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Synthesis and *in vitro* anticancer activity of ferrocenyl-aminoquinoline-carboxamide conjugates

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ABSTRACT

The syntheses and characterization of new multifunctional aminoquinoline-carboxamides and their ferrocene derivatives are reported, as well as their cytotoxicity against human colon adenocarcinoma (Caco-2, HTB-37), human breast carcinoma (HTB-129) and a normal cell line as a control (human normal breast epithelial cells MCF-10A, CRL-10317). All tested compounds showed higher activity against HTB-129 cells than against Caco-2 cells. The ferrocenyl-chloroquine amide conjugates displayed higher activity against both cancer cells than did their parent organic compounds.

1. Introduction

Organometallic compounds that incorporate a ferrocene have attracted considerable attention in medicinal organometallic chemistry; ferrocene has shown itself to be an excellent choice to design new drugs [1–3] due to its small size, aromaticity, hydrophobicity, stability toward air and moisture, and redox behavior. Ferrocene derivatives have shown antimalarial [1,4–17], anticancer [1,2,18–23], antimycobacterial [18,19], antiviral [19], and antibacterial activity [24]. One strategy for improving the activity of biologically active molecules is to incorporate ferrocene into the framework. Some relevant examples are ferrocifen [25,26] and ferroquine derivatives [27,28] (Scheme 1) that have shown higher anticancer and antimalarial activity, respectively, than do their precursor compounds. The biological activity of ferrocenium salts [29] and ferrocene derivatives [1] may be augmented by its capacity to generate hydroxyl radicals ($OH^{\bullet}$) in physiological solutions, inducing oxidative damage to DNA.

Our general aim is the development of methods that provide easy access to anticancer and antimalarial drugs that include ferrocene in their structure and aminoquinolineamide conjugates. Recently, we reported the cytotoxic and antimalarial activities of various ferrocenyl carbohydrate chloroquine conjugates in MDA-MB-435S breast cancer and HTB-37 colon carcinoma cells [17]. The IC_{50} values showed higher anticancer activity in the ferrocene functionalized with chloroquine and carbohydrate groups than in their compounds without chloroquine. In this current work, we report the synthesis and characterization of new aminoquinoline-carboxamides and ferrocenyl-chloroquine amides (Scheme 2) and their *in vitro* anticancer and cytotoxicity activity. We expect that the insertion of amide or ferrocenyl amide groups into the 2-position of chloroquine should improve the biological activity against cancer.

2. Experimental

2.1. Materials and instrumentation

Reagents and analytical grade materials were obtained from commercial suppliers and used without further purification Solvents were dried and distilled prior to use. 3-Chloroaniline, dimethylacetylene dicarboxylate, ferrocene carboxaldehyde, phosphorous(V) oxychloride ($POCl_3$), sodium borohydride ($NaBH_4$), anhydrous ethylenediamine (ETD), anhydrous 1,3-diaminopropane and diphenyl ether (Ph_2O) were purchased from *Sigma–Aldrich*. Reactions in the microwave reactor were performed in a *Biotage* version 2.5. The following instruments were used for physical characterization of the compounds: elemental analyses, *Carlo Erba* Elemental Analyser EA 1108; electrospray ionization mass spectrometry (ESI-MS) spectra, *Micromass* LCT and *Waters* LC–MS; and NMR spectra, Bruker Avance 300 (^1H: 300 MHz and ^{13}C: 75 MHz). Some compounds were separated using a CombiFlash Rf system, *Teledyne Isco*.

2.2. X-ray crystallography

The crystal of **5** was obtained from ethyl acetate. The crystal was mounted on glass fiber, and measurement was made on a

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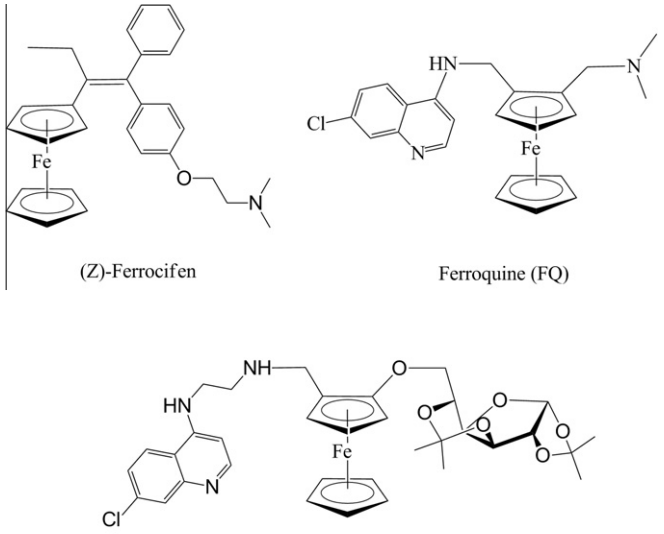
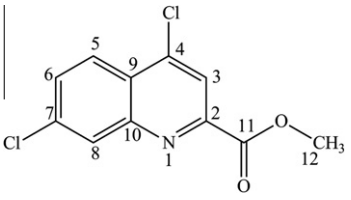


Scheme 1. Ferrocene derivatives with biological activity.

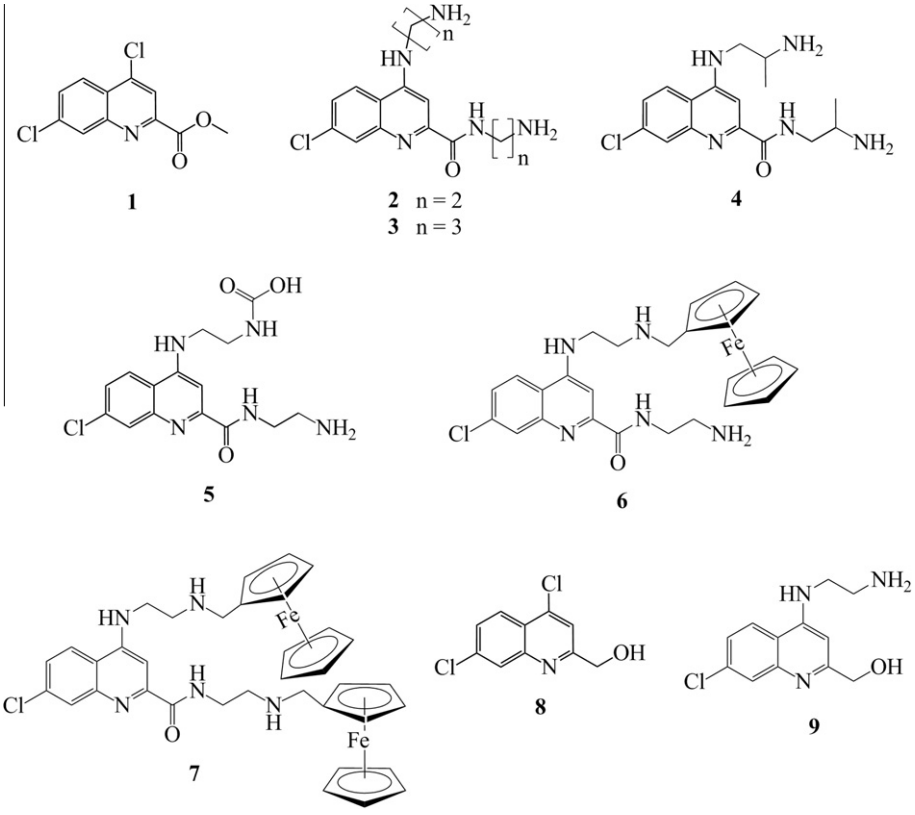
Bruker X8 APEX II instrument using graphite-monochromated Mo Kα radiation. The data were collected at 100 K. The structure was solved with direct methods using SHELX-97 [30]. The material crystallized with two crystallographically independent molecules in the asymmetric unit; the N37-C38-C39-N40 fragment of one molecule is disordered and was modeled in two orientations. Additionally, the material crystallizes with one disordered molecule of methanol in the asymmetric unit. All non-hydrogen atoms were refined anisotropically. All N–H hydrogen atoms were located in difference maps and refined isotropically; however the isotropic thermal parameters were linked to the nitrogens to which they are bonded. The O–H hydrogens were added using the HFIX 147 command. All other hydrogen atoms were placed in calculated positions.

2.3. Synthesis of compounds in Scheme 2

2.3.1. Synthesis of quinoline ester – methyl 4,7-dichloroquinoline-2-carboxylate **1**



Compound **1** was prepared using procedures previously reported [29,30]. 3-Chloroaniline (20.4 mL, 194.3 mmol) was dissolved in MeOH (200 mL), and dimethylacetylene dicarboxylate (25 mL, 203.4 mmol) was then added dropwise (exothermic reaction). The red-brown mixture was heated at reflux for 2 h, and then cooled at rt. After evaporating methanol in vacuum, the solid was dissolved in Ph_2O (50 mL), and added dropwise over 40 min into a solution of Ph_2O (250 °C) with further stirring for 4 h. After, it was cooled at rt. The reaction mixture was filtered and washed with hexane and ether. A yellow solid was obtained (24 g), redissolved in pyridine (150 mL) and refluxed for 1.5 h. The solution was then cooled and the resulting precipitate was filtered, washed with ether, dried under vacuum, and then refluxed with $POCl_3$ (9 mL) for 1 h. After cooling, the excess $POCl_3$ was removed under vacuum, and 1 M NaOH solution was then added to adjust the reaction mixture to pH 8,



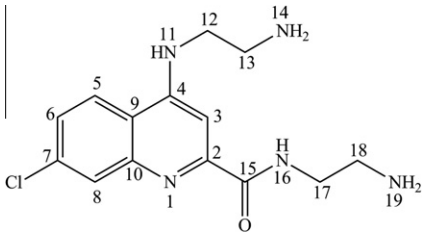
Scheme 2. Compounds **1**–**9**.

resulting in the formation of a precipitate. This was triturated and washed with water, and dissolved in chloroform to recrystallize. White very thin needles were obtained (yield: 6.7 g, 14%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K, δ, ppm): 8.36 (1H, H8), 8.29 (1H, H3), 8.25 (1H, H5), 7.72 (1H, H6), 4.11 (3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 164.9 (C=O), 148.9 (C2), 148.7 (C10), 144.2 (C4), 137.6 (C7), 130.8 (C6), 130.0 (C8), 126.1 (C9), 125.6 (C5), 121.6 (C3), 53.7 (CH_3). HRMS (ESI): $(C_{11}H_8NO_2Cl_2)^+$ Calc.: 255.9932, found: 255.9934. *Anal.* Calc. for $C_{11}H_7Cl_2NO_2$: C, 51.59; H, 2.76; N, 5.47. Found: C, 51.88; H, 2.90; N, 5.77%.

2.3.2. General method for the preparation of aminoquinolin-carboxamides **2**–**4** and carbamic acid **5**

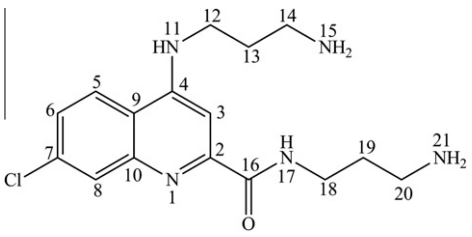
Compound **1** and the corresponding diamine were dissolved in a 2–5 mL microwave vial using a heat gun to achieve complete dissolution. The reaction was carried out in the microwave with different reaction times and temperatures to yield a yellow solution. After cooling to room temperature, the microwave vial was opened. In a separation funnel the reaction mixture and 1 M NaOH solution were mixed. The aqueous phase was extracted with $CHCl_3$ (3 × 20 mL). The combined organic phases were dried over $MgSO_4$ and after filtration, the solvent was removed in vacuum. Yellow oils were obtained in all cases.

2.3.2.1. N-(2-Aminoethyl)-4-((2-aminoethyl)amino)-7-chloroquinoline-2-carboxamide **2**.



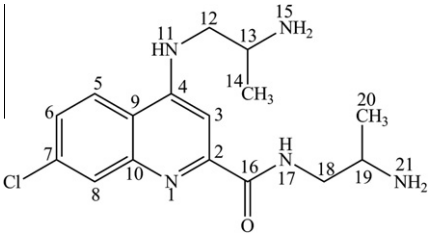
Compound **1** (200 mg, 0.78 mmol) and ethylenediamine (1 mL, 15.0 mmol) were used with microwave conditions 140 °C and 30 min. Adding 10 mL of 1 M NaOH yielded a yellow oil (178 mg, 74%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K, δ, ppm): 8.30 (1H, NH16), 7.56 (1H, H5), 7.47 (1H, H8), 6.94 (1H, H6), 6.88 (1H, H3), 6.51 (1H, NH11), 3.13 (2H, H17), 2.98 (2H, H12), 2.63 (2H, H18), 2.56 (2H, H13), 2.11 (4H, NH14, NH19). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 165.4 (O=C), 151.5 (C2), 151.3 (C4), 147.7 (C10), 134.9 (C7), 128.3 (C8), 125.5 (C5), 122.3 (C6), 117.8 (C9), 96.3 (C3), 45.2 (C17), 42.5 (C12), 41.6 (C18), 40.1 (C13). HRMS (ESI): $(C_{14}H_{19}N_5OCl)^+$ Calc.: 308.1278, found: 308.1286.

2.3.2.2. N-(3-Aminopropyl)-4-((3-aminopropyl)amino)-7-chloroquinoline-2-carboxamide **3**.



Compound **1** (210 mg, 0.82 mmol) and 1,3-diaminopropane (1 mL, 12.3 mmol) were used with microwave conditions 140 °C and 15 min. Adding 10 mL of 1 M NaOH yielded an impure yellow oil (197 mg) that was redissolved in a small volume of $CHCl_3$. After evaporation of the solvent in air, a precipitate was formed and removed by filtration. The resulting yellow solution was evaporated to yield a yellow oil (101 mg, 37%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K, δ, ppm): 8.37 (1H, NH17), 7.73 (1H, H8), 7.65 (1H, H5), 7.51 (1H, NH11), 7.18 (1H, H6), 7.10 (1H, H3), 3.34 (2H, H13), 2.68 (2H, H12), 2.61 (4H, H14, H20), 3.43 (2H, H19), 3.10 (2H, H18), 1.56 (4H, NH15, NH21). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 165.6 (O=C), 151.9 (C2), 151.5 (C4), 147.9 (C10), 135.1 (C7), 128.6 (C8), 125.8 (C5), 122.4 (C6), 118.0 (C9), 96.0 (C3), 43.5 (C18), 41.3 (C12), 39.6 (C20), 37.1 (C14), 33.4 (C19), 30.0 (C13). HRMS (ESI): $(C_{16}H_{23}N_5OCl)^+$ Calc.: 336.1591, found: 336.1578.

2.3.2.3. N-(2-Aminopropyl)-4-((2-aminopropyl)amino)-7-chloroquinoline-2-carboxamide **4**.



Compound **1** (220 mg, 0.86 mmol) and 1,2-diaminepropane (1 mL, 11.7 mmol) were used with microwave conditions 140 °C and 15 min. Adding 10 mL of 1 M NaOH yielded a yellow oil (245 mg, 85%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K, δ, ppm): 8.46 (1H, NH17), 7.77 (1H, H8), 7.75 (1H, H5), 7.20 (1H, H6), 7.12 (1H, H3), 6.39 (1H, NH11), 4.70 (4H, $2NH_2$), 3.33–2.92 (6H, H12, H13, H18, H19), 1.04 (6H, $2CH_3$). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 165.5 (O=C), 151.5 (C2), 151.3 (C4), 147.8 (C10), 135.2 (C7), 128.4 (C8), 125.9 (C5), 122.0 (C6), 117.8 (C9), 96.6 (C3), 54.2 (C12), 50.2 (C18), 47.5 (C13), 46.9 (C19), 22.4 (C14), 21.4 (C20). HRMS (ESI): $(C_{16}H_{23}N_5OCl)^+$ Calc.: 336.1591, found: 336.1589.

2.3.2.4. (2-((2-((2-Aminoethyl)carbamoyl)-7-chloroquinolin-4-yl)amino)ethyl)carbamic acid **5**.

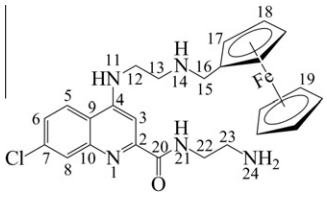
4,7-Dichloroquinoline-2-carboxylate (300 mg, 1.2 mmol) and ethylenediamine (15 mL, 224.4 mmol) were used with microwave conditions 165 °C and 30 min. Adding 30 mL of 1 M NaOH yielded a yellow oil that was dissolved in a small volume of hot ethyl acetate. When this solution was cooled to RT, a precipitate formed to which methanol:hexane (4:6) was added to form a yellow solution. This was evaporated at RT and light yellow crystals were obtained (yield: 292 mg, 71%) and analyzed by X-ray diffraction. The yellow crystals were scarcely soluble in methanol and insoluble in $CDCl_3$ and DMSO-d_6; they were soluble enough in MeOH to be characterized in solution by low mass spectrometry: MS ES$^+$ (MeOH): $[C_{15}H_{18}ClN_5O_3]^+$ (350 *m*/*z*, 20%). *Anal.* Calc. $C_{15}H_{18}ClN_5O_3{\cdot}2H_2O$: C, 46.46; H, 5.72; N, 18.06. Found: C, 46.06; H, 5.63; N, 18.14%.

2.3.3. Synthesis of ferrocenyl-chloroquine conjugates **6** and **7**

Compound **2** (170 mg, 0.55 mmol) and ferrocene carboxaldehyde (118 mg, 0.55 mmol) were dissolved in dry methanol (20 mL) in Schlenk-type glassware under Ar; a red-orange solution was obtained and stirred for 16 h, after which time a red-brown solution was obtained. $NaBH_4$ (31 mg, 0.82 mmol) was added. The solution changed color to dark brown, and was stirred for 1.5 h at RT. The reaction was quenched by addition of 1 M NaOH solution (10 mL). Brine (NaCl conc., 20 mL) was added and the aqueous phase was extracted using chloroform (3 × 40 mL). The combined organic phases were dried over $MgSO_4$ and after filtration, the solvent was removed in vacuum. The crude product was

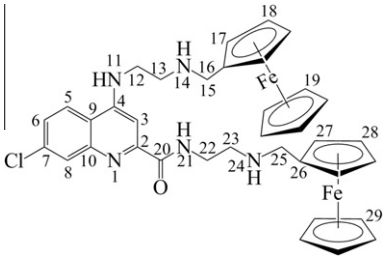
purified by column chromatography on silica with a 3:2 mixture of chloroform/methanol as eluent (Rf = 0.17) for **6** and in 4:1 chloroform/methanol (Rf = 0.41) for **7**.

2.3.3.1. N-(2-Aminoethyl)-7-chloro-4-((2-((ferrocenylmethyl)amino) ethyl)amino)quinoline-2-carboxamide 6.



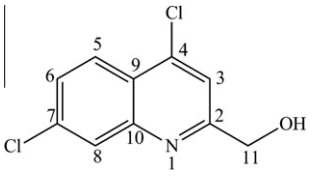
An orange-red oil was obtained (yield: 64 mg, 23%). In the ^{1}H NMR spectrum (300 MHz, $CDCl_3$, 300 K), the signals were very broad and not available to be assignable: 8.54, 7.91–7.78, 7.39–7.29, 6.25, 4.30–2.96, 2.25. ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 165.7 (O═C), 151.7 (C2, C4), 148.1 (C10), 135.5 (C7), 128.8 (C8), 126.4 (C5), 122.2 (C6), 118.4 (C9), 96.8 (C3), 86.8 (C16), 68.7 (C17, C19), 68.3 (C18), 50.8 (C22), 48.9 (C15), 47.1 (C12), 42.7 (C23), 39.6 (C13). HRMS (ESI): $(C_{25}H_{29}ClFeN_5O)^+$ Calc.: 504.1457, found: 504.1449.

*2.3.3.2. 7-Chloro-N-(2-((ferrocenylmethyl)amino)ethyl)-4-((2-((ferrocenylmethyl)amino)-ethyl))amino)quinolone-2-carboxamide **7**.*



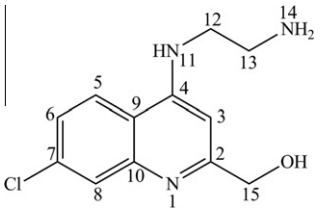
An orange-yellow oil was obtained (yield: 34 mg, 9%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K, δ, ppm): 8.58 (1H, HN21), 7.95 (1H, H5), 7.73 (1H, H8), 7.40 (1H, H6), 7.32 (1H, H3), 6.12 (1H, HN11), 4.24 (4H, H18, H28), 4.20 (4H, H17, H27), 4.14 (10H, H19, H29), 3.62 (2H, H25), 3.61 (2H, H15), 3.59 (2H, H22), 3.58 (1H, HN24), 3.41 (2H, H12), 3.05 (2H, H23), 2.98 (2H, H13), 1.89 (1H, HN14). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 165.6 (O═C), 151.6 (C2), 151.4 (C4), 148.0 (C10), 135.5 (C7), 129.1 (C8), 126.3 (C5), 121.8 (C6), 117.9 (C9), 97.1 (C3), 87.0 (C26), 86.6 (C16), 68.7 (C29), 68.6 (C19), 68.2 (C17, C27), 68.0 (C18, C28), 48.6 (C15), 48.5 (C22), 48.4 (C25), 46.9 (C12), 42.2 (C13), 42.2 (C23). HRMS (ESI): $(C_{36}H_{39}ClFe_2N_5O)^+$ Calc.: 702.1589, found: 702.1583.

*2.3.4. Reduction of **1** to (4,7-dichloroquinolin-2-yl)methanol **8***



Compound **1** (600 mg, 2.3 mmol) and finely powdered $NaBH_4$ (530 mg, 14.0 mmol) were stirred together in THF (30 mL) to give a colorless suspension which was then heated at 70 °C for 30 min. The light yellow mixture, after 10 min of heating, changed to green-yellow. Then, with stirring and no heating, under Ar, dry methanol (8 mL) was added dropwise over 30 min (exothermic reaction) to give a dark brown reaction mixture. This was stirred at 70 °C for 4 h. The reaction mixture was then cooled, quenched with aq. NH_4Cl (15 mL) and stirred for an additional 2 h. The organic layer was separated and the aqueous phase extracted with ethyl acetate (30 mL). The organic phases were dried over $MgSO_4$ and concentrated to give **8** as a white solid (yield: 370 mg, 70%). ^{1}H NMR (300 MHz, $CDCl_3$, 300 K, δ, ppm): 8.99 (1H, H5), 7.82 (1H, H8), 7.65 (1H, H3), 7.45 (1H, H6), 4.78 (1H, OH), 4.73 (2H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 162.9 (C2), 147.3 (C10), 143.1 (C4), 136.2 (C7), 127.5 (C8), 126.4 (C6), 124.9 (C5), 123.3 (C9), 118.5 (C3), 63.7 (C11). HRMS (ESI): $(C_{10}H_8Cl_2NO)^+$ Calc.: 227.9983, found: 227.9983.

*2.3.5. (4-((2-Aminoethyl)amino)-7-chloroquinolin-2-yl)methanol **9***



Compound **9** was obtained using the general method to prepare the aminoquinoline-carboxamides **2**–**4**, using a mixture of compound **8** (83 mg, 0.36 mmol) and ethylenediamine (1 mL, 15 mmol) at 140 °C for 30 min. A dark red oil was obtained. The target product was not soluble in $CHCl_3$ and therefore the crude mixture was freed of impurities by washing with $CHCl_3$; the obtained solid was dried under vacuum (yield: 37 mg, 41%). It was very soluble in methanol. ^{1}H NMR (300 MHz, CD_3OD, 300 K, δ, ppm): 8.00 (1H, H5), 7.71 (1H, H8), 7.30 (1H, H6), 6.69 (1H, H3), 4.68 (2H, H15), 4.66 (NH_2), 3.56 (1H, NH), 3.42 (OH), 3.40 (2H, H12), 2.97 (2H, H13). ^{13}C NMR (75 MHz, $CDCl_3$, 300 K, δ, ppm): 163.3 (C2), 152.0 (C4), 148.2 (C10), 135.2 (C7), 126.1 (C8), 124.5 (C5), 122.8 (C6), 116.9 (C9), 94.4 (C3), 64.9 (C15), 45.3 (C12), 39.8 (C13). HRMS (ESI): $(C_{12}H_{15}ClN_3O)^+$ Calc.: 252.0904, found: 252.0902.

2.4. Biological activity evaluation (in vitro)

2.4.1. Cell viability assays

The MTT assay measures the reduction of a tetrazolium component by the mitochondria into insoluble purple formazan crystals in metabolically active cells [33]. The amount of color produced is directly proportional to the number of viable cells. Cellular responses of two tumour cell lines (human colon adenocarcinoma Caco-2, ATCC #HTB-37 and human breast carcinoma, ATCC #HTB-129) and one normal cell line as a control (human normal breast epithelial cells MCF-10A, ATCC CRL-10317) after exposure to compounds **1**–**8** were determined.

The cells were purchased from ATCC. Caco-2 cells were incubated in *Eagle* Minimum Essential Medium (MEM-α), 10% fetal bovine serum (FBS), 50 IU/mL penicillin, 50 μg/mL streptomycin at 37 °C; 5% CO_2. The HTB-129 cells were grown in *Leibovitz's* L-15 medium with 2 mM L-glutamine, supplemented with 0.01 mg/mL human recombinant insulin, Zn solution (GIBCO 12585-014); 10% FBS; 50 IU/mL penicillin, 50 μg/mL streptomycin at 37 °C with atmospheric air. The MCF-10A cells were grown in *Dulbecco's*

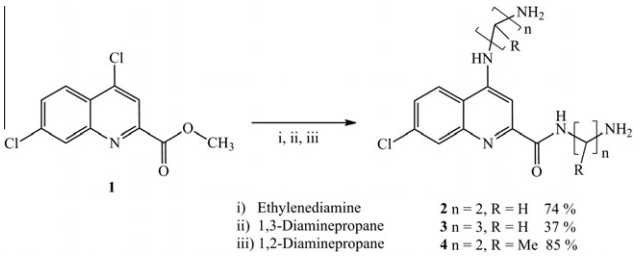
modified Eagle medium F12, 5% FBS, 50 IU/mL penicillin, 50 μg/mL streptomycin, 2 mM glutamine, 0.01 μg/mL human epidermal growth factor, 0.5 μg/mL hydrocortisone, 10 μg/mL insulin at 37 °C, 5% CO_2. The cell lines were grown in *Falcon* BD T-25 vented flasks. All cell culture reagents were purchased from Invitrogen. Cell viability assays were performed in 96-well flat bottom plates (*Becton Dickinson*, Franklin Lakes, NJ). Each cell line monolayer was detached from flasks with 0.25% trypsin and 0.38 g/L EDTA and the cells were re-suspended in fresh culture medium at a density of 1×10^5 cells/mL. An aliquot of 100 μL of cell suspension was used in each well. For blank control 200 μL of medium without cells was used. To prevent evaporation 200 μL of sterile distilled water was added to the plate's outer wells. Each plate was incubated for 24 h in correspondence with cell line growth requirement before treatment with experimental compounds.

Compounds **1**–**8** were dissolved in DMSO and serially diluted in culture medium in concentrations from 100 to 0.1 μg/mL and added to the cells (compound **9** was not available in large enough quantities for testing. For "no treatment" control 100 μL of culture medium was used instead. For the "DMSO effect" control 100 μL 0.5% DMSO dissolved in medium was added to the control wells. The plate was incubated as previously described for 72 h. Then 50 μL of MTT solution (2.5 mg/mL) was added to all of the plate's experimental wells, except those containing water. The plates were incubated for 3 h and medium was carefully aspirated from each well. DMSO (150 μL) was added to lyse the cells and solubilize the formazan crystals and the plate was placed on an *Orbit* P4 shaker for 3 min (*Labnet International*, Woodbridge, NJ). The samples were analyzed on a DTX 880 (*Beckman Coulter Inc.*) plate reader at a wavelength of 570 nm. Cell viability at each drug concentration was expressed as a percentage of untreated controls and IC_{50} values were determined by non-linear regression analysis using Prism5 (*GraphPad*, CA, USA).

3. Results and discussion

3.1. Synthesis and spectroscopic characterization

Methyl 4,7-dichloroquinoline-2-carboxylate **1** was prepared from 3-chloroaniline and dimethylacetylene dicarboxylate, using procedures modified from the literature [31,32]. The target aminoquinoline-carboxamides **2**–**4** were prepared by substitution reaction of compound **1** with an excess of the corresponding diamine *via* microwave-assisted conditions, as shown in Scheme 3. The reactions were carried out at 140 °C for 15–30 min, and the purification of the products was accomplished by addition of 1 M NaOH and extraction with chloroform. As expected, the chlorine was not the only substituent in the quinoline ring (4-position) subject to nucleophilic displacement, but the carboxylate group also suffered a nucleophilic attack, given the formation of aminoquinoline carboxamides **2**–**4**. When symmetrical bidentate amines were used, such as ethylenediamine or 1,3-diaminopropane, just one compound was obtained in each case. The reaction of compound **1** with the asymmetric 1,2-diaminopropane gave structural isomers, observed by NMR in a 5:1 ratio, isomer **4** the major product; they were analyzed as a mixture. In all cases the products were obtained as yellow oils, in moderate yields. The amides **2**–**4** were very soluble in $CHCl_3$, and stable towards air and moisture.



Scheme 3. Synthesis of diamides **2**–**4**. Microwave conditions: (i) 140 °C, 30 min; (ii and iii) 140 °C, 15 min.

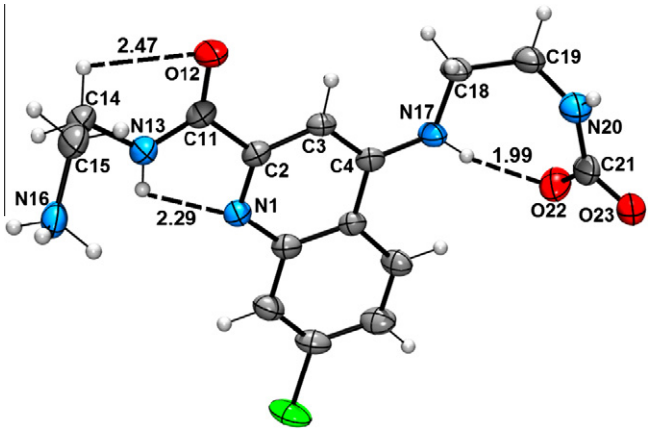


Fig. 1. Displacement ellipsoid plot (50% probability) showing one of the two independent molecules in the crystal of **5**. Intramolecular hydrogen bonds found in **5** are depicted.

These new amides were reacted with ethyl acetate (EtAcO) in alkaline conditions to form carbamic acids, as was probed with the amide **2**. Amide **2** was converted into its acid derivative by ammonolysis and the ester was hydrolysed in alkaline solution to give **5**. The solid product formed in ethyl acetate was completely solubilized by addition of MeOH and hexane (4:6), and then further purified by recrystallization from these solvents. Crystals of compound **5** were characterized in the solid state by X-ray diffraction and elemental analysis. It too poorly soluble for characterization in solution; only the low resolution mass spectrum was obtained from a solution in MeOH, from which the crystals were obtained.

The solid state structure of **5** is shown in Fig. 1, and selected bond distances and angles are listed in Table 1. Compound **5** is a zwitterionic species in the solid state, with N16 protonated and the carboxylate group deprotonated. The delocalization of electrons in the fragment O–C–O is deduced from the similar C–O bond lengths (C21–O22 = 1.271(5) Å and C21–O23 = 1.272(5) Å). The positive charge on nitrogen N16 can be deduced from the covalent bond length C15–N16 (1.485(5) Å) being longer than other N–C bonds in the molecule (from 1.326(5) to 1.459(5) Å).

Table 1
Selected bond distances and angles for zwitterion **5**.

Bond distance (Å)			
C2–N1	1.331(4)	C11–N13	1.326(5)
C2–C3	1.392(5)	C14–N13	1.459(5)
C2–C11	1.519(5)	C15–N16	1.485(5)
C3–C4	1.382(4)	C18–N17	1.451(4)
C4–N17	1.347(4)	C19–N20	1.431(5)
C4–C9	1.440(4)	C21–O22	1.271(5)
C10–N1	1.375(4)	C21–O23	1.272(5)
C11–O12	1.225(4)	C21–N20	1.365(5)
Bond angles (°)			
N1–C2–C11	116.4(3)	N16–C15–C14	113.1(3)
N17–C4–C3	122.3(3)	N20–C19–C18	113.4(3)
N17–C4–C9	120.4(3)	O22–C21–O23	123.0(3)
O12–C11–N13	124.3(3)	O22–C21–N20	119.3(4)
O12–C11–C2	120.5(3)	O23–C21–N20	117.6(4)
N13–C11–C2	115.1(3)	C4–N17–C18	123.8(3)

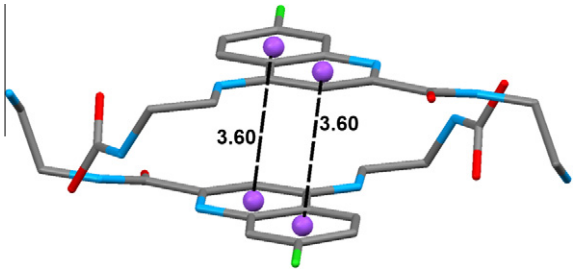


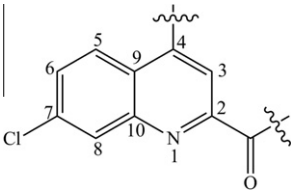
Fig. 2. π-Stacking in **5**, the distance between the ring centroids is shown (3.60 Å).

The crystal structure of **5** shows four weak cooperative intramolecular interactions: two short contacts (O22···H17 = 1.99(5) Å and N1···H13 = 2.29(5) Å; $\sum r_{vdW}$ = 2.8 Å) [34], and another O···H–C hydrogen bond with an aromatic proton (O12···H14 = 2.47 Å). In the lattice structure of **5**, the molecules are linked through π stacking, Fig. 2, between quinoline moieties (distance of 3.6 Å), which is considered a weak interaction [35]. This kind of arrangement has been observed in similar structures of quinoline derivatives [36,37]. Also, dimers of **5** were observed in the lattice, formed by hydrogen bonding interactions of the amide with the carboxylate oxygen atoms.

The ferrocenyl-aminoquinoline-carboxamide conjugates **6** and **7** result from the reaction of aminoquinoline-carboxamides **2** and ferrocene carboxaldehyde in a 1:1 M ratio, Scheme 4. They were obtained as a mixture and separated by column chromatography on silica gel using chloroform and methanol as eluent. Ferrocene conjugate **6** was obtained as the main product. The ferrocene conjugate **6** is orange-red and **7** is orange-yellow oil. In order to avoid the double amine substitution in **1** to have only one ferrocene unit, the ester **1** was converted into alcohol. The reduction of **1** was carried out with $NaBH_4$ in dry THF, the reaction product was the (4,7-dichloroquinolin-2-yl)methanol **8**. Then, it was treated with an excess of ethyleneamine, as solvent at 140 °C for 30 min, giving the (4-((2-aminoethyl)amino)-7-chloroquinolin-2-yl)methanol **9**.

The structures of compound **1**–**4** and **6**–**9** were deduced in solution by ^1H and ^{13}C NMR spectroscopy and confirmed by mass spectrometry. The ^1H and ^{13}C NMR spectra of compounds **1**–**4** and **6**–**9** presented all the expected chemical shifts (*vide supra*), with the exception of the ^1H NMR spectrum of **6**, which showed very broad signals. The most relevant chemical shifts are shown in Table 2. In the ^{13}C NMR spectra of these compounds, the chemical shifts around 165.0 ppm correspond to carbonyl groups. In compounds **2**–**4** and **6**–**9**, the C3 signal was found at lower frequencies than in the starting material **1** due to the interchange of chlorine atom by amine group (~25 ppm). The signal of C4 appeared ~7 ppm at high frequency with respect to compound **1**, corroborated the presence of amine groups. A similar effect was observed in C2 (~2 ppm at high frequencies). The high-resolution mass spectra of the compounds showed the corresponding molecular ions, further confirming their identity.

Table 2
^{13}C NMR data (ppm) of compounds **1**–**4** and **6**–**9**.



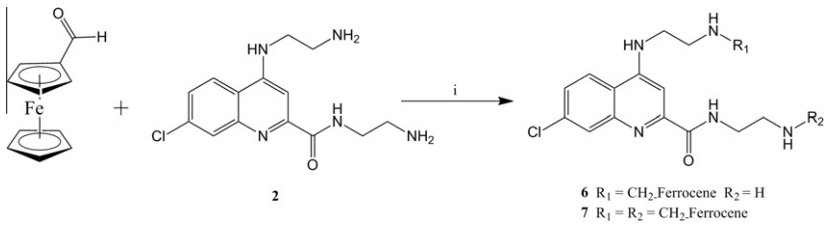
Compound	1	2	3	4	6	7	8	9
C=O	164.9	165.4	165.6	165.5	165.7	165.6	–	–
C2	148.9	151.5	151.9	151.5	151.7	151.6	162.9	163.3
C3	121.6	96.3	96.0	96.6	96.8	97.1	118.5	94.4
C4	144.2	151.3	151.5	151.3	151.7	151.4	143.1	152.0

3.2. Cytotoxicity activity evaluation (in vitro)

The *in vitro* antiproliferative effect of compounds **1**–**8** were evaluated in two different cancer cell lines, namely human colon carcinoma (Caco-2, HTB-37), and human breast cancer (MDA-MB-435S, HTB-129), while the determination of compound cytotoxicity was done on non-cancerous cell lines (human normal breast epithelial cells (MCF-10A). Six wells per condition for each of the three cell lines. Cisplatin was used as reference in the cell proliferation assay. Also, a selectivity index (SI) was determined, based on the ratio of IC_{50} values obtained against the normal cells (cytotoxicity) and the IC_{50} values obtained against the cancer cells. The IC_{50} values (μM) are listed in Table 3.

The data revealed that compounds **1**–**8** were more active against HTB-129 cells than against Caco-2 cells. A general trend in the IC_{50} values of HTB-129 was that compounds containing ferrocene moieties were more active than their organic parent compound, chloroquinecarboxamide **2**. Compound **7** with two ferrocene groups in its structure was slightly more potent than was compound **6**, with one ferrocene. In compounds **2** and **3**, which differ in the side chain length between the amine groups by one CH_2 unit, the cytotoxicity was ethyl-**2** > propyl-**3**. For the organic compound **2**, replacement of the OCH_3 of **1** by an ethylenediamine chain improved the cytotoxicity of the compound, **2** > **1**. In this cell line, compounds **4** and **8** were more potent than cisplatin, which was used as reference (compound **4** was a racemate).

When compounds **1**–**8** were tested against Caco-2 cells, it was found that only **1**, **6**–**8** (143.16, 0.53, 0.33 and 28.5 μM, respectively) possessed greater potencies in comparison to cisplatin (395.8 μM). A similar trend in activity against HTB-129 cells of the ferrocenyl-aminoquinoline-carboxamide conjugates **6** and **7** was observed against Caco-2 cells, the presence of two ferrocene groups in the structure of **7** improved its activity by a factor of two in comparison with its analog with one ferrocene unit. So, both compounds **6**–**7** were again the most potent against Caco-2 of all



Scheme 4. Synthesis of ferrocenyl derivatives **6** and **7**. Conditions: (i) MeOH, RT, 16 h, then $NaBH_4$, RT, 1.5 h.

Table 3
In vitro cytotoxicity results.

Compound	IC_{50} (μM)			SI[a]	
	HTB-129	Caco-2	MCF-10A	HTB-129	Caco-2
Cisplatin	50.50(±0.04)	395.80(±0.19)	12.80(±0.02)	0.25	0.03
1[b]	16.83(±0.01)	143.16(±1.46)	170.02(±0.97)	10.11	1.19
2	3.31(±0.01)	984.14	16.51(±0.01)	5.00	0.02
3	16.73	3739.99	93.08(±0.04)	5.57	0.02
4	2.11(±0.01)	–	4.50(±0.02)	2.14	–
5	5.12(±0.02)	–	54.44(±0.02)	10.66	–
6	0.59(±0.02)	0.53(±0.02)	0.32(±0.01)	0.54	0.60
7	0.28(±0.02)	0.33(±0.02)	0.24(±0.02)	0.85	0.72
8	23.50(±0.02)	28.50(±0.02)	8.55(±0.14)	0.36	0.30
$CQ(PO_4)_2$[c]	39.1	112	69.7		
FQ[c]	8.7	101	34.3		

[a] Selectivity index (SI) = IC_{50} MCF-10A/IC_{50} cancer cell line.
[b] Compound **1** was slightly soluble in DMSO and the maximum concentration was 50 μg/mL.
[c] IC_{50} values reported by Herrmann et al. [17].

the studied compounds. Also, they were more active than chloroquine diphosphate (112 μM) and ferroquine (101 μM) [17]. Ferrocenyl derivatives **6** and **7** had lower IC_{50} values against HTB-129 and Caco-2 cancer cell lines than the previously described ferrocene conjugates with carbohydrates in the framework [17].

The cytotoxicity of the compounds **1**–**8** was measured against the MCF-10A cell line. It was found that compounds **4** and **6**–**8** showed higher cytotoxicity than did cisplatin. The same trend was observed in the HTB-129 cells with non-cancerous cells; the introduction of the methylene group to increase the length of aliphatic chain between amine groups decreased the cytotoxicity. In spite of being highly potent against colon and breast cancer cells, the ferrocenyl derivatives were more cytotoxic than the precursor organic compound **2** (16.51 μM) and the cisplatin (12.8 μM). Nevertheless, the SI data in Table 3 showed that they are slightly more selective against both cancer cells than was cisplatin.

4. Conclusions

The syntheses, characterization and cytotoxic activities of some new aminoquinoline-carboxamides and ferrocenyl-quinoline conjugates are reported. They were synthesized by microwave-based reactions of methyl 4,7-dichloroquinoline-2-carboxylate (**1**) with ethylenediamine, 1,3-diaminepropane and 1,2-diaminepropane, with good yields and in shorter reaction times than with traditional methods. These aminoquinoline-carboxamides have shown to be excellent precursors for novel substituted ferrocene-conjugates of chloroquine. All compounds were tested *in vitro* against cancer and normal cells and in general, they showed good activity against human breast cancer cells. In general, the ferrocenyl derivatives were highly potent against colon and breast cancer cells but not very selective.

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Appendix A. Supplementary material

Crystal data for the structure of compound **5**. Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2012.06.039.

References

[1] M.F.R. Fouda, M.M. Abd-Elzaher, R.A. Abdelsamaia, A.A. Labib, Appl. Organometal. Chem. 21 (2007) 613.
[2] C. Ornelas, New J. Chem. 35 (2011) 1973.
[3] G. Gasser, N. Metzler-Nolte, Curr. Opin. Chem. Biol. 16 (2012) 84.
[4] D.D. N'Da, J.C. Breytenbach, P.J. Smith, C. Lategan, Arzneimittel-Forschung 61 (2011) 358.
[5] J. Quirante, F. Dubar, A. González, C. Lopez, M. Cascante, R. Cortés, I. Forfar, B. Pradines, C. Biot, J. Organomet. Chem. 696 (2011) 1011.
[6] N. Chavain, H. Vezin, D. Dive, N. Touati, J.-F. Paul, E. Buisine, C. Biot, Mol. Pharm. 5 (2008) 710.
[7] C.L. Ferreira, C.B. Ewart, C.A. Barta, S. Little, V. Yardley, C. Martins, E. Polishchuk, P.J. Smith, J.R. Moss, M. Merkel, M.J. Adam, C. Orvig, Inorg. Chem. 45 (2006) 8414.
[8] V. Singh, L. Tyagi, M. Singhal, C.S. Sharma, M.L. Kori, Syst. Rev. Pharm. 1 (2010) 182.
[9] C. Biot, B. Pradines, D. Dive, Drug Discov. Infect. Dis. 2 (2011) 397.
[10] F. Dubar, T.J. Egan, B. Pradines, D. Kuter, K.K. Ncokazi, D. Forge, J. -F Paul, C. Pierrot, H. Kalamou, J. Khalife, E. Buisine, C. Rogier, H. Vezin, I. Forfar, C. Slomianny, X. Trivelli, S. Kapishnikov, L. Leiserowitz, D. Dive, C. Biot, ACS Chem. Biol. 6 (2011) 275.
[11] C.J. Marfurt, F. Chalfein, P. Prayoga, F. Wabiser, E. Kenangalem, K.A. Piera, B. MacHunter, E. Tjitra, N.M. Anstey, R.N. Price, Antimicrob. Agents Chemother. 55 (2011) 4461.
[12] C. Biot, D. Dive, Bioorganometallic Chemistry and Malaria, E-Publishing, Springer-Verlag Berling Heidelberg, 2010. pp. 155–193.
[13] F. Dubar, J. Khalife, J. Brocard, D. Dive, C. Biot, Molecules 13 (2008) 2900.
[14] C. Biot, D. Taramelli, I. Forfar-Bares, L.A. Maciejewski, M. Boyce, G. Nowogrocki, J.S. Brocard, N. Basilico, P. Olliaro, T.J. Egan, Mol. Pharm. 2 (2005) 185.
[15] C. Biot, L. Delhaes, L.A. Maciejewski, M. Mortuaire, D. Camus, D. Dive, J.S. Brocard, Eur. J. Med. Chem. 35 (2000) 707.
[16] M. J. Adam, C. Orvig, P. Salas, C. Herrmann. Carbohydrate–Metallocene–Antimalarial Conjugates. USA provisional, PCT, and utility patent applications filed February 2010.
[17] C. Herrmann, P. Salas-Fernandez, B.O. Patrick, C. de Kock, P.J. Smith, M.J. Adam, C. Orvig, Dalton Trans. 41 (2012) 6431.
[18] A. Mahajan, L. Kremer, S. Louw, Y. Guérardel, K. Chibale, C. Biot, Bioorg. Med. Chem. Lett. 21 (2011) 2866.
[19] B. Lal, A. Badshah, A.A. Altaf, N. Khan, S. Ullah, Appl. Organometal. Chem. 25 (2011) 843.
[20] R. Miao, J. Wei, M. Lv, Y. Cai, Y. Du, X. Hui, Q. Wang, Eur. J. Med. Chem. 46 (2011) 5000.
[21] J. Spencer, J. Amin, M. Wang, G. Packham, S.S. Alwi, G.J. Tizzard, S.J. Coles, R.M. Paranal, J.E. Bradner, T.D. Heightman, ACS Med. Chem. Lett. 2 (2011) 358.
[22] C. Wu, H. Ye, W. Bai, Q. Li, D. Guo, G. Lv, H. Yan, X. Wang, Bioconjugate Chem. 22 (2011) 16.
[23] A. Mooney, A.J. Corry, C.N. Ruairc, T. Mahgoub, D. O'Sullivan, N. O'Donovan, J. Crown, S. Varughese, S.M. Draper, D.K. Rai, P.T.M. Kenny, Dalton Trans. 39 (2010) 8228.
[24] M. Patra, G. Gasser, N. Metzler-Nolte, Dalton Trans. 41 (2012) 6350.
[25] P. Pigeon, S. Top, A. Vessières, M. Huchè, M. Görmen, M.E. Arbi, M.A. Plamont, M.J. McGlinchey, G. Jaouen, New J. Chem. 35 (2011) 2212.
[26] S. Top, A. Vessières, C. Cabestaing, I. Laios, G. Leclercq, C. Provot, G. Jaouen, J. Organomet. Chem. 637–639 (2001) 500.
[27] C. Biot, G. Glorian, L.A. Maciejewski, J.S. Brocard, O. Domarle, G. Blampain, P. Millet, A.J. Georges, H. Abessolo, D. Dive, J. Lebibi, J. Med. Chem. 40 (1997) 3715.
[28] C. Biot, B. Pradines, D. Dive, in: K. Becker (Ed.), Ferroquine: A Concealed Weapon, Wiley-VCH, 2011, pp. 397–411.
[29] D. Osella, M. Ferrali, P. Zanello, F. Laschi, M. Fontani, C. Nervi, G. Cavigiolio, Inorg. Chim. Acta 306 (2000) 42.
[30] G.M. Sheldrick, Acta Crystallogr. 64 (2008) 112.

[31] D. De, L.D. Byers, D.J. Krogstad, J. Heterocycl. Chem. 34 (1997) 315.
[32] N.D. Heindel, I.S. Bechara, P.D. Kennewell, J. Molnar, C.J. Ohnmacht, S.M. Lemke, T.F. Lemke, J. Med. Chem. 11 (1968) 1218.
[33] T. Mosmann, J. Immunol. Methods 65 (1983) 55 (MTT = 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide).
[34] S.S. Batsanov, Inorg. Mater. 37 (2001) 871.
[35] C. Janiak, J. Chem. Soc., Dalton Trans. (2000) 3885.
[36] J. Janse van Rensburg, A. Muller, A. Roodt, Acta Crystallogr., E 63 (2007) m3015.
[37] S. Furuseth, J. Karlsen, A. Mostad, C. Roemming, R. Salmen, H.H. Toennesen, Acta Chem. Scand. 44 (1990) 741.