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1 **Synthesis and characterization of 8-aminoquinolines, substituted by**
2 **electron-donating groups, as high affinity copper chelators for the**
3 **treatment of Alzheimer's disease**
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34 **Abstract**
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36 The deregulation of copper homeostasis generates copper-amyloid aggregation and
37 strongly participate to neuron damage in the brains of patients with Alzheimer's disease
38 (AD). Therefore, copper chelators able to regulate copper homeostasis should be
39 considered as potential therapeutic agents. Based on a bidentate amine side chain
40 attached at the 2-position of a 8-aminoquinoline motif, a series of
41 low-molecular-weight copper chelators have been designed to act as specific
42 tetradentate Cu(II) chelators. The affinity of these ligands for Cu(II) and their
43 selectivity for Cu(II) with respect to Zn(II) is reported. These ligands provide a square
44 planar tetradentate coordination sphere that should be suitable to extract copper(II)
45 from copper-amyloid, and are therefore expected to regulate copper homeostasis *in*
46 *vivo*.
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Introduction

The progressive and devastating cognitive decline of Alzheimer's disease (AD) described by Alzheimer a century ago^[1] is related to the abnormal deposition of two proteins, amyloid proteins (A β) and hyperphosphorylated tau protein. Current AD therapy is based on four acetylcholine esterase inhibitors and memantine, a weak antagonist of the *N*-methyl-D-aspartate receptor, that are not really effective, and the pipeline of new drugs is rather unproductive.^[2] Therefore, it is essential to explore new efficient drugs for AD, which can slow down or stop the AD loss of memory at the early phase. There is extensive evidence linking metal dysregulation to tau protein pathology and A β -aggregation in AD brain.^[3-5] Copper ions are concentrated in AD amyloid plaques, as high as 400 μ M, close to 6 times higher than in normal brain.^[6] The activation of dioxygen by copper-amyloid complexes, in the presence of endogenous reductants, induces the catalytic generation of reactive oxygen species (ROS).^[7-10] The bioinorganic pharmacology of AD should therefore be developed, as a potential drug target. Few specific ligands able to restore the homeostasis of copper have been considered as potential drugs for the treatment of AD.^[11-15] These copper chelators are expected to extract copper ions from copper-amyloid complex and translate them to copper-carrier proteins or copper enzymes inside the brain. Bush and coworkers developed hydroxyquinoline derivatives clioquinol (CQ) and PBT2. Unfortunately, CQ and PBT2 are unable to efficiently extract copper from Cu-A β , and they generate ternary Cu-A β -CQ or Cu-A β -PBT2 complexes that retain a damaging redox activity.^[16] Based on our previous work on bis-8-aminoquinolines,^[17-18] we have recently designed a series of mono-8-aminoquinoline derivatives (named TDMQ) with a bidentate amine side chain. When the length of the side chain is suitable, TDMQs offer a N₄-tetradentate square planar coordination sphere able to specifically chelate copper(II) in complexes having a TDMQ/Cu = 1/1 stoichiometry and, therefore, to extract this metal ion from copper-amyloid.^[19] Copper is then released from the Cu-TDMQ complex in the presence of glutathione which is a physiological provider of copper for copper-carrier proteins,^[19] without interacting with zinc which is abundant in the brain,^[20] but preferred a tetrahedral coordination. In order to document the structure-selectivity

1 relationship in the series of TDMQ ligands retaining the already characterized
2 N_4 -tetradentate square planar coordination sphere, and to enlarge the scope of these
3 putative drugs, we report here the synthesis and characterization of new derivatives
4 bearing electron donating groups [O-CH₃ or N(CH₃)₂] at several positions of the
5 quinoline nucleus. The chelation of copper(II) by these ligands, and their selectivity
6 with respect to zinc, is also reported and compared with that ones of the previously
7 reported TDMQ chelators.
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10 **Materials and methods**

11 **1. General methods.**

12 Chemical reagents were purchased from common suppliers and used as received.
13 Reactions were monitored by thin-layer chromatography (TLC) performed with 0.2
14 mm silica gel 60F plates and visualized by ultraviolet light. Column chromatography
15 purification of products was carried out on silica gel (300-400 mesh). ¹H NMR and
16 ¹³C NMR spectra was recorded on a Bruker AVANCE III 400 MHz spectrometer
17 (400.0 MHz for ¹H NMR, 100.6 MHz for ¹³C NMR) and Bruker AVANCE III 500
18 MHz spectrometer (500.0 MHz for ¹H NMR, 125.7 MHz for ¹³C NMR).
19 Tetramethylsilane (TMS) was used as an external standard (0 ppm) for the ¹H NMR
20 spectra. The ¹³C NMR were referred to the chemical shift of the solvent (δ = 39.5 ppm
21 for DMSO-*d*₆ and 77.0 ppm for CDCl₃).
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45 **2. Syntheses.**

46 **Synthesis of TDMQ31** (Scheme 1, R₅ = R₇ = H, R₆ = OCH₃).

47 *6-Methoxy-2-methyl-8-nitroquinoline (D-TDMQ31)*. To a solution of
48 4-methoxy-2-nitroaniline (C-TDMQ31, Scheme 2, R₆ = OCH₃) in HCl (12 M, 10 mL)
49 at 0 °C, acetaldehyde was added dropwise under stirring. The reaction medium was
50 kept at 0 °C for 15 min, gradually raised to 80 °C, and then stirred at 80 °C for 1 h.
51 The resulting mixture was poured into ice-cold water and neutralized with aqueous
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1 ammonium hydroxide. After extraction with CH₂Cl₂, the organic phase was dried over
2 MgSO₄, filtered, and concentrated under reduced pressure. The crude mixture was
3 purified by silica gel flash column chromatography (ethyl acetate/hexane, 1/20, v/v).
4 After evaporation of the eluent, D-TDMQ31 was obtained as a yellow solid (41%). ¹H
5 NMR (CDCl₃) : δ = 7.99 (d, *J* = 8.6 Hz, 1H), 7.62 (d, *J* = 2.8 Hz, 1H), 7.35 (d, *J* = 8.6
6 Hz, 1H), 7.24 (d, *J* = 2.8 Hz, 1H), 3.96 (s, 3H), 2.72 ppm (s, 3H).

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13 *6-Methoxy-8-nitro-2-vinylquinoline (E-TDMQ31)*. To a solution of D-TDMQ31
14 (Scheme 2, R₅ = R₇ = H, R₆ = OCH₃) in *N,N*-dimethylacetamide (5 mL) was added
15 FeCl₃ and K₂S₂O₈. The mixture was stirred at 110 °C for 30 min and quenched with
16 water. After extraction with CH₂Cl₂, the organic phase was dried over MgSO₄, filtered,
17 and concentrated under reduced pressure. The crude mixture was purified by silica gel
18 flash column chromatography (hexane/dichloromethane, 50/1, v/v). After evaporation
19 of the eluent, compound E-TDMQ31 was obtained as a red solid (17%). ¹H NMR
20 (CDCl₃) : δ = 8.05 (d, *J* = 8.8 Hz, 1H), 7.64 (d, *J* = 2.8 Hz, 1H), 7.61 (d, *J* = 8.8 Hz,
21 1H), 7.24 (d, *J* = 2.8 Hz, 1H) 7.00-6.93 (m, 1H), 6.34 (d, *J* = 17.6 Hz, 1H), 5.65 (d, *J*
22 = 10.8 Hz, 1H), 3.97 ppm (s, 3H).

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34 *N¹-(2-(6-Methoxy-8-nitroquinolin-2-yl)ethyl)-N³,N³-dimethylpropane-1,3-diamine*
35 (*F-TDMQ31*). To a mixture of E-TDMQ31 and K₂CO₃ in 1,4-dioxane (10 mL) was
36 added *N,N*-dimethyl-1,2-ethanediamine. The reaction mixture was stirred at room
37 temperature for 12 h, and quenched with water. After extraction with CH₂Cl₂, the
38 organic phase was dried over MgSO₄, filtered, and concentrated under reduced
39 pressure. The crude mixture was purified by silica gel flash column chromatography
40 (ethyl acetate/isopropanol/25% ammonium hydroxide, 5/1/0.2, v/v/v). After
41 evaporation of the eluent, F-TDMQ31 was obtained as brown solid (21%). ¹H NMR
42 (CDCl₃) : δ = 8.01 (d, *J* = 8.6 Hz, 1H, H₄), 7.64 (d, *J* = 2.6 Hz, 1H), 7.36 (d, *J* = 8.6
43 Hz, 1H), 7.24 (d, *J* = 2.6 Hz, 1H), 3.96 (s, 3H), 3.18-3.12 (m, 4H), 2.77 (t, *J* = 6.6 Hz,
44 2H), 2.45 (t, *J* = 6.6 Hz, 2H), 2.22 ppm (s, 6H).

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57 *N¹-(2-(8-Amino-6-methoxyquinolin-2-yl)ethyl)-N³,N³-dimethylpropane-1,3-diamine*
58 (*G-TDMQ31*). Iron (131.6 mg, 2.36 mmol) and acetic acid (3 mL) were added to a
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1 solution of F-TDMQ31 in ethanol (9 mL). The mixture was stirred at 80 °C under
2 reflux for 1 h. The reaction mixture was poured into an aqueous saturated NaCl
3 solution (200 mL) and extracted with CH₂Cl₂ (3 x 100 mL). The combined organic
4 layers were dried over MgSO₄, filtered and concentrated under reduced pressure. The
5 crude product was purified by silica gel flash chromatography (ethyl acetate/hexane,
6 1/10, v/v). Evaporation of the solvent afforded G-TDMQ31 was obtained as yellow
7 oil (70%). ¹H NMR (CDCl₃) : δ = 7.86 (d, *J* = 6.8 Hz, 1H), 7.21 (d, *J* = 6.8 Hz, 1H),
8 6.55 (d, *J* = 2.0 Hz, 1H), 6.45 (d, *J* = 2.0 Hz, 1H), 5.02 (s, 2H), 3.86 (s, 3H), 3.11 (s,
9 4H), 2.76 (t, *J* = 4.8 Hz, 2H), 2.43 (t, *J* = 4.8 Hz, 2H), 2.22 ppm (s, 6H).

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20 *TDMQ31*. To a solution of G-TDMQ31 in dry dichloromethane was added excess
21 hydrogen chloride solution (2.0 M in diethyl ether). The precipitate was filtered,
22 washed with diethyl ether and dried under reduced pressure to give TDMQ31 that was
23 obtained as a yellow solid (96 %). ¹H NMR (DMSO-*d*₆) : δ = 9.58 (brs, 2H,
24 CH₂-NH₂⁺-CH₂), 8.26 (d, *J* = 8.4 Hz, 1H, H4), 7.52 (d, *J* = 8.4 Hz, 1H, H3), 7.14 (s,
25 1H, H5), 7.07 (s, 1H, H7), 3.87 (s, 3H, C6-OCH₃), 3.62 (t, *J* = 6.4 Hz, 2H,
26 C2-CH₂-CH₂), 3.59 [m, 4H, NH₂⁺-CH₂-CH₂-N⁺H(CH₃)₂], 3.45 (t, *J* = 6.4 Hz, 2H,
27 C2-CH₂), 2.86 ppm [s, 6H, NH⁺(CH₃)₂]; ¹³C NMR (100 MHz, DMSO-*d*₆) : δ = 158.3
28 (C6), 155.4 (C2), 144.7 (C8), 135.2 (C4), 134.7 (C8a), 128.1 (C4a), 122.4 (C3), 101.5
29 (C7), 94.5 (C5), 59.1 [CH₂-NH⁺(CH₃)₂], 55.2 (C6-OCH₃), 49.1 (C2-CH₂-CH₂), 47.2
30 (NH⁺(CH₃)₂), 45.6 (NH₂⁺-CH₂-CH₂), 38.1 ppm (C2-CH₂); mp: 153 °C; IR (KBr): ν =
31 804, 865, 1027, 1166, 1199, 1265, 1340, 1396, 1471, 1504, 1608, 1650, 2948, 2977,
32 3236, 3401, 3465 cm⁻¹; HRMS (ESI) calculated for C₁₆H₂₅ON₄: 289.20235 ([M +
33 H]⁺), found: 289.20229; Elemental analysis for C₁₆H₂₄ON₄•3.0HCl•1.9•
34 0.4C₂H₅OC₂H₅•0.2CH₂Cl₂ (apparent MW = 471.5444): calculated C, 45.34; H, 7.52;
35 N, 11.88; found C, 45.34; H, 7.40; N, 11.75.

36 37 38 39 40 41 42 43 44 45 46 47 48 49 50 51 52 53 **Synthesis of TDMQ32.**

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56 *5-Methoxy-2-methyl-8-nitroquinoline (D-TDMQ32)*. The procedure was similar to
57 that described for the preparation of D-TDMQ31, but starting from
58 5-methoxy-2-nitroaniline (C-TDMQ32, Scheme 1, R₅ = OCH₃). D-TDMQ32 was
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1 obtained as yellow solid (70%). ¹H NMR (CDCl₃): δ = 8.48 (d, *J* = 8.6 Hz, 1H), 8.01
2 (d, *J* = 8.6 Hz, 1H), 7.37 (d, *J* = 8.6 Hz, 1H), 6.78 (d, *J* = 8.6 Hz, 1H), 4.07 (s, 3H),
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5 2.77 ppm (s, 3H).
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7 *5-Methoxy-8-nitro-2-vinylquinoline (E-TDMQ32)*. To a solution of D-TDMQ32
8 (Scheme 2, R₆ = R₇ = H, R₅ = OCH₃) in *N,N*-dimethylformamide (5 mL) was added
9 FeCl₃ (37.2 mg, 0.23 mmol) and TBHP (660 μL). The mixture was stirred at 140°C
10 for 3 hours and quenched with water. After extraction with CH₂Cl₂, the organic phase
11 was dried over MgSO₄, filtered, and concentrated under reduced pressure. The crude
12 mixture was purified by silica gel flash column chromatography
13 (hexane/dichloromethane, 10/1, v/v). After evaporation of the eluent, compound
14 E-TDMQ32 was obtained as a yellow solid (50%). ¹H NMR (CDCl₃): δ = 8.41 (d, *J*
15 = 8.6 Hz, 1H, H4), 8.01 (d, *J* = 8.6 Hz, 1H), 7.48 (d, *J* = 8.6 Hz, 1H), 6.94-6.87 (m,
16 1H), 6.66 (d, *J* = 8.6 Hz, 1H), 6.36 (d, *J* = 17.6 Hz, 1H), 5.62 (d, *J* = 10.8 Hz, 1H),
17 3.96 ppm (s, 3H).
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30 *N¹-(2-(5-Methoxy-8-nitroquinolin-2-yl)ethyl)-N³,N³-dimethylpropane-1,3-diamine*
31 (*F-TDMQ32*). The procedure was similar to that described for the preparation of
32 F-TDMQ31, but starting from E-TDMQ32 (Scheme 2, R₆ = R₇ = H, R₅ = OCH₃) and
33 the reaction time was 1 hour. F-TDMQ32 was obtained as a yellow solid (65%). ¹H
34 NMR (CDCl₃): δ = 8.48 (d, *J* = 8.8 Hz, 1H), 8.12 (d, *J* = 8.4 Hz, 1H), 7.38 (d, *J* = 8.8
35 Hz, 1H), 6.78 (d, *J* = 8.4 Hz, 1H), 4.07 (s, 3H), 3.20-3.15 (m, 4H), 2.79 (t, *J* = 6.4 Hz,
36 2H), 2.46 (t, *J* = 6.4 Hz, 2H), 2.22 ppm (s, 6H).
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45 *N¹-(2-(8-Amino-5-methoxyquinolin-2-yl)ethyl)-N³,N³-dimethylpropane-1,3-diamine*
46 (*G-TDMQ32*). The procedure was similar to that described for the preparation of
47 G-TDMQ31, but starting from F-TDMQ32 (Scheme 2, R₆ = R₇ = H, R₅ = OCH₃).
48 G-TDMQ32 was obtained as a yellow solid (60%). ¹H NMR (CDCl₃): δ = 8.40 (d, *J* =
49 8.4 Hz, 1H), 7.26 (d, *J* = 8.4 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.68 (d, *J* = 8.0 Hz,
50 1H), 3.92 (s, 3H), 3.19-3.18 (m, 4H), 2.80 (t, *J* = 6.0 Hz, 2H), 2.46 (t, *J* = 6.0 Hz, 2H),
51 2.21 ppm (s, 6H).
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60 *TDMQ32*. To a solution of G-TDMQ32 in dry dichloromethane was added an excess
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1 hydrogen chloride solution (2.0 M in diethyl ether). The precipitate was filtered,
2 washed with diethyl ether and dried under reduced pressure to give TDMQ32 that was
3 obtained as a yellow solid (99 %). ¹H NMR (D₂O): δ = 8.61 (d, J = 8.6 Hz, 1H, H4),
4 7.69 (d, J = 8.2 Hz, 1H, H7), 7.53 (d, J = 8.6 Hz, 1H, H3), 7.02 (d, J = 8.2 Hz, 1H,
5 H6), 4.00 (s, 3H, OCH₃), 3.74 (t, J = 6.8 Hz, 2H, C2-CH₂-CH₂-NH₂⁺), 3.58-3.54 [m,
6 4H, NH₂⁺-CH₂-CH₂-NH⁺(CH₃)₂], 3.45 (t, J = 6.8 Hz, 2H, C2-CH₂), 2.93 ppm [s, 6H,
7 N(CH₃)₂]; mp: 146 °C; IR (KBr): ν = 827, 1020, 1085, 1194, 1272, 1411, 1483, 1602,
8 1645, 2638, 2746, 2873, 2975, 3450 cm⁻¹; HRMS (ESI) calculated for C₁₆H₂₅ON₄:
9 289.20251 ([M + H]⁺), found: 289.20229; Elemental analysis for
10 C₁₆H₂₄ON₄•3.0HCl•2.5H₂O•0.2C₂H₅OC₂H₅•0.15CH₂Cl₂ (apparent MW = 470.3732):
11 calculated C, 43.28; H, 7.35; N, 11.91; found C, 43.30; H, 7.04; N, 11.89.
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24 **Synthesis of TDMQ33.**

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26 *7-Methoxy-2-methylquinoline (B-TDMQ33)*. The procedure was similar to that
27 described for the preparation of D-TDMQ31, but starting from 3-methoxyaniline
28 (A-TDMQ33, Scheme 2, R₃ = OCH₃). B-TDMQ33 was obtained as a yellow oil
29 (36%). ¹H NMR (CDCl₃): δ = 7.93 (d, J = 8.6 Hz, 1H), 7.62 (d, J = 8.6 Hz, 1H), 7.36
30 (s, 1H), 7.13 (d, J = 8.0 Hz, 1H), 7.11 (d, J = 8.0 Hz, 1H), 3.92 (s, 3H), 2.70 ppm (s,
31 3H).
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39 *7-Methoxy-2-methyl-8-nitroquinoline (D-TDMQ33)*. To a stirred solution of
40 B-TDMQ33 in neat sulfuric acid (10.0 mL) was added fuming nitric acid (5.0 mL)
41 dropwise over a 1 h period at ambient temperature. The resulting mixture was stirred
42 for an additional hour, and was then poured into ice. The mixture was allowed to
43 warm to ambient temperature, neutralized with aqueous ammonium hydroxide. After
44 extraction with CH₂Cl₂, the organic phase was dried over MgSO₄, filtered, and
45 concentrated under reduced pressure. The crude mixture was purified by silica gel
46 flash column chromatography (ethyl acetate/hexane, 1/30, v/v). Evaporation of the
47 solvent afforded D-TDMQ33 as a yellow solid (32 %). ¹H NMR (CDCl₃): δ = 7.90 (d,
48 J = 8.4 Hz, 1H), 7.75 (d, J = 9.0 Hz, 1H), 7.22 (d, J = 9.0 Hz, 1H), 7.14 (d, J = 8.4 Hz,
49 1H), 3.94 (s, 3H), 2.60 ppm (s, 3H).
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1 *7-Methoxy-8-nitro-2-vinylquinoline (E-TDMQ33)*. The procedure was similar to that
2 described for the preparation of E-TDMQ32, but starting from D-TDMQ33 (Scheme
3 2, R₆ = R₅ = H, R₇ = OCH₃). E-TDMQ33 was obtained as yellow solid (34%). ¹H
4 NMR (500.0 MHz, CDCl₃): δ = 8.45 (d, *J* = 8.5 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H),
5 7.81 (d, *J* = 8.5 Hz, 1H), 7.74 (d, *J* = 9.0 Hz, 1H), 6.97~6.91 (m, 1H), 6.43 (d, *J* =
6 17.5 Hz, 1H), 5.75 (d, *J* = 10.5 Hz, 1H), 4.08 ppm (s, 3H).

7 *N¹-(2-(7-Methoxy-8-nitroquinolin-2-yl)ethyl)-N³,N³-dimethylpropane-1,3-diamine*
8 (*F-TDMQ33*). The procedure was similar to that described for the preparation of
9 F-TDMQ31, but starting from E-TDMQ33 (Scheme 2, R₆ = R₅ = H, R₇ = OCH₃) and
10 the catalyst was replaced by Cs₂CO₃, the reaction time was 48 hours. F-TDMQ33 was
11 obtained as a yellow oil (75%). ¹H NMR (500.0 MHz, DMSO - *d*₆): δ = 8.40 (d, *J* =
12 8.5 Hz, 1H), 8.21 (d, *J* = 9.0 Hz, 1H), 7.73 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 8.5 Hz,
13 1H), 4.07 (s, 3H), 3.13 (t, *J* = 7.0 Hz, 2H), 3.04 (t, *J* = 7.0 Hz, 2H), 2.73 (t, *J* = 6.0 Hz,
14 2H), 2.38 (t, *J* = 6.0 Hz, 2H), 2.14 ppm (s, 6H).

15 *N¹-(2-(8-Amino-7-methoxyquinolin-2-yl)ethyl)-N³,N³-dimethylpropane-1,3-diamine*
16 (*G-TDMQ33*). The procedure was similar to that described for the preparation of
17 G-TDMQ31, but starting from F-TDMQ33 (Scheme 2, R₆ = R₅ = H, R₇ = OCH₃).
18 G-TDMQ33 was obtained as a yellow solid (80%). ¹H NMR (500.0 MHz, CDCl₃): δ
19 = 7.92 (d, *J* = 8.4 Hz, 1H), 7.20 (d, *J* = 8.4 Hz, 1H), 7.12 (s, 1H), 7.10 (s, 1H), 4.96 (s,
20 2H, C8-NH₂), 3.98 (s, 3H), 3.14 (s, 4H), 2.76 (t, *J* = 6.2 Hz, 2H), 2.43 (t, *J* = 6.2 Hz,
21 2H), 2.21 ppm (s, 6H).

22 *TDMQ33*. To a solution of G-TDMQ33 in dry dichloromethane was added an excess
23 of hydrogen chloride solution (2.0 M in diethyl ether). The precipitate was filtered,
24 washed with diethyl ether and dried under reduced pressure to give TDMQ33 as a
25 yellow solid (99 %). ¹H NMR (400.0 MHz, D₂O): δ = 8.33 (d, *J* = 8.4 Hz, 1H, H4),
26 7.92 (d, *J* = 9.2 Hz, 1H, H5), 7.57 (d, *J* = 9.2 Hz, 1H, H6), 7.41 (d, *J* = 8.4 Hz, 1H,
27 H3), 4.05 (s, 3H, OCH₃), 3.72 (t, *J* = 6.8 Hz, 2H, C2-CH₂-CH₂), 3.56-3.52 [m, 4H,
28 NH₂⁺-CH₂-CH₂-NH⁺(CH₃)₂], 3.44 (t, *J* = 6.8 Hz, 2H, C2-CH₂), 2.92 ppm [s, 6H,
29 NH⁺(CH₃)₂]; mp: 97 °C; IR (KBr): ν = 786, 848, 1002, 1045, 1147, 1268, 1456, 1513,
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1 1625, 1643, 2642, 2750, 2869, 2956, 3083, 3436 cm^{-1} ; HRMS (ESI) calculated for
2 $\text{C}_{16}\text{H}_{25}\text{ON}_4$: 289.20249 ($[\text{M} + \text{H}]^+$), found: 289.20229; Elemental analysis for
3 $\text{C}_{16}\text{H}_{24}\text{ON}_4 \cdot 3.0\text{HCl} \cdot 1.8\text{H}_2\text{O} \cdot 0.3\text{C}_2\text{H}_5\text{OC}_2\text{H}_5 \cdot 0.02\text{CH}_2\text{Cl}_2$ (apparent MW = 454.1344):
4 calculated C, 45.54; H, 7.47; N, 12.34; found C, 45.53; H, 7.56; N, 12.63.
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9 **Synthesis of TDMQ38.**

10 *N*¹,*N*¹-Dimethyl-2-nitrobenzene-1,3-diamine (C-TDMQ38). To a solution of
11 3-chloro-2-nitroaniline in dry *N,N*-dimethylformamide was added K_2CO_3 and
12 dimethylamine. The mixture was stirred at 140 °C for 12 h and quenched with water.
13 After extraction with CH_2Cl_2 , the organic phase was dried over MgSO_4 , filtered, and
14 concentrated under reduced pressure. The crude mixture was purified by silica gel
15 flash column chromatography (hexane/ethyl acetate, 10/1, v/v). After evaporation of
16 the eluent, compound C-TDMQ38 was obtained as a yellow solid (99%). ¹H NMR
17 (CDCl_3): δ = 7.07 (dd, J = 8.8 Hz, 1H), 6.25 (d, J = 8.8 Hz, 1H), 6.18 (d, J = 8.8 Hz,
18 1H), 5.0 (s, 2H), 2.84 ppm (s, 6H).
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30 *N,N,2-Trimethyl-8-nitroquinolin-7-amine* (D-TDMQ38). The procedure was similar to
31 that described for the preparation of D-TDMQ31, but starting from C-TDMQ38.
32 D-TDMQ38 was obtained as a yellow solid (54%). ¹H NMR (CDCl_3): δ = 7.88 (d, J =
33 8.4 Hz, 1H), 7.66 (d, J = 9.2 Hz, 1H), 7.18 (d, J = 9.2 Hz, 1H), 7.13 (d, J = 8.4 Hz,
34 1H), 3.05 (s, 6H), 2.66 ppm (s, 3H).
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41 *N,N-Dimethyl-8-nitro-2-vinylquinolin-7-amine* (E-TDMQ38). The procedure was
42 similar to that described for the preparation of E-TDMQ32, but starting from
43 D-TDMQ38 and the reaction time was 5 hours. E-TDMQ38 was obtained as a yellow
44 solid (22%). ¹H NMR (CDCl_3): δ = 7.89 (d, J = 8.4 Hz, 1H, H6), 7.60 (d, J = 9.2 Hz,
45 1H), 7.31 (d, J = 8.4 Hz, 1H), 7.13 (d, J = 9.2 Hz, 1H), 6.86 (dd, J = 10.8, 17.6 Hz,
46 1H), 6.27 (d, J = 17.6 Hz, 1H), 5.54 (d, J = 10.8 Hz, 1H), 3.01 ppm (s, 6H).
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53 *N*¹-(2-(7-(Dimethylamino)-8-nitroquinolin-2-yl)ethyl)-*N*²,*N*²-dimethylethane-1,2-
54 diamine (F-TDMQ38). The procedure was similar to that described for the preparation
55 of F-TDMQ33, but starting from E-TDMQ32. F-TDMQ38 was obtained as a yellow
56 oil (70%). ¹H NMR (CDCl_3): δ = 7.91 (d, J = 8.4 Hz, 1H), 7.67 (d, J = 9.2 Hz, 1H),
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1 7.20 (d, $J = 9.2$ Hz, 1H), 7.15 (d, $J = 8.4$ Hz, 1H), 3.12-3.09 (m, 4H), 3.07 (s, 6H),
2
3 2.76 (t, $J = 6.6$ Hz, 2H), 2.45 (t, $J = 6.6$ Hz, 2H), 2.23 ppm (s, 6H).

4 *2-(2-((2-(Dimethylamino)ethyl)amino)ethyl)- N^7, N^7 -dimethylquinoline-7,8-diamine*

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7 (*G-TDMQ38*). The procedure was similar to that described for the preparation of
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9 *G-TDMQ31*, but starting from *F-TDMQ38*. *G-TDMQ38* was obtained as a yellow
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11 solid (60%). $^1\text{H NMR}$ (CDCl_3): $\delta = 7.96$ (d, $J = 8.4$ Hz, 1H), 7.34 (d, $J = 8.8$ Hz, 1H),
12
13 7.18 (d, $J = 8.4$ Hz, 1H), 7.13 (d, $J = 8.8$ Hz, 1H), 5.23 (s, 2H, C8-NH₂), 3.26 (m, 4H),
14
15 2.90 (t, $J = 5.8$ Hz, 2H), 2.80 (s, 6H), 2.55 (t, $J = 5.8$ Hz, 2H), 2.26 ppm (s, 6H).

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17 *TDMQ38*. To a solution of *G-TDMQ38* in dry dichloromethane was added an excess
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19 of hydrogen chloride solution (2.0 M in diethyl ether). The precipitate was filtered,
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21 washed with diethyl ether and dried under reduced pressure to give *TDMQ38* as a
22
23 yellow solid (99 %). $^1\text{H NMR}$ (D_2O): $\delta = 8.26$ (d, $J = 8.6$ Hz, 1H, H6), 7.64 (d, $J =$
24
25 9.0 Hz, 1H, H4), 7.54 (d, $J = 8.6$ Hz, 1H, H5), 7.48 (d, $J = 9.0$ Hz, 1H, H3), 3.75 (t, J
26
27 = 7.2 Hz, 2H, C2-CH₂-CH₂), 3.56-3.54 [m, 4H, NH₂⁺-CH₂-CH₂-NH⁺(CH₃)₂], 3.43 (t,
28
29 $J = 7.2$ Hz, 2H, C2-CH₂), 3.32 [s, 6H, C7-(CH₃)₂], 2.93 ppm [s, 6H, NH⁺(CH₃)₂]; mp:
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31 127 °C; IR (KBr): $\nu = 728, 848, 999, 1118, 1267, 1394, 1471, 1635, 2458, 2657,$
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33 2967, 3344, 3423 cm^{-1} ; HRMS (ESI) calculated for C₁₇H₂₈N₅: 302.23392 ([M + H]⁺),
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35 found: 302.23392; Elemental analysis for
36
37 C₁₇H₂₇N₅•3.0HCl•2.2H₂O•0.25C₂H₅OC₂H₅•0.13CH₂Cl₂ (apparent MW = 480.0179):
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39 calculated C, 45.36; H, 7.80; N, 14.59; found C, 45.28; H, 7.85; N, 14.64.

40 41 42 **Synthesis of *TDMQ39*.**

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44 *N¹, N¹-Dimethyl-4-nitrobenzene-1,3-diamine (C-TDMQ39)*. To a solution of
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46 5-chloro-2-nitroaniline in dry *N,N*-dimethylformamide was added K₂CO₃ and
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48 dimethylamine. The mixture was stirred at 150 °C for 12 h and quenched with water.
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50 After extraction with CH₂Cl₂, the organic phase was dried over MgSO₄, filtered, and
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52 concentrated under reduced pressure. The crude mixture was purified by silica gel
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54 flash column chromatography (hexane/ethyl acetate, 10/1, v/v). After evaporation of
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56 the eluent, compound *C-TDMQ39* was obtained as a yellow solid (99%). $^1\text{H NMR}$
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58 (CDCl_3): $\delta = 8.01$ (d, $J = 9.6$ Hz, 1H, H3), 6.14 (dd, $J = 9.6$ Hz, 2.6 Hz, 1H, H4), 6.12
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1 (d, $J = 2.6$ Hz, 1H, H6), 3.05 ppm [s, 6H, C5-N(CH₃)₂].

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3 *N,N*,2-Trimethyl-8-nitroquinolin-5-amine (*D-TDMQ39*). The procedure was similar to
4 that described for the preparation of *D-TDMQ31*, but starting from *C-TDMQ39*.
5 *D-TDMQ39* was obtained as a yellow solid (64%). ¹H NMR (CDCl₃): $\delta = 8.35$ (d, $J =$
6 8.6 Hz, 1H, H4), 8.01 (d, $J = 8.6$ Hz, 1H, H6), 7.32 (d, $J = 8.6$ Hz, 1H, H7), 6.87 (d, J
7 = 8.6 Hz, 1H, H3), 3.08 [s, 6H, C5-N(CH₃)₂], 2.75 ppm (s, 3H, C2-CH₃).

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13 *N,N*-Dimethyl-8-nitro-2-vinylquinolin-5-amine (*E-TDMQ39*). The procedure was
14 similar to that described for the preparation of *E-TDMQ32*, but starting from
15 *D-TDMQ39*. *E-TDMQ39* was obtained as a yellow solid (20%). ¹H NMR (CDCl₃): δ
16 = 8.36 (d, $J = 8.8$ Hz, 1H, H6), 7.98 (d, $J = 8.6$ Hz, 1H, H4), 7.49 (d, $J = 8.8$ Hz, 1H,
17 H7), 6.91 (dd, 1H, C2-CH), 6.82 (d, $J = 8.6$ Hz, 1H, H3), 6.36 (d, $J = 17.6$ Hz, 1H,
18 C2-CH-CH_{trans}), 5.64 (d, $J = 10.8$ Hz, 1H, C2-CH-CH_{cis}), 2.93 ppm [s, 6H,
19 C5-N(CH₃)₂].

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28 *N*¹-(2-(5-(Dimethylamino)-8-nitroquinolin-2-yl)ethyl)-*N*²,*N*²-dimethylethane-1,2-diam
29 ine (*F-TDMQ39*). The procedure was similar to that described for the preparation of
30 *F-TDMQ33*, but starting from *E-TDMQ39*. *F-TDMQ39* was obtained as a yellow oil
31 (70%). ¹H NMR (CDCl₃): $\delta = 8.37$ (d, $J = 8.8$ Hz, 1H), 8.09 (d, $J = 8.6$ Hz, 1H), 7.33
32 (d, $J = 8.8$ Hz, 1H), 6.89 (d, $J = 8.6$ Hz, 1H), 3.26-3.20 (m, 4H), 3.01 (s, 6H), 2.84 (t,
33 $J = 6.4$ Hz, 2H), 2.51 (t, $J = 6.4$ Hz, 2H), 2.24 ppm (s, 6H).

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41 2-(2-((2-(Dimethylamino)ethyl)amino)ethyl)-*N*⁵,*N*⁵-dimethylquinoline-5,8-diamine
42 (*G-TDMQ39*). The procedure was similar to that described for the preparation of
43 *G-TDMQ31*, but starting from *F-TDMQ39*. *G-TDMQ39* was obtained as a brown
44 solid (80%). ¹H NMR (MHz, CDCl₃): $\delta = 8.48$ (d, $J = 8.6$ Hz, 1H), 7.27 (d, $J = 8.6$ Hz,
45 1H), 6.99 (d, $J = 8.0$ Hz, 1H), 6.86 (d, $J = 8.0$ Hz, 1H), 3.36 (s, 4H), 3.00 (t, $J = 5.6$
46 Hz, 2H), 2.77 (s, 6H), 2.64 (t, $J = 6.0$ Hz, 2H), 2.02 ppm (s, 6H).

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TDMQ39. To a solution of *G-TDMQ39* in dry dichloromethane was added an excess
of hydrogen chloride solution (2.0 M in diethyl ether). The precipitate was filtered,
washed with diethyl ether and dried under reduced pressure to give *TDMQ39* as a
black solid (99 %). ¹H NMR (D₂O): $\delta = 8.42$ (d, $J = 8.8$ Hz, 1H, H7), 7.71 (d, $J = 8.8$

1 Hz, 1H, H6), 7.65 (d, $J = 8.8$ Hz, 1H, H4), 7.24 (d, $J = 8.8$ Hz, 1H, H3), 3.76 (t, $J =$
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3 7.0 Hz, 2H, C2-CH₂-CH₂), 3.57-3.51 [m, 4H, NH₂⁺-CH₂-CH₂-NH⁺(CH₃)₂], 3.46 (t, J
4 = 7.0 Hz, 2H, C2-CH₂), 3.34 [s, 6H, C5-N(CH₃)₂], 2.93 ppm [s, 6H, NH⁺(CH₃)₂]; mp:
5 100 °C; IR (KBr): $\nu = 806, 1051, 1124, 1186, 1259, 1480, 1633, 1664, 2892, 2954,$
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7 2973, 3440 cm⁻¹; HRMS (ESI) calculated for C₁₇H₂₈N₅: 302.23376 ([M + H]⁺), found:
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9 302.23392; Elemental analysis for
10
11 C₁₇H₂₇N₅•3.0HCl•3.5H₂O•0.3C₂H₅OC₂H₅•1.0CH₂Cl₂ (apparent MW = 581.0351):
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13 calculated C, 39.69; H, 7.29; N, 12.05; found C, 39.60; H, 7.28; N, 12.04.
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20 **3. UV-visible titrations.**

21 All solvents and commercially available reagents were purchased from Fluka and TCI,
22 they were used without further purification. CuSO₄•5H₂O and ZnSO₄•7H₂O were
23 used as source of metal ions. UV-visible spectra were recorded from 200 nm to 500
24 nm, at room temperature, on an Evolution 300 spectrophotometer equipped with a
25 magnetic stirrer. Preparation of TDMQ ligands is described in the Synthesis section.
26 Except otherwise noted, the stock solutions were as follows: TDMQ ligand (5 mM in
27 H₂O), CuSO₄•5H₂O (5 mM in H₂O), ZnSO₄•7H₂O (5 mM in H₂O) and Tris-HCl
28 buffer 50 mM, pH 7.4. For each experiment, the solution of metal ions was added to
29 the solution of TDMQ ligand in buffer. Final concentrations in the cuvette were:
30 [buffer] = 50 mM, [TDMQ] = 40 μM. Final volume was 1.5 mL.
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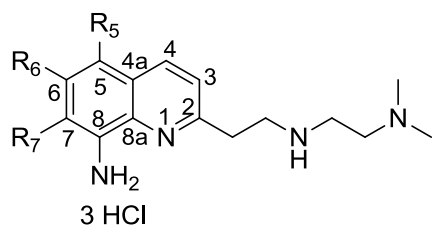
43 Note that, according to the Recommendations of IUPAC for Nomenclature of
44 inorganic chemistry, M²⁺ stands for the metal cation (salt), and M(II) stands for the
45 oxidation state of the metal involved in a complex.
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51 **Results and discussion**

52 **1. Syntheses.**

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57 These new TDMQ chelators consist of an 8-aminoquinoline scaffold substituted by
58 methoxy- or dimethylamino- groups at variable positions. The structure of these
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ligands is summarized in Scheme 1. To investigate the role of the substitution position, the 5-OCH₃, 6-OCH₃, 7-OCH₃ derivatives were prepared (TDMQ31, 32, 33, respectively). To investigate the role of the different donating group, TDMQ33 (7-OCH₃) was compared to TDMQ38 [7-N(CH₃)₂], and TDMQ32 (5-OCH₃) was compared to TDMQ39 [5-N(CH₃)₂].

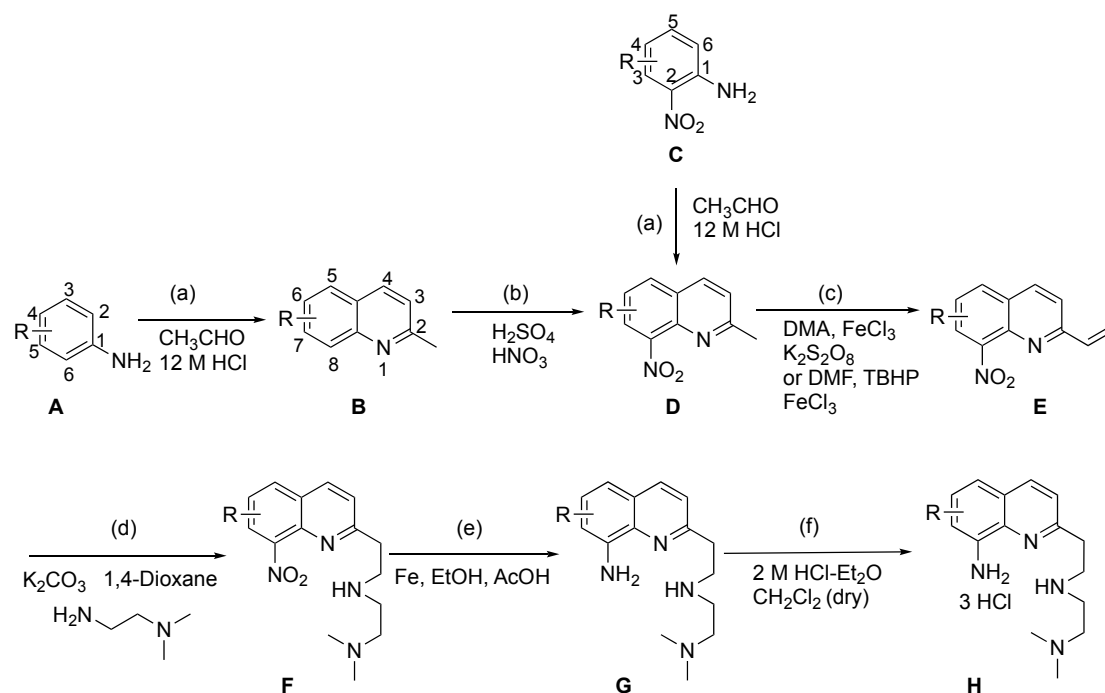


TDMQn	R ₅	R ₆	R ₇
n =			
31	H	OCH ₃	H
32	OCH ₃	H	H
33	H	H	OCH ₃
38	H	H	N(CH ₃) ₂
39	N(CH ₃) ₂	H	H
20 ^a	Cl	H	Cl

^a The previously reported TDMQ20 is given for comparison.

Scheme 1. Structures of TDMQ ligands having different electron withdrawing substituents on the quinoline ring. The previously published TDMQ20^[11] is given for comparison.

The synthesis procedure of TDMQ31-39 is summarized in Scheme 2. Starting from the suitable aniline (Compound A) or 2-nitroaniline (C), the quinoline ring was generated by reaction with acetaldehyde in concentrated hydrochloric acid. The side chain is then introduced by vinylation at position 2 of the 8-nitroquinoline (E), and further reaction with *N,N*-dimethylethylenediamine (F). The 8-nitro group is then reduced by iron/acetic acid to yield the TDMQ base (G). This compound is then protonated by HCl to yield the water-soluble chloride salt (H).



Scheme 2. Synthesis of TDMQ31, TDMQ32, TDMQ33, TDMQ38 and TDMQ39. (a) $\text{CH}_3\text{CHO}/12 \text{ M HCl}$, 80°C , 1 h; (b) $\text{HNO}_3/\text{H}_2\text{SO}_4$, r.t. 1 h; (c) FeCl_3 , $\text{K}_2\text{S}_2\text{O}_8$, DMA, 110°C , 30 min for TDMQ31; DMF, TBHP, FeCl_3 , 140°C , 3 h for TDMQ32, TDMQ33 and TDMQ39, and 5 h for TDMQ38; (d) $\text{NH}_2(\text{CH}_2)_2\text{N}(\text{CH}_3)_2$, K_2CO_3 , 1,4-dioxane, 12 h for TDMQ31, and 1 h for TDMQ32; Cs_2CO_3 , 48 h for TDMQ33, TDMQ38 and TDMQ39; (e) Fe , $\text{C}_2\text{H}_5\text{OH}$, CH_3COOH , 80°C , 1 h; (f) HCl 2 M in Et_2O , CH_2Cl_2 , 1 h.

2. Affinity of TDMQs for copper(II) and zinc(II).

The abilities of TDMQ31, 32, 33, 38, and 39 to chelate $\text{Cu}(\text{II})$ and $\text{Zn}(\text{II})$ were monitored by using UV-visible titration, in the range 200-600 nm, as previously reported.^[21] Spectral modifications upon addition of aliquots of concentrated aqueous solutions of Cu^{2+} or Zn^{2+} sulfates in a TDMQ buffered solution were recorded and considered significant of the metal complexation by the TDMQ ligands.

Upon addition of copper sulfate, a similar behavior was observed for ligands 31, 32, 33, 38, 39. For example, titration of TDMQ33 ($40 \mu\text{M}$ in Tris-HCl buffer, pH 7.4) by Cu^{2+} is depicted in Figure 1. From 0.1 to 1.0 mole equivalent (mol equiv) of Cu^{2+} , spectral changes were immediately observed. The absorbance at 255 nm of the free

ligand decreased in favor of bands at 241 nm and 335 nm assigned to the Cu^{II} -TDMQ33 complex. Isosbestic point were detected at 247 nm, 274 nm and 363 nm, well defined in the range 0-1 mol equiv of Cu^{2+} (Figure 1), in agreement with the formation of a single Cu(II) complex. The plot of absorbance at 335 nm with respect to the Cu^{2+} /TDMQ33 mole ratio (Figure 1, Insert) clearly indicate that the metal chelation was complete in these experimental conditions (no equilibrium in solution), and that the metal/ligand stoichiometry of the complex was 1/1, as previously reported for another series of N_4 -tetradentate chelators, bis(8-aminoquinolines).^[17,18,22] No further changes were observed when an excess of Cu^{2+} was added (up to 2-50 mol equivalents with respect to the ligand). Results obtained with TDMQ31, 32, 38 and 39 were similar as for TDMQ33, significant of the formation of high affinity Cu(II) complexes with L/M stoichiometries = 1/1.^[23]

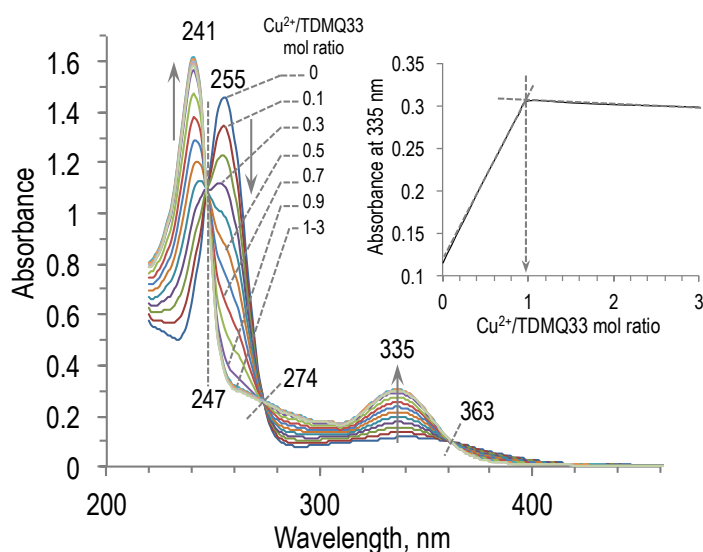
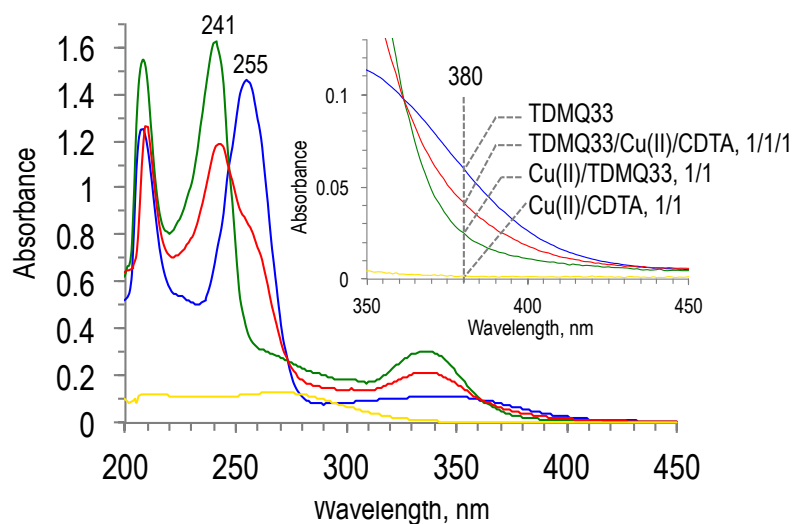


Figure 1. Titration of TDMQ33 (40 μM in Tris-HCl buffer 50 mM, pH 7.4) by $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$. The reaction was carried out from 0 to 50 mol equiv of Cu^{2+} with respect to TDMQ33. Insert: Absorbance at 355 nm as a function of the Cu^{2+} /TDMQ33 mole ratio.

In these conditions, the affinity of TDMQ ligands for Cu(II) cannot be calculated from titration curves. We therefore used 1,2-cyclohexylenedinitrilotetraacetic acid

1 {CDTA, $\log K_{app} [\text{Cu}^{\text{II}}\text{-CDTA}]$ at pH 7.4 = 17.1^[24]} as competitive chelator, and the
 2 proportion of Cu-TDMQ at equilibrium in a mixture containing TDMQ/Cu(II)/CDTA
 3 = 1/1/1 was evaluated by UV-visible spectroscopy in Tris-HCl buffer pH 7.4.^[17] In
 4 these conditions (40 μM), the proportions of Cu(II) chelated by TDMQ31, 32, 33, 38
 5 and 39 were 0.52, 0.53, 0.52, 0.68, and 0.26, respectively. As an example, results
 6 obtained for TDMQ33 are depicted in Figure 2. Consequently, the $\log K_{app}$ values for
 7 [Cu-L] were 17.2 for TDMQ31, 32 and 33, 17.8 for TDMQ 38, and 16.2 for
 8 TDMQ39 (Table 1).
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 37 Figure 2. Determination by UV-visible spectroscopy of $\log K_{app}$ of $\text{Cu}^{\text{II}}\text{-TDMQ33}$ by
 38 competition with CDTA in Tris-HCl buffer pH 7.4. The blue trace is the spectrum of
 39 TDMQ33 alone (40 μM), the red trace is the spectrum of TDMQ33/Cu/CDTA = 1/1/1,
 40 the green trace is the spectrum of Cu/TDMQ33 = 1/1, the yellow trace is the spectrum
 41 of Cu/CDTA = 1/1.
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Table 1. Affinity constants of TDMQ ligands for Cu(II) and Zn(II); selectivity for Cu(II) with respect to Zn(II).

n =	M=Cu(II) ^b	M=Zn(II)	Cu(II)/Zn(II) ^c
31	17.2	6.0 ^c	11.2
32	17.2	6.3 ^c	10.9
33	17.2	6.2 ^c	11.0
38	17.8	5.6 ^c	12.2
39	16.2	9.9 ^d	6.6
20 ^a	16.5	4.2	12.3

^a Given for comparison. Data are from Reference 19. ^b Measured by UV-Vis spectroscopy in the presence of CDTA as competitor. ^c Calculated from UV-visible titration of TDMQ ligand by Zn²⁺. ^d Measured by UV-Vis spectroscopy in the presence of EGTA as competitor. ^e Calculated as $\{\log K_{app} [Cu^{II}\text{-TDMQ}]\} - \{\log K_{app} [Zn^{II}\text{-TDMQ}]\}$.

Titration of ligands TDMQ31, 32, 33, 38, and 39 (40 μM) with Zn²⁺ was performed in a similar way as titration by Cu²⁺, but using ZnSO₄·7H₂O (up to 100 mol equiv) as a metal source. The reactions were monitored by UV-vis spectroscopy. In all cases, chelation of Zn(II) by the TDMQ ligand was observed. For example, upon addition of Zn²⁺ into a solution of TDMQ33 (40 μM in Tris-HCl buffer, pH 7.4), the absorbance of the free ligand (255 nm) disappeared in favor of absorption bands at 244 nm and 335 nm assigned to the Zn^{II}-TDMQ33 complex (Figure 3). Isosbestic points detected at 246 nm, 288 nm and 357 nm are in agreement with the formation of a single Zn^{II}-TDMQ33 complex. Significantly different from titration by Cu²⁺, TDMQ33 was not saturated upon addition of a single Zn²⁺ mole equivalent, but an excess of metal ion was required to achieve full metallation. The plot of absorbance at 335 nm with respect to the Zn²⁺/TDMQ33 mole ratio (Figure 3, Insert) clearly indicate the formation of a Zn^{II}-TDMQ33 complex with 1/1 stoichiometry. In the presence of equimolar concentrations of Zn²⁺ and TDMQ33, Zn^{II}-TDMQ, free TDMQ and Zn²⁺ coexist in solution. The proportion of Zn-TDMQ33 (88% with respect to overall amount of TDMQ33) allowed to calculate the affinity of Zn(II) for TDMQ33: $\log K_{app}[Zn^{II}\text{-TDMQ33}] = 6.2$. Details of the calculation are given in Reference 25.

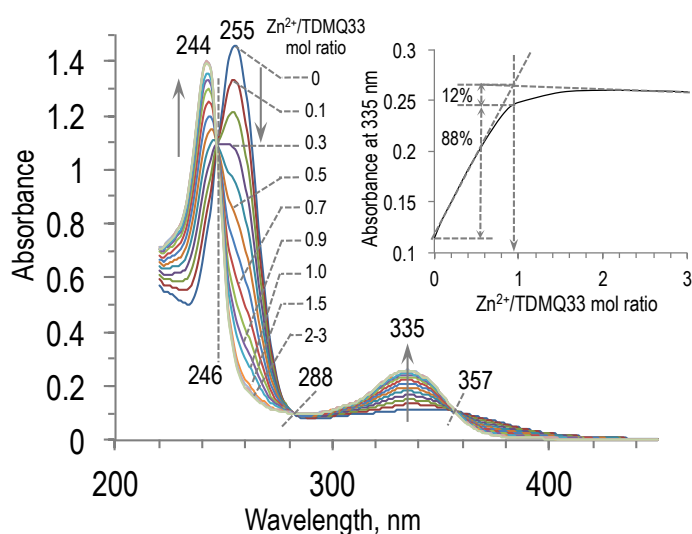


Figure 3. Titration of TDMQ33 (40 μM) by $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0-100 mole equiv) in Tris-HCl buffer 50 mM, pH 7.4. Insert: Absorbance at 335 nm as a function of the Zn^{2+} /TDMQ33 mole ratio.

Titration of TDMQ31, 32, and 38 by Zn^{2+} provided similar results, with metallation values of 85%, 90%, and 77%, respectively, upon addition of 1 mol equiv of Zn^{2+} . Then, the calculated values of $\log K_{app}[\text{Zn}^{\text{II}}\text{-TDMQ}]$ were 6.0, 6.3, and 5.6 (reported in Table 1).

Chelation of Zn(II) by TDMQ39 was more complicated (Figure 4). Plot of the absorbance at 259 nm with respect to the Zn/TDMQ39 mole ratio (Figure 4b) suggested the formation of a lower affinity transitory complex with stoichiometry $\text{Zn}/\text{TDMQ39} = 1/2$, followed by irreversible formation of a 1/1 complex in these conditions. This feature was confirmed by the absence of any clear isosbestic point in the range 310-340 nm (Figure 4a, Insert). The affinity of TDMQ39 for Zn in the 1/1 complex was measured by competitive chelation using ethylene glycol-bis(2-aminoethylether)-*N,N,N',N'*-tetraacetic acid (EGTA) as competitive chelator, and was found to be $\log K_{app}[\text{Zn}^{\text{II}}\text{-TDMQ39}] = 9.9$ at pH 7.4 (Figure 5).

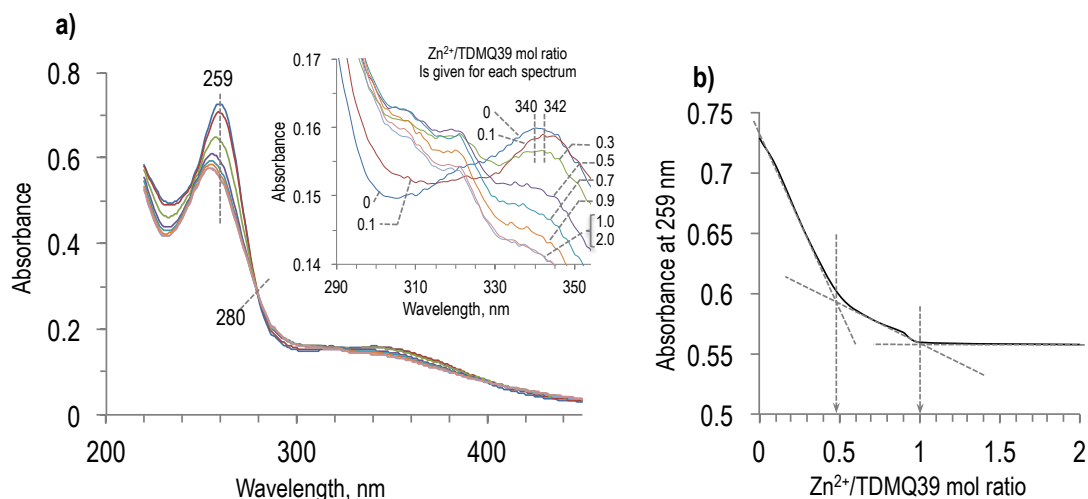


Figure 4. (a) Titration of TDMQ39 (40 μM) by ZnSO₄•7H₂O (0-100 mol equiv) in Tris-HCl buffer 50 mM, pH 7.4; Insert: Expansion of the region 290-390 nm. (b) Absorbance at 335 nm as a function of the Zn²⁺/TDMQ39 mole ratio.

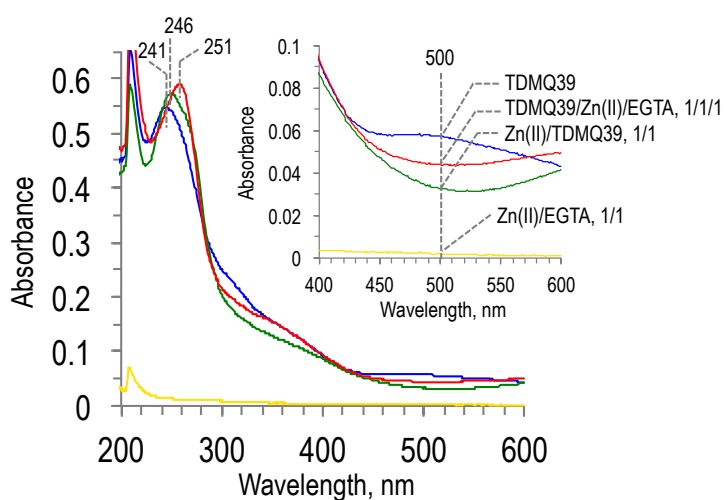


Figure 5. Determination by UV-visible of log K_{app} [Zn^{II}-TDMQ39] by competition with EGTA in Tris-HCl buffer pH 7.4. Blue trace is the spectrum of TDMQ39 alone (40 μM), the red trace is the spectrum of TDMQ39/Zn/EGTA = 1/1/1, the green trace is the spectrum of TDMQ39/Zn = 1/1, the yellow trace is the spectrum of Zn/EGTA = 1/1. log K_{app} [Zn^{II}-EGTA] = 9.4 at pH 7.4.^[24]

1 This study indicate that TDMQ derivatives substituted by a methoxy group on the
2 quinoline ring (TDMQ31-33) exhibit the same high affinity for Cu(II) ($\log K_{app} =$
3 17.2), and similar lower affinities for Zn(II) ($\log K_{app}$ in the range 6.0-6.3), giving rise
4 to high selectivity values for Cu(II) with respect to Zn(II) (close to 11 orders of
5 magnitude), whatever the position of the substituents on the quinoline ring.
6 Substitution by dimethylamine at C7 (TDMQ38) resulted in a slight increase of the
7 affinity for Cu (17.8) with respect to its methoxy analogue (17.2 for TDMQ33), while
8 the affinity for Zn slightly decreased (5.6 for TDMQ38 compared to 6.2 for
9 TDMQ33), resulting in a higher Cu/Zn selectivity. Substitution by dimethylamine at
10 C5 (TDMQ39) resulted in a decrease of the affinity for Cu (16.2) with respect to
11 substitution at C7 by 1.6 log unit. More importantly, TDMQ39 exhibited a rather high
12 affinity for Zn(II), with a $\log K_{app}$ value of 9.9. Consequently, the Cu/Zn selectivity
13 was drastically reduced for TDMQ39 compared to other TDMQ ligands.
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28 These data have been compared with those previously reported for TDMQ20,
29 with the same side chain, but was based on a 5,7-dichloroquinoline nucleus^[19] (Table
30 1). $\log K_{app}$ [Cu^{II}-TDMQ20] was 16.5, very similar to that of TDMQ39
31 (5-dimethylamino-, $\log K_{app} = 16.2$) and not far from the affinities of the methoxy-
32 derivatives TDMQ31-33 (17.2), consistent with a coordination of Cu(II) in a
33 N₄-square planar environment. For Zn(II), the affinity of TDMQ20 was 4.2,
34 significantly lower than that of all TDMQ derivatives bearing electron donating
35 substituents. This feature suggests that the substituent induced electronic effects does
36 not clearly influence the affinity of TDMQ chelators for Cu(II), and slightly increased
37 the affinity for Zn(II). The drastically different affinity of TDMQ38 and TDMQ39 (7-
38 and 5-dimethylamino derivatives, respectively) for Zn(II) (5-log units of difference)
39 should be noted. It may be due to the significantly different basicity induced by the
40 N(CH₃)₂ substitution at C5 or C7 on the 8-aminoquinoline nucleus. All in all, for
41 selective chelation of Cu in the context of AD pharmacology, TDMQ38 should be
42 preferred.
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59 **Conclusion**

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3 The present study reports a series of new 8-aminoquinoline chelators containing
4 electron donating groups, and their affinities for Cu(II) and Zn(II). These compounds
5 exhibit high affinity for Cu(II) ($\log K_{app} > 16$), consistent with the formation of
6 N_4 -square planar complexes with a Cu/TDMQ = 1/1 stoichiometry, as already
7 reported by X-ray crystallography for TDMQ20, another 8-aminoquinoline chelator
8 bearing the same side chain at C2^[19]. The affinity for Zn(II) is low, and associated
9 with a very high selectivity for Cu with respect to Zn (≥ 11). The single exception is
10 that of TDMQ39 which bears a $N(CH_3)_2$ substituent at C5. These results confirm that
11 metal selectivity in the TDMQ series is more driven by the capacity of these chelators
12 to specifically chelate Cu in a N_4 -square planar environment with the suitable side
13 chain than by electronic factors induced by different substituents on the quinoline
14 ring.
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28 On the basis of its selectivity for Cu with respect to Zn, TDMQ38 is one of the
29 promising chelators for regulation of copper homeostasis, with the previously
30 reported TDMQ20, suggesting that such low-molecular-weight copper chelators
31 should be able to regulate the homeostasis of copper in the brains of patients,^[18] and
32 should then be considered as anti-AD drug candidates. In addition, the C7- $N(CH_3)_2$
33 substituent of TDMQ38 might improve the pharmacological behavior of TDMQ38
34 with respect to TDMQ20. Further investigation on TDMQ38 is therefore in progress.
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7 **Key words**

Alzheimer; 8-Aminoquinoline; Copper homeostasis; Tetradentate chelator

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13 **Mots-clés**

Alzheimer; 8-Aminoquinoléine; Chélateur tétradentate; Homéostasie du cuivre

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26 state should be indicated in brackets for the full name of the complex, or as a
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