactivation (see Table 2). While the B_4 (**16**, **17**) can be considered as inactive along with compounds having the A_2B_2 type of structure (**13**, **15**), one of the mono-substituted compounds (**14**) exhibited some activity, providing a 70% cell photoinactivation at 30 μM concentration in the standard assay employed. These



Scheme 4. (i) *n*-Pentanol, $Zn(OCOCH_3)_2$, DBU, △, 48 h.

Table 2. *C. albicans* photoinactivation by **11**–**17**

Compound	Growth inhibition (%)
11	1
12	10
13	1
14	70
15	1
16	1
17	1

findings indicate that the activity against *C. albicans* presented by mono-substituted compounds may be related to their amphiphilic character. This behavior, strongly noticed for mono-substituted phthalocianines, was previously found during a structure activity relationship study on phthalocyanine quaternary ammonium salt derivatives (Roncucci, personal communication).

In conclusion, in this paper we have presented the synthesis of some new unsymmetrical substituted phthalocyanines; and their antimicrobial activity tested on *C. albicans* as a model. These results confirm the importance of the amphiphilic character conferred by an hydrophilic mono substituent to the activity of this class compounds and a further indication and stimulation in the search for new structures with improved activity for a perspective use as antimicrobial PDT agents.

3. Experimental

3.1. General

Melting points were measured using a Reichert Termovar apparatus and are uncorrected. NMR spectra were recorded on a Varian Inova 300 Instrument in the Fourier transform mode at 21±0.5°C. ^1H (300 MHz) and ^{13}C NMR (75.43 MHz) chemical shifts (δ) are in ppm relative to TMS as secondary reference standard; coupling constants are in Hz. Mass spectra were recorded on a VG70 70E apparatus. ESI-MS spectra were obtained on a micromass ZMD Waters instrument (4 kV and 150 V). IR spectra were

obtained by a Perkin–Elmer FT-IR Spectrometer Spectrum 2000. UV spectra were performed on an Uvicon 860 Kontron instrument. Silica gel (Merck F254) and silica gel 60 (Merck 230–400 mesh) were used for analytical TLC and flash chromatography, respectively.

3.1.1. 4,5-Bis(phenylsulfanyl)phthalonitrile 2. Thiophenol (1.24 mL, 12 mmol) and K_2CO_3 (2.5 g, 16 mmol) were added, under stirring, to a solution of 4,5-dichlorophthalonitrile (**1**) (789 mg, 4 mmol) in acetone (24 mL) and the mixture was allowed to stir at room temperature until disappearance (by TLC) of **1** (15 h). The solvent was removed under reduced pressure and the residue was treated with H_2O (450 mL), filtered, washed with H_2O until pH 7, dried in vacuo and crystallized from methanol. Colourless crystals; 1.24 g (90%), mp 195°C [lit.[14] mp 159°C]. IR (KBr) cm^{-1} 3095, 3028, 2228, 1578, 1575, 1504, 1219, 1005; ^1H NMR ($CDCl_3$) δ 7.54 (10H, m, 2×H–SAr); 6.98 (2H, s, H-3 and H-6) [lit.[14] 7.55 (10H, m, 2×H–SAr); 6.99 (2H, s, H-3 and H-6)].

3.1.2. 4-Chloro-5-[4-(hydroxymethyl)phenoxy]phthalonitrile 4. 4-Hydroxybenzylic alcohol (**3**) (496 mg, 4 mmol) and K_2CO_3 (2.5 g, 16 mmol) were added, under stirring, to a solution of 4,5-dichlorophthalonitrile (**1**) (789 mg, 4 mmol) in acetone (24 mL) and the mixture was refluxed until disappearance (TLC) of **1** (24 h). After cooling to room temperature, the solvent was removed under reduced pressure and the residue was treated with H_2O (300 mL), filtered, washed with H_2O until pH 7 and dried in vacuo to give pure compound **4** (1.04 g, 95%). An analytical sample was obtained by flash chromatography (eluant: ethyl acetate/petroleum ether 40–60°C=1:2). White powder, mp 132–135°C. IR (KBr) cm^{-1} 3269, 3092, 3029, 2917, 2853, 2233, 1580, 1485, 1383, 1302, 1273, 1164, 1048, 1006; ^1H NMR (DMSO-d_6) δ 8.57 (1H, s, H-6); 7.60 (1H, s, H-3); 7.43 (2H, AA′ part of AA′XX′ system, H-Ar); 7.15 (2H, XX′ part of AA′XX′ system, H-Ar); 5.28 (1H, t, ^3J=5.6 Hz, OH); 4.53 (2H, d, ^3J=5.6 Hz, CH_2). ^{13}C NMR (DMSO-d_6) δ 156.6 (s, C-4); 152.5 (s, C-1′); 140.1 (s, C-4′); 136.1 (d, C-6); 129.0 (s, C-5); 128.4 (d, C-3′and C-5′); 122.6 (d, C-3); 119.2 (d, C-2′ and C-6′); 115.2 (s, C-2); 114.9 (s, CN); 114.8 (s, CN); 109.7 (s, C-1); 62.2 (t, CH_2). EI-MS *m/z* (%): 284 (M^+, 50); 267 (10); 255 (16); 107 (100); 89 (14); 79 (80); 77 (73); 51 (47). HRMS: 284.03577, $C_{15}H_9N_2O_2Cl$ requires 284.03526.

3.1.3. 4-[4-(Hydroxymethyl)phenoxy]-5-(phenylsulfanyl)-phthalonitrile 5. Thiophenol (0.12 mL, 1 mmol), K_2CO_3 (2.5 g, 16 mmol) and triethylamine (0.15 mL, 1.1 mmol) were added, under stirring, to a solution of 4-chloro-5-[4-(hydroxymethyl)phenoxy]phthalonitrile (**4**) (285 mg, 1 mmol) in acetone (12 mL) and the mixture was allowed at room temperature until disappearance (TLC) of **4** (24 h). The solvent was removed under reduced pressure and the residue was treated with H_2O (200 mL), filtered, washed with H_2O until pH 7, dried in vacuo and purified by flash chromatography (eluant: ethyl acetate/petroleum ether 40–60°C=1:2). White powder; 143 mg (40%), mp 168–170°C. IR (KBr) cm^{-1} 3380, 3092, 3020, 2928, 2867, 2226, 1576, 1505, 1476, 1384, 1270, 1204, 1004; ^1H NMR (DMSO-d_6) δ 7.65–7.51 (5H, m, H–SAr); 7.42 (2H, AA′ part of AA′XX′ system, H-Ar); 7.40 (1H, s, H-6); 7.24 (1H, s, H-3); 7.12 (2H, XX′ part of AA′XX′ system, H-Ar); 5.27 (1H, t, ^3J=5.6 Hz, OH); 4.53 (2H, d, ^3J=5.6 Hz, CH_2). ^{13}C NMR (DMSO-d_6) δ 156.1 (s, C-4); 152.4 (s, C-1′); 140.0 (s, C-4′); 136.8 (s, C-1″); 134.5 (d, C-2″ and C-6″); 131.6 (d, C-6); 130.4 (d, C-3″ e C-5″); 130.1 (d, C-4″); 128.4 (d, C-3′ and C-5′); 127.9 (s, C-5); 120.3 (d, C-3); 119.2 (d, C-2′ and C-6′); 115.4 (s, CN), 115.3 (s, CN); 112.4 (s, C-2); 109.4 (s, C-1); 62.2 (t, CH_2). EI-MS *m/z* (%): 358 (M^+, 54); 196 (14); 164 (10); 107 (25); 89 (10); 79 (52); 77 (100); 69 (12); 63 (18); 51 (55). HRMS: 358.07825, $C_{21}H_{14}N_2O_2$ requires 358.07760.

3.1.4. 4-[4-(Hydroxymethyl)phenoxy]phthalonitrile 7. 4-Hydroxybenzylic alcohol (**3**) (371 mg, 3 mmol) and K_2CO_3 (2.5 g, 16 mmol) were added, under stirring, to a solution of 4-nitrophthalonitrile (**6**) (346 mg, 2 mmol) in dimethylsulfoxide (4 mL). The mixture was allowed at room temperature until disappearance (TLC) of **6** (48 h), poured into H_2O (250 mL) and extracted with dichloromethane (9×30 mL). The extracts were dried over sodium sulphate and evaporated. Compound **7** was obtained by flash chromatography (eluant: ethyl acetate/petroleum ether 40–60°C=2:1). White powder; 337 mg (68%), mp 85–87°C. IR (KBr) cm^{-1} 3490, 3101, 3074, 3046, 2937, 2880, 2232, 1590, 1565, 1504, 1485, 1417, 1284, 1203, 1039, 1008; ^1H NMR (DMSO-d_6) δ 8.09 (1H, d, ^3J=8.8 Hz, H-6); 7.76 (1H, d, ^4J=2.5 Hz, H-3); 7.43 (2H, AA′ part of AA′XX′ system, H-Ar); 7.34 (1H, dd, ^3J=8.8 Hz, ^4J=2.5 Hz, H-5); 7.16 (2H, XX′ part of AA′XX′ system, H-Ar); 5.28 (1H, t, ^3J=5.6 Hz, OH); 4.53 (2H, d, ^3J=5.6 Hz, CH_2). ^{13}C NMR (DMSO-d_6) δ 161.2 (s, C-4); 152.2 (s, C-1′); 140.1 (s, C-4′); 136.2 (d, C-6); 128.5 (d, C-3′ and C-5′); 122.4 (d, C-5); 121.7 (d, C-3); 120.0 (d, C-2′ and C-6′); 116.6 (s, C-2); 115.8 (s, CN); 115.3 (s, CN); 107.9 (s, C-1); 62.2 (t, CH_2). EI-MS *m/z* (%): 250 (M^+, 52); 221 (18); 107 (97); 100 (16); 89 (9); 79 (100); 77 (62); 64 (19); 51 (45). HRMS: 250.07449, $C_{15}H_{10}N_2O_2$ requires 250.07423.

3.1.5. 3-[4-(Hydroxymethyl)phenoxy]phthalonitrile 9. 4-Hydroxybenzylic alcohol (**3**) (371 mg, 3 mmol) and K_2CO_3 (2.5 g, 16 mmol) were added, under stirring, to a solution of 3-nitrophthalonitrile (**8**) (346 mg, 2 mmol) in dimethylsulfoxide (4 mL). The mixture was allowed at room temperature until disappearance (TLC) of **8** (22 h) and poured into H_2O (300 mL). The precipitate was collected by filtration, washed with H_2O until pH 7, dried in vacuo and purified by flash chromatography (eluant: ethyl acetate/petroleum ether 40–60°C=2:1). White powder; 298 mg (66%), mp 122–123°C. IR (KBr) cm^{-1} 3261, 3078, 3039, 2923, 2881, 2231, 1585, 1560, 1506, 1469, 1290, 1207, 1164, 1010; ^1H NMR (DMSO-d_6) δ 7.85–7.78 (2H, m, H-5 and H-6); 7.44 (2H, AA′ part of AA′XX′ system, H-Ar); 7.23 (1H, dd, ^3J=7.6 Hz, ^4J=2.4 Hz, H-4); 7.20 (2H, XX′ part of AA′XX′ system, H-Ar); 5.28 (1H, t, ^3J=5.6 Hz, OH); 4.53 (2H, d, ^3J=5.6 Hz, CH_2). ^{13}C NMR (DMSO-d_6) δ 159.8 (s, C-3); 152.6 (s, C-1′); 140.0 (s, C-4′); 135.7 (d, C-5); 128.3 (d, C-3′ and C-5′); 127.8 (d, C-6); 121.7 (d, C-4); 119.4 (d, C-2′ and C-6′); 115.7 (s, C-1); 115.3 (s, CN); 113.0 (s, CN); 104.9 (s, C-2); 62.1 (t, CH_2). EI-MS *m/z* (%): 250 (M^+, 100); 233 (14); 221 (84); 107 (18); 106 (40); 79 (13); 78 (15); 77 (43); 69 (27); 51 (20). HRMS: 250.07443, $C_{15}H_{10}N_2O_2$ requires 250.07423.

3.2. General procedure for the cyclization involving 4,5-bis(phenylsulfanyl)phthalonitrile 2

DBU (0.15 mL, 1 mmol) was added, under stirring, to a solution of 4,5-bis(phenylsulfanyl)phthalonitrile **2** (311 mg, 0.9 mmol) and the opportune phthalonitrile **B** (0.1 mmol) in *n*-pentanol (50 mL) and the mixture was warmed to reflux before addition of $Zn(OAc)_2$ (46 mg, 0.25 mmol). The green solution was allowed to reflux for 48 h, and then cooled to room temperature. The precipitate was collected by filtration and dried under vacuum (see Table 1 for **B**, ESI-MS and UV data). The NMR spectra of these blue crudes have not sufficient resolution to allow a correct interpretation of product composition. Only by ESI-MS spectra we obtained an indication about the mixture components.

3.3. General procedure for the cyclization involving phthalonitrile 10

DBU (0.15 mL, 1 mmol), was added, under stirring, to a solution of phthalonitrile **10** (0.3 mmol) and the opportune phthalonitrile **B** (0.1 mmol) in *n*-pentanol (50 mL) and the mixture was warmed to reflux before to add $Zn(OAc)_2$ (46 mg, 0.25 mmol). The green solution was allowed to reflux for 48 h, and then cooled to room temperature. The solvent was removed under reduced pressure and the residue was treated with H_2O and MeOH to give a blue precipitate that was collected by filtration and dried under vacuum. The crude was separated by chromatography (THF/cyclohexane=1:1 as eluant). The fastest running band was constituted by the zinc phthalocyanine (A_4), then was separated the structure A_3B and, finally the compounds A_2B_2. Washing with methanol further purified the second fraction containing A_3B.

3.3.1. Reaction with 4-chloro-5-[4-(hydroxymethyl)phenoxy]phthalonitrile 4 as partner B. The ESI-MS spectrum of the mixture showed the presence of the species $A_4/A_3B/A_2B_2/AB_3$.

Spectroscopic data for zinc phthalocyanine A4. This compound (blue powder, 40%) is commercially available. ^{1}H NMR (DMSO-d_6) δ 9.22 (8H, m, H-phthalo); 8.19 (8H, m, H-phthalo). UV/Vis (DMF) λ_{max}: 668 nm. ESI-MS ($MeOH/CHCl_3$): 577 (A_4+H^+).

Spectroscopic data for zinc 2-chloro-3-[4-(hydroxymethyl)-phenoxy]phthalocyanine (*A3B*, **11**). Blue powder (11%), mp >300°C dec. IR (KBr) cm^{-1} 3360, 3042, 2918, 2849, 1598, 1504, 1482, 1455, 1401, 1334, 1286, 1245, 1201, 1161, 1112, 1092, 1004; ^{1}H NMR (DMSO-d_6) δ 9.10 (2H, m, H-phthalo); 8.97–8.94 (3H, m, H-phthalo); 8.68–8.61 (2H, m, H-phthalo); 8.25–8.22 (2H, m, H-phthalo); 8.17–8.07 (5H, m, H-phthalo); 7.67 (2H, AA′ part of AA′XX′ system, H-Ar); 7.53 (2H, XX′ part of AA′XX′ system, H-Ar); 5.41 (1H, t, ^3J=5.6 Hz, OH); 4.72 (2H, d, ^3J=5.6 Hz, CH_2). UV/Vis (DMF) λ_{max}: 672 nm. ESI-MS ($MeOH/CHCl_3$): 733 (**11**+H$^+$, ^{35}Cl isotope).

3.3.2. Reaction with 4-[4-(hydroxymethyl)phenoxy]phthalonitrile 7 as partner B. The ESI-MS spectrum of the mixture showed the presence of the species $A_4/A_3B/A_2B_2/AB_3$.

Spectroscopic data for zinc 2-[4-(hydroxymethyl)phenoxy]phthalocyanine (*A3B*, **12**). Blue powder (13%), mp >300°C dec. IR (KBr) cm^{-1} 3358, 3081, 3050, 2922, 2829, 1602, 1503, 1485, 1403, 1333, 1286, 1232, 1165, 1085; ^{1}H NMR (DMSO-d_6) δ 9.10–9.02 (5H, m, H-phthalo); 8.92–8.89 (2H, m, H-phthalo); 8.40 (1H, m, H-phthalo); 8.17–8.04 (6H, m, H-phthalo); 7.71 (1H, dd, ^3J=8.3 Hz, ^4J=2.2 Hz, H-3); 7.64 (2H, AA′ part of AA′XX′ system, H-Ar); 7.52 (2H, XX′ part of AA′XX′ system, H-Ar); 5.38 (1H, t, ^3J=5.6 Hz, OH); 4.70 (2H, d, ^3J=5.6 Hz, CH_2). UV/Vis (DMF) λ_{max}: 671 nm. ESI-MS ($MeOH/CHCl_3$): 699 (**12**+H$^+$).

Spectroscopic data for zinc phthalocyanines (*A2B2*, **13**). Blue powder (5%), mp >300°C dec. ^{1}H NMR (DMSO-d_6) δ 9.28–8.92 (6H, m, H-phthalo); 8.68–8.43 (2H, m, H-phthalo); 8.27–8.12 (4H, m, H-phthalo); 7.87–7.73 (2H, m, H-phthalo); 7.63 (2H, AA′ part of AA′XX′ system, H-Ar); 7.52 (2H, XX′ part of AA′XX′ system, H-Ar); 5.38 (1H, t, ^3J=5.2 Hz, OH); 4.70 (2H, d, ^3J=5.2 Hz, CH_2). UV/Vis (DMF): λ_{max}: 674 nm. ESI-MS ($MeOH/CHCl_3$): 821 (**13**+H$^+$).

3.3.3. Reaction with 3-[4-(hydroxymethyl)phenoxy]phthalonitrile 9 as partner B. The ESI-MS spectrum of the mixture showed the presence of the species $A_4/A_3B/A_2B_2/AB_3$.

Spectroscopic data for zinc 1-[4-(hydroxymethyl)phenoxy]phthalocyanine (*A3B*, **14**). Blue powder (15%), mp >300°C dec. IR (KBr) cm^{-1} 3357, 3380, 3043, 2958, 2927, 2871, 1697, 1600, 1329, 1252, 1201, 1115, 1091; ^{1}H NMR (DMSO-d_6) δ 9.33–9.23 (5H, m, H-phthalo); 9.20 (1H, m, H-phthalo); 8.91 (1H, m, H-phthalo); 8.25–8.13 (7H, m, H-phthalo); 7.80 (1H, m, H-phthalo); 7.49 (4H, AA′BB′, H-Ar); 5.18 (1H, t, ^3J=5.4 Hz, OH); 4.53 (2H, d, ^3J=5.4 Hz, CH_2). UV/Vis (DMF) λ_{max}: 674 nm. ESI-MS ($MeOH/CHCl_3$): 699 (**14**+H$^+$).

Spectroscopic data for zinc phthalocyanines (*A2B2*, **15**). Blue powder (5%), mp >300°C dec. UV/Vis (DMF): λ_{max}: 673 nm. ESI-MS ($MeOH/CHCl_3$): 821 (**15**+H$^+$).

3.3.4. Zinc 2,6(7),10(11),14(15)-tetrakis[4-(hydroxymethyl)phenoxy]phthalocyanine (16). Operating as point 3.3 by using 4-[4-(hydroxymethyl)phenoxy]phthalonitrile **7** only. Green solid (yield: 31%) mp >300°C dec.

Spectroscopic data. IR (KBr) cm^{-1} 3353, 2950, 1602, 1507, 1485, 1394, 1335, 1230, 1161; ^{1}H NMR (DMSO-d_6) δ 9.25–9.05 (4H, m, H-phthalo); 8.70–8.60 (4H m, H-phthalo); 7.90–7.70 (4H, m, H-phthalo); 7.60–7.40 (16H, m, H-phenoxy); 5.35–5.20 (4H, m, OH); 4.70–4.40 (4H, m, CH_2). UV/Vis (DMF) λ_{max}: 672 nm. ESI-MS ($MeOH/CHCl_3$): 1067 (**16**+H$^+$).

3.3.5. Zinc 1,5(8),9(12),13(16)-tetrakis[4-(hydroxymethyl)phenoxy]phthalocyanine (17). Operating as point 3.3 by using 3-[4-(hydroxymethyl)phenoxy]phthalonitrile **9** only. Green solid (yield: 43%) mp >300°C dec.

Spectroscopic data. IR (KBr) cm^{-1} 3355, 3083, 3040, 2930, 2885, 1605, 1505, 1483, 1330, 1238, 1163; ^1H NMR (DMSO-d_6) δ 9.25–8.60 (4H, m, H-phthalo); 8.25–7.95 (4H m, H-phthalo); 7.80–7.45 (4H, m, H-phthalo); 7.45–7.15 (16H, m, H-phenoxy); 5.25–5.10 (4H, m, OH); 4.60–4.45 (4H, m, CH_2). UV/Vis (DMF) λ_{max}: 689 nm. ESI-MS (MeOH/$CHCl_3$): 1067 (**17**+H^+).

3.4. Antimicrobial activity

3.4.1. Phthalocyanine formulation. The phthalocyanines were solubilized in DMSO at 1 mg/mL concentration (stock solutions), aliquoted and stored at −20°C. Fresh aliquots from the stock solutions were diluted in PBS containing 5% DMSO in order to obtain the final working phthalocyanine solutions.

3.4.2. Microorganisms and growth conditions. *C. albicans* (strain 10231 ATCC) was grown in Fluid Sabouraud Medium (Difco Laboratories, Detroit, MI) at 37°C under aerobic conditions. Cells from culture in the stationary growth phase were harvested, washed with PBS, and diluted to a final concentration of 10^6 cells/mL corresponding to an absorbance of 0.12 at 630 nm, according to a previously established method.[15]

3.4.3. Phthalocyanines treatment and irradiation procedure. Irradiated and unirradiated samples were prepared by adding suitable volumes of the phthalocyanine solutions to the cell suspensions; the final dye concentrations were in the 0.01–30 μM range. Samples were incubated at 37°C in the dark for 60 min, then irradiated with red light (50 mW/cm^2) for 10 min. All the irradiation studies were performed by using a Waldmann halogen light source, which was equipped with a set of bandpass filters to isolate the 600–700 nm spectral interval, thus matching the absorption maximum of the photosensitizer used.

3.4.4. *C. albicans* survival assays. Irradiated and unirradiated cells samples were serially 10-fold diluted with PBS; each dilution was plated onto specific agar medium. After incubation of the plates at 37°C for 24 h, the number of colony-forming units (cfu/mL) was counted. *C. albicans* survival values were corrected with controls (cells with or without photosensitizer and no light) and reported as percentage.

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