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# Low-dimensional compounds containing bioactive ligands. Part XVI: Halogenated derivatives of 8-quinolinol N-oxides and their copper(II) complexes

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ABSTRACT

Four N-oxides, 8-quinolinol N-oxide (8-HQNO), 5,7-dichloro-8-quinolinol N-oxide (HdClQNO), 5,7-dibromo-8-quinolinol N-oxide (HdBrQNO) and 7-iodo-8-quinolinol N-oxide (HIQNO) as well as their six copper complexes, $CuCl_2(8\text{-}HQNO)_2(H_2O)$ (**1**), $CuCl_2(HdClQNO)_2(H_2O)_2$ (**2**), $Cu(dClQNO)_2(CHCl_3)$ (**3**), $Cu(dClQNO)_2(H_2O)$ (**4**), $\{[Cu(dBrQNO)_2]\bullet 2H_2O\}_n$ (**5**) and $CuCl_2(HIQNO)_2(H_2O)_4$ (**6**) were synthesised as possible anticancer agents. Crystal structures of N-oxides contain planar molecules held together *via* hydrogen bonds involving oxygen atoms of N-oxide groups as acceptors. Crystal structure of **5** represents the first structure of a copper(II) complex with an N-oxide ligand derived from 8-HQNO and is formed by infinite chains. In the chain, the Cu(II) atom coordinates to six oxygen atoms from two bidentate chelating dBrQNO ligands occupying apexes of elongated tetragonal bipyramid with bridging oxygen atoms of N-oxide groups in axial positions. Antiproliferative activity of prepared N-oxides as well as their complexes was studied using *in vitro* MTT assay against the MDA-MB-231, HCT-116 and A549 cancer cell lines and their selectivity was verified on MSCs cells. Among the tested cancer cell lines, A549 and MDA-MB-231 cells were the most sensitive to the tested complexes. Complex **1** showed the highest cytotoxicity against both tumor cell lines. At concentration, which could be tested in animal models, **1** induced cell death in more than 50% of cancer cells and in 20% of MSCs indicating its selectivity.



## 1. Introduction

The study of anticancer and antimicrobial activity of coordination compounds with biologically active metals and bioactive ligands is at the forefront of the field of coordination chemistry. Ligands with marked biological activity include *N*- and *O*-donor ligands such as quinoline and its derivatives [1]. One of the quinoline derivatives, 8-quinolinol (8-hydroxyquinoline, 8-HQ) and its derivatives like halogenderivatives, exhibit significant biological activities, particularly antifungal, antibacterial and antitumor activities [2]. It has been found that the activities of these bioactive molecules can be enhanced upon their coordination to different biologically active metals, like copper [3,4]. Coordination of halogenderivatives of 8-HQ (e.g. 5-chloro-7-iodo-8-quinolinol (HCQ)), which exhibit highest biological activities, to copper(II) has a beneficial effect in the treatment of Alzheimer's disease [5,6]. Such complexes exhibit increased antimicrobial activity against gram-positive and gram-negative microorganisms [7] and increased antiproliferative effects [3].

Our previous work focused on the synthesis of square-planar complexes of 3*d* and 4*d* metals with 8-HQ derivatives simulating the structure of cisplatin or other platinum anticancer drugs has shown that many of the prepared Pd(II) ionic compounds interact with DNA and exhibit more pronounced antitumor activity than cisplatin [8,9,10]. Aromatic amine *N*-oxides are also an important structural class of many pharmaceutical products. Compounds containing *N*-oxides have been described as antiproliferative agents in the sixties [11,12]. It is known that 8-quinolinol N-oxide (8-HQNO) is an inhibitor of bacteria [13] and a potential precursor

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for antimalarial and anticancer drugs [13,14,15]. More recent research revealed that N-oxides like tirapazamine or AQ4N, which have advanced into clinical trials, may represent a newer class of cytotoxic drugs with selective toxicity towards hypoxic mammalian cells since they form a toxic radical only at low concentrations of oxygen [12,16].

Interesting results of biological activity of 8-quinolinol N-oxides (HRQNO) as well as of copper(II) complexes with halogenderivatives of 8-quinolinol motivated us to prepare copper(II) complexes with 8-HQNO and its halogenderivatives. There is a documented interest in Cu(II) complexes with HRQNO in literature for more than sixty years. Synthesis and spectroscopic studies of complexes with stoichiometries 1:1, 1:2, and 1:4 (metal to ligand) have been reported [17–21]. In the case of 1:1 complexes, spectroscopic studies and conductivity measurements indicate the formation of an octahedral Cu(II)(glicynato) complex with neutral 8-HQNO acting as a chelating ligand [17] or deprotonated dClQNO coordinating in a chelating mode in the Cu(II)(dClQNO)(OAc)$(H_2O)_2$ molecular complex [18]. In the case of 1:2 complexes, chelates with deprotonated ligands 8-QNO and dBrQNO were presumed to have formed [19,20]. Complexes of 1:4 stoichiometry with neutral 8-HQNO were reported for several 3*d* metals including Cu(II) [21]. Based on the results of elemental analysis, magnetic susceptibility, electric conductivity, and IR spectra, the ligand 8-HQNO coordinates *via* the phenolic O atom and the N- oxide O atom is not involved in coordination. However, there are no known crystal structures that could support the proposed geometry of the isolated Cu(II) complexes deduced from the indirect methods. Three crystal structures of complexes with 8-HQNO are known, two with Co(II) and one with Mn(II) central atoms [22– 24]. In all three cases, the neutral 8-HQNO coordinates terminally via the N-oxide O atom in contrary to findings in [21]. This clearly indicates the need for structurally characterized complexes with HRQNO ligands to better understand the coordination ability of such ligands.

The lack of structural data is evident even for the ligands themselves. While the structure of 8-HQNO was published already in 1971 [25], no structures of its derivatives are known. This motivated us to study structures of HRQNO ligands. According to the work of Ramaiah and Srinivasan [26], we similarly prepared 8-quinolinol N-oxide, which we used in the preparation of other derivatives. HdClQNO, HIQNO and HdBrQNO were prepared by halogenation of 8-HQNO according to the literature [27– 29].

In this paper, we present the synthesis of six copper(II) complexes with four different 8-quinolinol N-oxides, study of their anticancer activity, which is compared with the 8-quinolinol N-oxides themselves. In addition, we are introducing the first crystal structure of a Cu(II) complex with an HRQNO ligand.

## 2. Materials and methods

### 2.1. Materials and chemicals

Reagents were obtained from the following commercial sources: 8-quinolinol, ≥ 99% from Merck, glacial acetic acid, p.a., hydrogen peroxide, 35% p.a., chloroform, 99% p.a., diethylether, 99% p.a., sodium bisulfite, p.a., petroleum ether, (50–70) °C p.a., acetone, 99.5% p.a., methanol, 99.8% p.a., dimethylformamide, 99 % p.a. and potassium carbonate, p.a. from Centralchem, sodium carbonate anhydrous, p.a., sodium sulfate anhydrous, p.a. and hydrochloric acid, 35% from MikroCHEM, iodine monochloride, 98% and iodine trichloride, ≥ 95% from Acros Organics, ethanol, 96% and potassium hydroxide, p.a. from ITES Vranov, and bromine, extra pure, copper(II) chloride dihydrate, ≥ 99% and triethylamine, ≥ 99% from Sigma Aldrich.

### Syntheses

#### Synthesis of 8-quinolinol N-oxide (8-HQNO)

According to [26], hydrogen peroxide (35%, 13.0 mL, 0.152 mol) was added slowly during a period of 60 min into a solution of 8-HQ (20.00 g, 0.1378 mol) in 81 mL of acetic acid at (70–80) °C. The mixture was heated at the same temperature for additional 2 h and the second portion (11.0 mL, 0.128 mol) of hydrogen peroxide was added dropwise. The resulting mixture was then heated for another 9 h after which its volume was reduced to about 1/4 using the rotary evaporator. Water (30 mL) was added and all volatile components were removed by the rotary evaporator. The residue was carefully neutralized with a saturated aqueous solution of $Na_2CO_3$ (about 3 table spoons). The solid mixture was repeatedly extracted with several small portions of chloroform (total volume of 210 mL). The chloroform extract was dried over anhydrous $Na_2SO_4$ and filtered. Chloroform was removed by a rotary evaporator and the resulting mixture was subjected to steam distillation in order to remove the unconverted 8-HQ. The residual aqueous solution was filtered while hot and after cooling to room temperature the product was isolated as fine pale yellow crystals of 8-HQNO.

8-HQNO – Calc. for $C_9H_7NO_2$ (161.16 g $mol^{-1}$): C, 67.08; H, 4.38; N, 8.69%. Found: C, 67.17; H, 4.29; N, 8.66 %. Yield: 5.55 g (25%).

$^1$H NMR (DMSO-$d_6$, 600 MHz): $\delta$ = 15.50 (s, 1H, OH), 8.53 (d, 1H, $J$ = 6.0 Hz, H-1), 8.09 (d, 1H, $J$ = 8.5 Hz, H-3), 7.55 (t, 1H, $J$ = 8.0 Hz, H-6), 7.50 (dd, 1H, $J$ = 8.5, 6.0 Hz, H-2), 7.43 (dd, 1H, $J$ = 8.0, 1.0 Hz, H-5), 7.00 (d, 1H, $J$ = 8.0 Hz, H-7) ppm.

$^{13}$C NMR (DMSO-$d_6$, 151 MHz): $\delta$ = 153.3 (C-8), 136.0 (C-1), 132.0 (C-4), 130.4 (C-6), 130.1 (C-3), 128.7 (C-9), 121.7 (C-2), 117.0 (C-5), 114.0 (C-7) ppm.

$^{15}$N NMR (DMSO-$d_6$, 61 MHz): $\delta$ = -106.2 (N-1) ppm.

IR (ATR, $cm^{-1}$): 3400(vw), 3063(w), 3028(w), 2621 (w), 2324(w), 1598(m), 1582(m), 1521(m), 1505(m), 1455(m), 1396(m), 1334(m), 1310(m), 1274(m), 1192(w), 1177(w), 1149(m), 1035(m), 1046(m), 885(m), 813(s), 787(m), 747(s), 709(m), 665(s), 612(m), 568(s), 499(m), 468(m).

#### Synthesis of 5,7-dichloro-8-quinolinol N-oxide (HdClQNO)

According to [27], 8-HQNO (2 g, 12.41 mmol) was dissolved in hydrochloric acid (35%, 15 mL); the solution was stirred vigorously. To this solution, a solution of iodine trichloride (2.89 g, 12.41 mmol) dissolved in hydrochloric acid (35%, 10 mL) was added dropwise. Stirring continued for 0.5 h after the last addition of iodine trichloride. The addition compound was then filtered by fritted disc funnel, rinsed with glacial acetic acid and diethylether. Intermediate product was decomposed by being added to water (25 mL) with mechanical stirring. The precipitate formed was filtered, washed with water and with 2% solution of sodium bisulfite. Yellow powder was recrystallized from acetone yielding yellow needles of HdClQNO in few days.

HdClQNO – Calc. for $C_9H_5Cl_2NO_2$ (230.05 g $mol^{-1}$): C, 46.99; H, 2.19; N, 6.09%. Found: C, 47.37; H, 2.37; N, 6.10%. Yield: 1.43 g (50%).

$^1$H NMR (DMSO-$d_6$, 600 MHz): $\delta$ = 8.74 (dd, 1H, $J$ = 6.1, 1.0 Hz, H-1), 8.25 (dd, 1H, $J$ = 8.8, 1.0 Hz, H-3), 8.03 (s, 1H, H-6), 7.71 (dd, 1H, $J$ = 8.8, 6.1 Hz, H-2) ppm.

$^{13}$C NMR (DMSO-$d_6$, 151 MHz): $\delta$ = 149.5 (C-8), 136.9 (C-1), 130.6 (C-6), 129.5 (C-9), 128.1 (C-4), 127.2 (C-3), 123.3 (C-2), 117.8 (C-5), 116.9 (C-7) ppm.

$^{15}$N NMR (DMSO-$d_6$, 61 MHz): $\delta$ = -108.3 (N-1) ppm.

IR (ATR, $cm^{-1}$): 3383(vw), 3074(m), 1592(w), 1574(m), 1511(m), 1445(m), 1392(s), 1353(m), 1315(m), 1281(m), 1246(w), 1207(w), 1189(m), 1121(s), 1045(w), 1064(m), 900(m), 873(m), 853(s), 821(w), 803(s), 741(s), 694(s), 647(s), 615(s), 558(m), 533(m), 507(w), 476(m), 430(m).

*Synthesis of 5,7-dibromo-8-quinolinol N-oxide (HdBrQNO)*

According to [28], 8-HQNO (0.8 g, 4.964 mmol) was dissolved in glacial acetic acid (10 mL) and bromine (1 mL, 4.964 mmol) dissolved in acetic acid (10 mL) was added dropwise to the solution. The resulting mixture was stirred for 1 h and the resulting yellow precipitate was filtered off and rinsed with petroleum ether. The precipitate of HdBrQNO was recrystallized in two ways a) from glacial acetic acid (formation of orange prisms – HdBrQNOa) and b) from acetone (formation of yellow needles – HdBrQNOb). The identity of both types of crystals was proved by NMR spectroscopy.

HdBrQNO – Calc. for $C_9H_5Br_2NO_2$ (318.95 g mol$^{-1}$): C, 33.89; H, 1.58; N, 4.39%. Found: C, 33.77; H, 1.67; N, 4.23%. Yield: 1.27 g (80%).

$^1$H NMR (DMSO-d$_6$, 600 MHz): $\delta$ = 8.72 (dd, 1H, $J$ = 6.1, 1.0 Hz, H-1), 8.23 (s, 1H, H-6), 8.19 (dd, 1H, $J$ = 8.8, 1.0 Hz, H-3), 7.72 (dd, 1H, $J$ = 8.8, 6.1 Hz, H-2) ppm.

$^{13}$C NMR (DMSO-d$_6$, 151 MHz): $\delta$ = 151.1 (C-8), 136.6 (C-1), 136.1 (C-6), 129.9 (C-3), 129.6 (C-4, C-9), 123.5 (C-2), 107.3 (C-5), 106.9 (C-7) ppm.

$^{15}$N NMR (DMSO-d$_6$, 61 MHz): $\delta$ = -108.7 (N-1) ppm.

IR (ATR, cm$^{-1}$): 3092(w), 3057(m), 1620(w), 1567(m), 1508(m), 1481(w), 1440(m), 1391(s), 1347(m), 1314(m), 1269(m), 1240(m), 1183(m), 1150(m), 1114(m), 1059(m), 898(m), 875(s), 829(m), 805(s), 750(s), 702(s), 643(s), 621(m), 600(s), 549(m), 528(s), 505(m), 474(m), 427(m).

*Synthesis of 7-iodo-8-quinolinol N-oxide (HIQNO)*

Iodine monochloride (4 g, 24.82 mmol) in cold ethanol (20 mL) was slowly added to warm (50 °C) solution of 8-HQNO (2 g, 12.41 mmol) in ethanol (25 mL) with vigorous stirring. Observed brownish-yellow precipitate was filtered and air dried. Subsequently, the precipitate was recrystallized from acetone forming brownish-yellow prismatic crystals of HIQNO.

HIQNO – Calc. for $C_9H_6INO_2$ (287.05 g mol$^{-1}$): C, 37.66; H, 2.11; N, 4.88%. Found: C, 37.31; H, 1.89; N, 4.65 %. Yield: 3.38 g (95%).

$^1$H NMR (DMSO-d$_6$, 600 MHz): $\delta$ = 8.59 (dd, 1H, $J$ = 6.0, 1.0 Hz, H-1), 8.13 (dd, 1H, $J$ = 8.5, 1.0 Hz, H-3), 7.94 (s, 1H, H-6), 7.55 (dd, 1H, $J$ = 8.5, 6.0 Hz, H-2), 7.26 (d, 1H, $J$ = 8.5 Hz, H-5) ppm.

$^{13}$C NMR (DMSO-d$_6$, 151 MHz): $\delta$ = 153.1 (C-8), 138.7 (C-6), 135.5 (C-1), 131.8 (C-4), 130.7 (C-3), 128.0 (C-9), 122.2 (C-2), 118.2 (C-5), 83.1 (C-7) ppm.

$^{15}$N NMR (DMSO-d$_6$, 61 MHz): $\delta$ = -110.0 (N-1) ppm.

IR (ATR, cm$^{-1}$): 3056(m), 1568(m), 1508(m), 1441(m), 1386(m), 1362(w), 1327(m), 1301(m), 1236(m), 1185(w), 1150(m), 1097(m), 1063(w), 1040(m), 913(m), 815(s), 752(m), 683(s), 638(m), 606(m), 588(w), 567(s), 537(m), 477(m), 429(m).

*Synthesis of $CuCl_2$(8-HQNO)$_2$($H_2O$) (1)*

Aqueous solution (50 mL) of 8-HQNO (0.161 g, 1 mmol) was adjusted to the pH of 9 by 0.5 mL of 1 mol dm$^{-3}$ aqueous KOH and heated under reflux for 1 h followed by the addition of $CuCl_2$ solution (0.085 g $CuCl_2$•$2H_2O$, 0.5 mmol) in 25 mL $H_2O$. The pH was again adjusted to 9 and the mixture was refluxed for 8 h. Yellow precipitate of **1** was filtered from a hot mixture and dried in the air.

$CuCl_2$(8-HQNO)$_2$($H_2O$) (**1**) – Calc. for $CuCl_2C_{18}H_{16}O_5N_2$ (474.78 g mol$^{-1}$): C, 45.53; H, 3.39; N, 5.90%. Found: C, 45.30; H, 3.35; N, 5.77%. Yield: 0.173 g (73 %).

IR (ATR, cm$^{-1}$): 3625-3200(m), 3063(vw), 3006(vw), 1567(s), 1511(w), 1452(m), 1423(w), 1387(s), 1347(m), 1307(s), 1297(m), 1198(w), 1136(w), 1111(w), 1052(m), 1033(s), 810(s), 791(m), 737(s), 715(s), 607(s), 573(w), 509(s).

*Synthesis of $CuCl_2$(HdClQNO)$_2$($H_2O$)$_2$ (2) and Cu(dClQNO)$_2$($CHCl_3$) (3)*

$Et_3N$ (0.073 mL, 1 mmol) was added to HdClQNO (0.23 g, 1 mmol) in chloroform (10 mL) to adjust the pH of the solution to ca. 7.5, observing a color change from yellow to orange. $CuCl_2$•$2H_2O$ (0.1705 g, 1 mmol) in methanol (5 mL) was added. Yellow-brown precipitate of **2** was filtered out after 2 h of stirring. Another portion of a yellow-brown precipitate of **3** was isolated after two days from the filtrate. Both products were air dried.

$CuCl_2$(HdClQNO)$_2$($H_2O$)$_2$ (**2**) – Calc. for $CuCl_6C_{18}H_{14}O_6N_2$ (630.58 g mol$^{-1}$): C, 34.29; H, 2.24; N, 4.44%. Found: C, 34.43; H, 1.97; N, 4.38%. Yield: 0.227 g (36%).

IR (ATR, cm$^{-1}$): 3449(vw), 3336(vw), 3082(w), 3059(w), 1761(vw), 1586(m), 1545(s), 1506(m), 1439(s), 1402(m), 1372(s), 1331(m), 1285(w), 1268(w), 1243(w), 1206(w), 1188(w), 1177(w), 1141(m), 1081(w), 1067(m), 919(w), 877(w), 860(s), 808(m), 765(s), 749(m), 734(m), 697(m), 658(m), 650(m), 625(m), 567(w), 540(m), 505(w), 473(m), 455(w), 432(m).

Cu(dClQNO)$_2$($CHCl_3$) (**3**) – Calc. for $CuCl_7C_{19}H_9O_4N_2$ (641.00 g mol$^{-1}$): C, 35.60; H, 1.42; N, 4.37 %. Found: C, 35.39; H, 1.81; N, 4.55 %. Yield: 0.205 g (32 %).

IR (ATR, cm$^{-1}$): 3102(w), 3082(m), 2940-2790 (w), 1759 (w), 1587(m), 1509(m), 1447(m), 1383(m), 1356(m), 1320(w), 1265(m), 1252(m), 1210(w), 1192(m), 1166(m), 1135(m), 1079(m), 1062(m), 995(w), 930(w), 877(m), 869(w), 856(s), 815(s), 738(s), 696(s), 649(s), 632(m), 617(m), 566(m), 548(m), 511(w), 485(w), 454(m).

*Synthesis of Cu(dClQNO)$_2$($H_2O$) (4)*

$K_2CO_3$ (0.069 g, 0.5 mmol) was added to HdClQNO (0.046 g, 0.2 mmol) in 15 mL DMF to adjust the pH of the solution to ca. 6.5. Unreacted $K_2CO_3$ was removed by filtration and $CuCl_2$•$2H_2O$ (0.017 g, 0.1 mmol) in 5 mL of ethanol was added to the filtrate. Mixture was stirring for 1 h and then filtered. After a few weeks, small hedgehog crystals of **4** were filtered out and dried in air.

Cu(dClQNO)$_2$($H_2O$) (**4**) – Calc. for $CuCl_4C_{18}H_{10}O_5N_2$ (539.64 g mol$^{-1}$): C, 40.06; H, 1.87; N, 5.19%. Found: C, 39.91; H, 2.01; N, 5.13%. Yield: 0.031 g (58%).

IR (ATR, cm$^{-1}$): 3476(m), 3352(w), 3084(vw), 3051(w), 1658(w), 1577(m), 1541(m), 1504(m), 1444(s), 1407(m), 1374(s), 1332(s), 1286(m), 1248(m), 1209(m), 1189(w), 1173(m), 1139(m), 1085(w), 971(w), 871(m), 858(s), 797(s), 762(s), 747(s), 729(m), 655(s), 626(m), 602(m), 536(s), 505(w), 476(w), 434(w).

*Synthesis of {[Cu(dBrQNO)$_2$]•$2H_2O$}$_n$ (5)*

$K_2CO_3$ (0.069 g, 0.5 mmol) was added to HdBrQNOa (0.064 g, 0.2 mmol) in 15 mL DMF to adjust the pH of the solution to ca. 6.5. Unreacted $K_2CO_3$ was removed by filtration and $CuCl_2$•$2H_2O$ (0.017 g, 0.1 mmol) in 5 mL of ethanol was added to the filtrate. Mixture was stirring for 1 h and then filtered. After a few weeks, small hedgehog crystals of **5** were filtered out and dried in air.

{[Cu(dBrQNO)$_2$]•$2H_2O$}$_n$ (**5**) – Calc. for $CuBr_4C_{18}H_{12}O_6N_2$ (735.46 g mol$^{-1}$): C, 29.40; H, 1.64; N, 3.81 %. Found: C, 29.85; H, 1.79; N, 3.96 %. Yield: 0.049 g (67 %).

IR (ATR, cm$^{-1}$): 3480(m), 3360(w), 3081(w), 3050(w), 2969(vw), 1747(w), 1651(m), 1573(m), 1537(s), 1500(m), 1436(s), 1403(s), 1368(s), 1327(s), 1275(w), 1246(m), 1208(m), 1188(m), 1171(m), 1132(m), 1080(m), 1059(s), 874(m), 852(m), 837(m), 795(s), 741(s), 703(w), 689(m), 650(s), 628(m), 596(m), 534(s), 471(m), 429(w).

*Synthesis of $CuCl_2$(HIQNO)$_2$($H_2O$)$_4$ (6)*

$CuCl_2$•$2H_2O$ (0.017 g, 0.1 mmol) in methanol (5 mL) was added drop by drop to the solution of HIQNO (0.057 g, 0.2 mmol) in methanol (15 mL) under stirring. After 1 h of stirring, a yellow precipitate was isolated by filtration and dried in air.

**Table 1**
Crystal data and structure refinement of N-oxides HRQNO and compound 5.

| Compound | 8-HQNO | HdClQNO | HdBrQNOa | HdBrQNOb | HIQNO | **5** |
|---|---|---|---|---|---|---|
| Empirical formula | $C_9H_7NO_2$ | $C_9H_5Cl_2NO_2$ | $C_9H_5Br_2NO_2$ | $C_9H_5Br_2NO_2$ | $C_9H_6INO_2$ | $C_{18}H_{12}Br_4CuN_2O_6$ |
| Formula weight | 161.16 | 230.04 | 318.96 | 318.96 | 287.05 | 735.48 |
| Temperature [K] | 295(2) | 295(2) | 295(2) | 295(2) | 295(2) | 100(2) |
| Wavelength [Å] | 0.71073 | 0.71073 | 0.71073 | 0.71073 | 0.71073 | 1.54184 |
| Crystal system | Monoclinic | Monoclinic | Monoclinic | Monoclinic | Monoclinic | Monoclinic |
| Space group | $P2_1/c$ | $P2_1/n$ | $C2/c$ | $P2_1/n$ | $P2_1$ | $P2_1/n$ |
| Unit cell dimensions [Å, °] | $a$ = 12.1271(10)<br>$b$ = 4.9276(4)<br>$c$ = 13.1529(10)<br>$\beta$ = 109.953(2) | $a$ = 3.7753(8)<br>$b$ = 14.457(3)<br>$c$ = 16.094(3)<br>$\beta$ = 93.894(4) | $a$ = 11.0376(4)<br>$b$ = 10.8788(4)<br>$c$ = 16.0618(6)<br>$\beta$ = 103.611(1) | $a$ = 3.9317(3)<br>$b$ = 14.3412(11)<br>$c$ = 16.4974(13)<br>$\beta$ = 91.494(2) | $a$ = 6.8648(7)<br>$b$ = 4.1463(4)<br>$c$ = 16.2241(16)<br>$\beta$ = 101.143(2) | $a$ = 3.8127(7)<br>$b$ = 28.262(13))<br>$c$ = 9.3713(19)<br>$\beta$ = 99.17(2) |
| Volume [Å$^3$] | 738.80(10) | 876.4(3) | 1874.47(12) | 929.89(12) | 453.09(8) | 996.9(5) |
| $Z$; density (calculated) [Mg m$^{-3}$] | 4; 1.449 | 4; 1.744 | 8; 2.260 | 4; 2.278 | 2; 2.104 | 2; 2.450 |
| Absorption coefficient [mm$^{-1}$] | 0.104 | 0.706 | 8.617 | 8.685 | 3.497 | 11.328 |
| $F$(000) | 336 | 464 | 1216 | 608 | 272 | 702 |
| Crystal shape, colour | prism, brownish yellow | plate, yellow | polyhedron, yellow | plate, yellowish green | prism, yellow | needle, brownish yellow |
| Crystal size [mm$^3$] | 0.48×0.44×0.12 | 0.40×0.21×0.05 | 0.09×0.08×0.07 | 0.44×0.135×0.04 | 0.25×0.14×0.12 | 0.013×0.007×0.007 |
| $\theta$ range for data collection [°] | 1.786 to 26.242 | 1.895 to 26.054 | 2.609 to 26.496 | 1.882 to 26.494 | 1.279 to 25.353 | 3.127 to 69.985 |
| Index ranges | -14 ≤ $h$ ≤ 15,<br>-6 ≤ $k$ ≤ 6,<br>-16 ≤ $l$ ≤ 16 | -4 ≤ $h$ ≤ 4,<br>-17 ≤ $k$ ≤ 17,<br>-19 ≤ $l$ ≤ 19 | -13 ≤ $h$ ≤ 13,<br>-13 ≤ $k$ ≤ 13,<br>-20 ≤ $l$ ≤ 20 | -4 ≤ $h$ ≤ 4,<br>-18 ≤ $k$ ≤ 18,<br>-20 ≤ $l$ ≤ 20 | -8 ≤ $h$ ≤ 8,<br>-4 ≤ $k$ ≤ 4,<br>-19 ≤ $l$ ≤ 19 | -4 ≤ $h$ ≤ 2,<br>-33 ≤ $k$ ≤ 32,<br>-11 ≤ $l$ ≤ 11 |
| Reflections collected/independent | 10255 / 1487<br>[$R$(int) = 0.0442] | 12551 / 1723<br>[$R$(int) = 0.0437] | 13904 / 1947<br>[$R$(int) = 0.0308] | 13655 / 1917<br>[$R$(int) = 0.0407] | 6034 / 1631<br>[$R$(int) = 0.0312] | 5267 / 1844<br>[$R$(int) = 0.1972] |
| Data/restraints/parameters | 1487 / 0 / 109 | 1723 / 0 / 127 | 1947 / 0 / 131 | 1917 / 0 / 127 | 1631 / 1 / 119 | 1844 / 0 / 142 |
| Goodness-of-fit on $F^2$ | 1.031 | 1.127 | 1.018 | 1.050 | 1.134 | 0.956 |
| Final $R$ indices [$I$>2$\sigma$($I$)] | $R1$ = 0.0510,<br>$wR2$ = 0.1401 | $R1$ = 0.0421,<br>$wR2$ = 0.1055 | $R1$ = 0.0257,<br>$wR2$ = 0.0614 | $R1$ = 0.0268,<br>$wR2$ = 0.0709 | $R1$ = 0.0341,<br>$wR2$ = 0.0783 | $R1$ = 0.1042,<br>$wR2$ = 0.2576 |
| $R$ indices (all data) | $R1$ = 0.0615,<br>$wR2$ = 0.1551 | $R1$ = 0.0552,<br>$wR2$ = 0.1119 | $R1$ = 0.0372,<br>$wR2$ = 0.0656 | $R1$ = 0.0338,<br>$wR2$ = 0.0747 | $R1$ = 0.0383,<br>$wR2$ = 0.0806 | $R1$ = 0.2336,<br>$wR2$ = 0.3466 |
| Largest diff. peak and hole [e Å$^{-3}$] | 0.283 and -0.123 | 0.323 and -0.200 | 0.435 and -0.269 | 0.736 and -0.324 | 1.084 and -0.327 | 1.397 and -1.423 |

$CuCl_2(HIQNO)_2(H_2O)_4$ (**6**) – Calc. for $CuCl_2I_2C_{18}H_{20}O_8N_2$ (780.62 g mol$^{-1}$): C, 27.69; H, 2.58; N, 3.59%. Found: C, 27.20; H, 2.25; N, 3.53%. Yield: 0.039 g (51%).

IR (ATR, cm$^{-1}$): 3600-3190(m), 3162(vw), 3100(vw), 3060(m), 1725(w),1640(m), 1583(m), 1507(m), 1435(m), 1377(s), 1328(m), 1238(m), 1210(w), 1190(w), 1152(w), 1101(m), 1063(w), 1039(s), 913(m), 821(s), 750(m), 683(s), 635(s), 605(m), 582(w), 568(m), 537(w), 501(m), 477(w), 455(m).

### 2.3. Physical measurements

The infrared spectra of the prepared complexes were recorded on a Nicolet 6700 FT-IR spectrophotometer from Thermo Scientific equipped with a diamond crystal Smart Orbit$^{TM}$ in the range 4000–400 cm$^{-1}$.

Elemental analyses of C, H and N were obtained on CHNOS Elemental Analyzer vario MICRO from Elementar Analysensysteme GmbH.

NMR spectra were recorded at room temperature on a Varian VNMRS spectrometer operating at 599.87 MHz for $^1$H, 150.84 MHz for $^{13}$C and 60.79 MHz for $^{15}$N. Spectra were recorded in DMSO-$d_6$ and the chemical shifts were referenced to the residual solvent signal ($^1$H NMR 2.50 ppm, $^{13}$C NMR 39.5 ppm) and external standard $CH_3NO_2$ ($^{15}$N NMR 0.00 ppm). The 2D gCOSY, gHSQC and gHMBC (optimized for a long-range coupling of 8 Hz) methods were employed.

### 2.4. X-ray data collection and structure refinement

Crystal structures of ligands HRQNO were determined using a Bruker APEX CCD diffractometer equipped with an APEX CCD detector. SMART V5.632 was used for data collection while SAINT V8.38A was used for cell refinement, data reduction and SADABS-2016/2 (Bruker, 2016/2) was used for absorption correction [30]. The data collection for **5** was carried out on a Rigaku XtaLAB Synergy S diffractometer equipped with Hybrid Pixel Array Detector (HyPix-6000HE). Oxford Cryosystems (Cryostream 800) cooling device was used for data collection at a temperature of 100 K. CrysAlisPro software was used for data collection and cell refinement, data reduction and absorption correction [31]. All structures were solved by SHELXT [32] and subsequent Fourier syntheses using SHELXL-2018 [33], implemented in WinGX program suit [34]. A geometric analysis was performed using SHELXL-2018 while DIAMOND [35] was used for molecular graphics. A summary of crystal data and structure refinement for ligands HRQNO and **5** is presented in Table 1.

### 2.5. In vitro antitumor activity

#### 2.5.1. Cell lines and cell culture

Human breast cancer cell line MDA-MB-231 and human lung carcinoma epithelial cell line A549 were purchased from American Type Culture Collection (ATCC, Manassas, USA). Human cancer colon cell line HCT-116 was kindly provided by Dr. Danijela Vignjevic (Institute Curie, Paris, France). Mesenchymal stem cells (MSCs)

were purchased from Invitrogen (Waltham, Massachusetts, USA). MDA-MB-231 and HCT-116 cells were maintained in RPMI 1640 (Sigma Aldrich, Munich, Germany) supplemented with 10% fetal bovine serum (FBS, Sigma), penicillin G (100 IU/mL), streptomycin (100 $\mu$g/mL), and in a humidified atmosphere of 95% air/5% $CO_2$ at 37 °C. The pH of RPMI 1640 medium was 8. The A549 cells and MSCs were cultured in Dulbecco's Modified Eagle Medium (DMEM) containing 10% FBS, 100 IU/mL penicillin G and 100 $\mu$g/mL streptomycin (Sigma). The pH of DMEM medium was 7.5. Cell number and viability were determined by trypan blue staining. MDA-MB-231, HCT-116, A549 cells and MSCs in passage 4 were used throughout these experiments.

*2.5.2. Assessment of cytotoxicity*

Effects of tested compounds on viability of MDA-MB-231, HCT-116, A549 cancer cells and MSCs were determined by MTT 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test. MDA-MB-231, HCT-116, A549 cells and MSCs were diluted with medium to 5 × $10^4$ cells/mL and aliquots (5 × $10^3$ cells / 100 $\mu$L) were placed in individual wells in 96-multiplates. After 24 h, cells were treated with selected concentrations of compounds for 3 days: medium was exchanged with 100 $\mu$L of different compounds, which had been serially diluted 2-fold in the medium to concentrations ranging from 1000 $\mu$M to 7.8 $\mu$M. Control wells were prepared by the addition of culture medium. Wells containing culture medium without cells were used as blanks. After incubation drug containing medium was discarded and replaced with serum free medium containing 15% of MTT (5 mg/mL) dye. After additional 4 h of incubation 37 °C in a 5% $CO_2$ incubator, medium with MTT was removed and DMSO (150 $\mu$L) with glycine buffer (20 $\mu$L) was added to dissolve the blue formazan crystals. The plates were shaken for 10 min. The optical density of each well was determined at 595 nm. The percentage of cell viability was determined by comparison with untreated controls according to formula: % of viable cells = (E-B)/(S-B) x 100 where B is for background of medium alone, S is for total viability/spontaneous death of untreated target cells, and E is for experimental well. The experiments, performed in triplicates, were repeated three times [36].

## 3. Results and discussion

### 3.1. Synthesis

In this work, we have focused on the preparation of halogen derivatives of 8-HQ, namely their N-oxides, and their copper complexes. We present optimized syntheses of HdClQNO and HdBrQNO by halogenation of 8-HQNO and preparation of a new N-oxide HIQNO. All four N-oxides were further used as ligands in the syntheses of copper complexes. The main goal was to increase the solubility of 8-HQ derivatives by converting them to N-oxides as well as their complexes in polar solvents, as solubility is an essential factor in biological activity assays.

At first, we have attempted to prepare halogen derivatives of N-oxides according to the procedure reported by Ramaiah and Srinivasan [26] by oxidizing the respective halogen derivative of 8-HQ. However, this process was unsuccessful because the oxidation resulted in the release of halogens as well as an overall breakdown of the halogen derivatives of 8-HQ. Therefore, we proceeded as in the case of the preparation of the 8-HQ halogen derivatives themselves [27,28]. By halogenation of 8-HQNO, that was prepared according to slightly modified synthesis reported in [26], we prepared three halogen derivatives of 8-HQNO, namely 5,7-dichloro-8-quinolinol N-oxide (HdClQNO), 5,7-dibromo-8-quinolinol N-oxide (HdBrQNO) and 7-iodo-8-quinolinol N-oxide (HIQNO) in a crystalline form. The formulae of HRQNO obtained from the elemental analysis were supported by the X-ray structural analysis as well as by $^1$H NMR and $^{13}$C NMR spectroscopy. To prepare copper complexes with the obtained N-oxides, we have employed several synthetic strategies. The first approach included the synthesis under reflux with an addition of a few drops of 1 mol dm$^{-3}$ KOH solution to adjust the pH of the reaction mixture. Adjusting the pH increased the solubility of 8-HQNO, which is insoluble in water. Although we tried to prepare complexes with all N-oxides under this reaction condition, only the synthesis of the $CuCl_2(8\text{-HQNO})_2(H_2O)$ (**1**) complex was successful.

Other syntheses were carried out at room temperature with addition of $Et_3N$ to the saturated solution of the ligand in chloroform to deprotonate the ligand, which was accompanied by a color change of the solution to deep yellow. The $CuCl_2$ methanolic solution was added precipitating $CuCl_2(HdClQNO)_2(H_2O)_2$ (**2**). After a few days a precipitate of $Cu(dClQNO)_2(CHCl_3)$ (**3**) was isolated from the mother liquor.

The third approach employed the addition of carbonate to deprotonate the ligand associated with the solution turning darker and subsequent addition of the $CuCl_2$ solution. Small hedgehog crystals of $Cu(dClQNO)_2(H_2O)$ (**4**) and $\{[Cu(dBrQNO)_2]\bullet 2H_2O\}_n$ (**5**) were isolated after a few weeks. Scheme 1 displays a preparation of **5** starting from the oxidation of 8-HQ through the bromation of 8-HQNO till the complexation of HdBrQNO to a copper atom.

Alternatively, mixing methanolic solutions of the respective reactants without the addition of any base, yielded $CuCl_2(HIQNO)_2(H_2O)_4$ (**6**) as the only complex that could be identified.

All the above-described approaches were attempted in the synthesis of complexes with all prepared N-oxides. However, only inseparable mixtures were isolated with the exception of the mentioned complexes.

### 3.2. X-ray structure analysis

Single crystal X-ray structure analysis revealed that all prepared 8-quinolinol N-oxides crystallized in monoclinic space groups. N-oxides HdClQNO and HdBrQNOb are isostructural compounds that crystalize in the $P2_1/n$ space group, while HdBrQNOa, that crystalizes in the $C2/c$ space group, is a polymorphic modification of HdBrQNOb. Finally, the structure of HIQNO was solved in the $P2_1$ space group while the structure of 8-HQNO belongs to the $P2_1/c$ space group. It has to be noted that the structure of 8-HQNO has been described previously without information on nonbonding interactions [25]. Therefore, we present its redetermined structure for the sake of comparison with structures of other ligands.

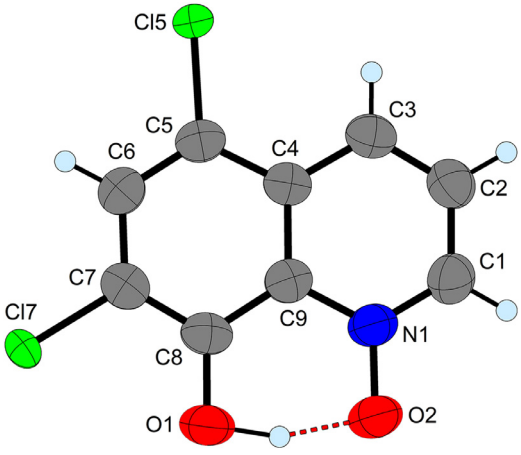
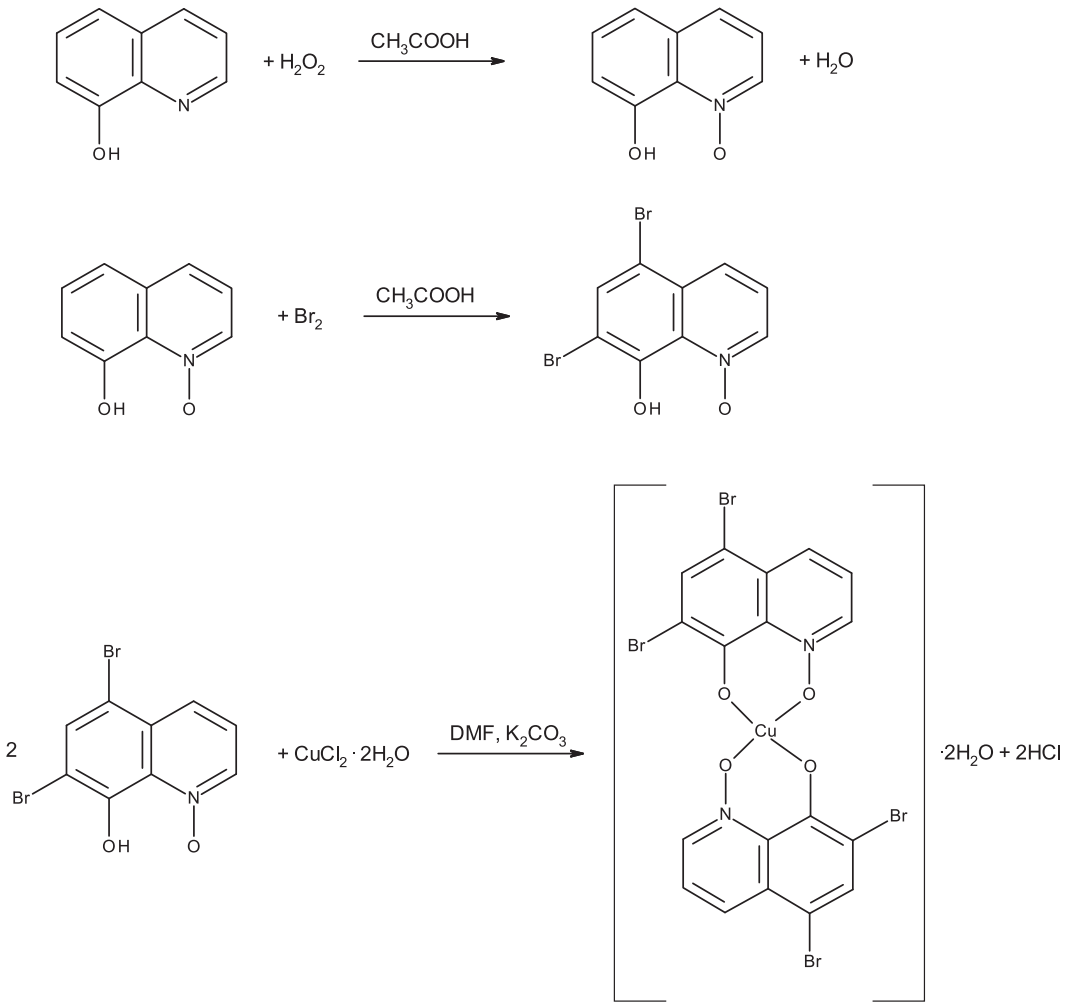



**Fig. 1.** Structure of HdClQNO. Displacement ellipsoids are drawn at the 50% probability level. Dashed line represents the intramolecular hydrogen bond.




**Scheme 1.** Reaction scheme showing the preparation of **5**.

**Table 2**
Selected bond distances and angles (Å, °) for HRQNO.

| Distance/Angle | 8-HQNO | HdClQNO | HdBrQNOa | HdBrQNOb | HIQNO |
|---|---|---|---|---|---|
| C1-N1 | 1.339(2) | 1.328(4) | 1.336(4) | 1.323(4) | 1.340(14) |
| C9-N1 | 1.3980(19) | 1.389(4) | 1.400(3) | 1.392(3) | 1.390(12) |
| N1-O2 | 1.3325(17) | 1.336(3) | 1.333(3) | 1.335(3) | 1.328(11) |
| C8-O1 | 1.3504(19) | 1.333(3) | 1.338(3) | 1.342(3) | 1.326(10) |
| C5-X5 | - | 1.749(3) | 1.894(3) | 1.899(3) | - |
| C7-X7 | - | 1.746(3) | 1.879(3) | 1.888(3) | 2.076(9) |
| C1-N1-O2 | 119.53(13) | 119.1(2) | 119.5(3) | 118.8(3) | 119.6(9) |
| C9-N1-O2 | 119.79(12) | 119.2(2) | 119.1(2) | 118.9(2) | 119.1(9) |
| C9-C8-O1 | 120.88(14) | 122.6(3) | 122.2(2) | 122.0(3) | 120.9(8) |

The structures of HRQNO (Fig. 1 and Figs. S1–S4) are formed by planar molecules with bond lengths (Table 2) being very close to those observed in 8-quinolinol and in the respective halogen derivatives of 8-quinolinol [38].

Although the bond lengths and angles in HdBrQNOa and HdBrQNOb are very similar, the polymorphic modifications differ in packing of the molecules. While the mean planes of the molecules at the borders of the unit cell make a dihedral angle with the mean planes of the molecules inside the unit cell of 44.5° in HdBrQNOa, all molecules in HdBrQNOb are coplanar (Fig. 2).

The molecules of HRQNO are stabilized by strong intramolecular O1-H1O1•••O2 hydrogen bonds and their structures are further stabilized by intermolecular van der Waals interactions between C1 and O2 atoms of the neighbouring molecules (Table 3). Due to these interactions, centrosymmetric dimers in 8-HQNO, HdClQNO, HdBrQNOa and HdBrQNOb are formed (Fig. S5) whereas a *zig-zag* chain along the *b*-axis is formed in HIQNO (Fig. S6).

Symmetry transformations used to generate equivalent atoms:

8-HQNO**:** (i) $-x + 1, -y + 2, -z + 1$;
HdClQNO**:** (i) $-x + 1, -y + 1, -z$;
HdBrQNOa: (i) $-x + 1, -y + 2, -z + 1$;
HdBrQNOb: (i) $-x + 1, -y + 1, -z$;
HIQNO: (i) $-x + 1, y$ -1/2, $-z$.

Moreover, stability of HdBrQNOa and HIQNO structures is further supported by halogen•••halogen interactions, as the intermolecular distances Br7•••Br7$^{ii}$ (3.5080(5) Å; (ii) $-x, y, -z + 1/2$) in HdBrQNOa, and I7•••I7$^{iii}$ and I7•••I7$^{iv}$ (3.8370(12) Å for both; (iii)

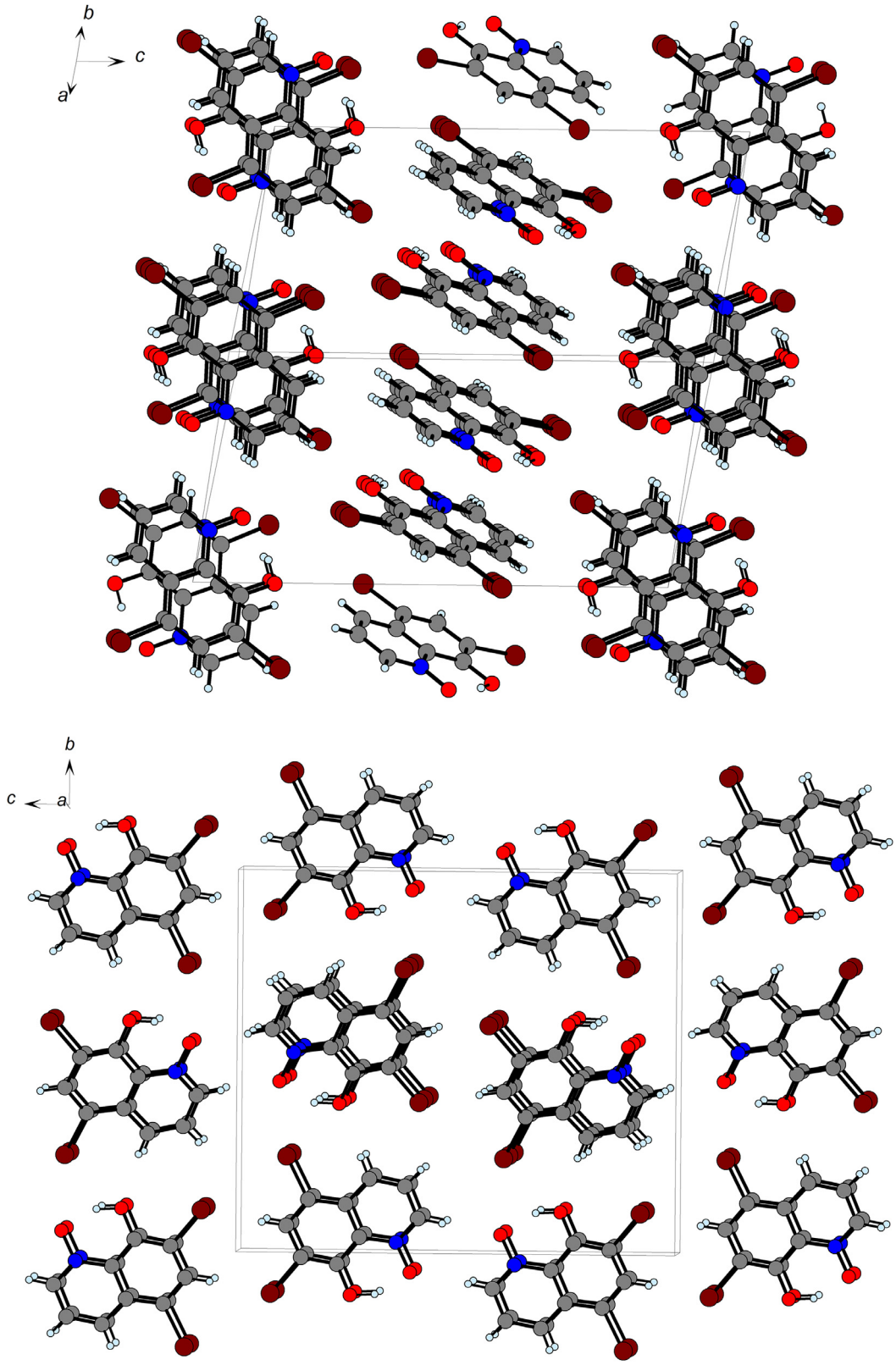



**Fig. 2.** Packing of the molecules HdBrQNOa (up) and HdBrQNOb (down) showing different arrangements of the molecules.

-$x$ + 2, $y$ + 1/2, -$z$ + 1; (iv) -$x$ + 2, $y$ - 1/2, -$z$ + 1) in HIQNO are shorter than the sum of Van der Waals radii of two bromine or two iodine atoms (3.70 and 3.96 Å, respectively). Due to these interactions the centrosymmetric dimers and chains formed by a system of hydrogen bonds in HdBrQNOa and HIQNO, respectively, are extended to 3D structure (Fig. S7) and wave like layers parallel with the *bc* plane (Fig. S8), respectively. No other significant interactions between molecules of HdBrQNOa, HIQNO and other HRQNO were found.

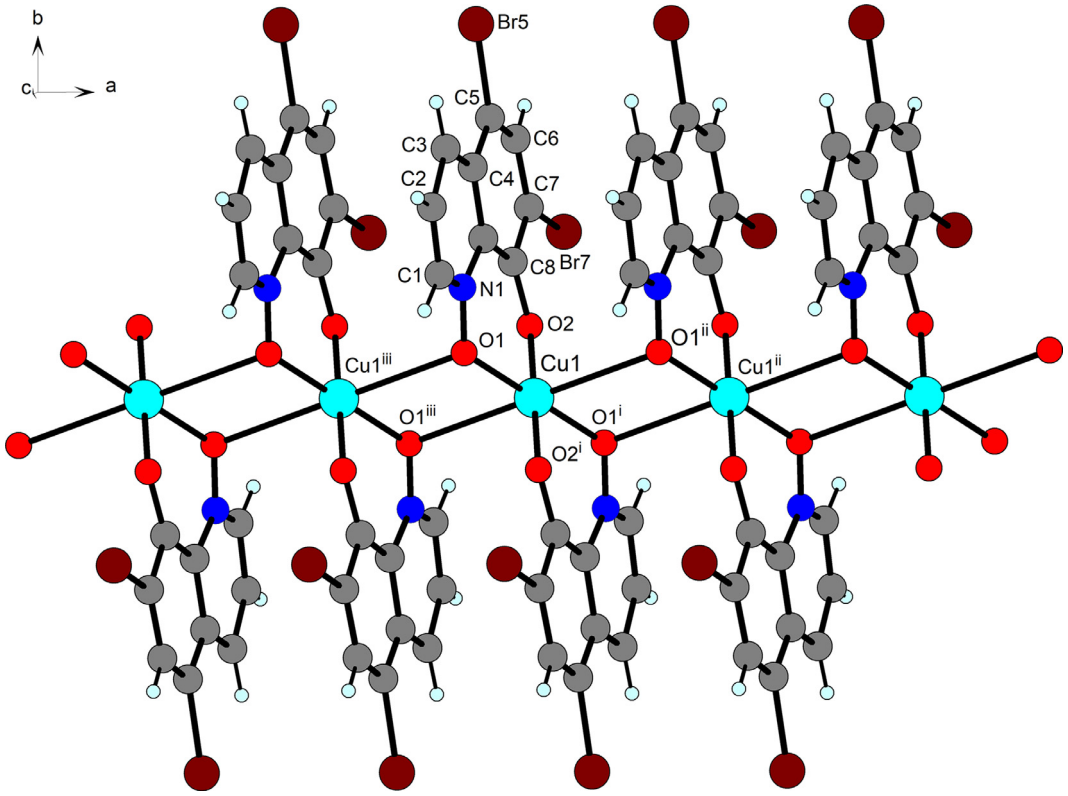



**Fig. 3.** Part of the chain in **5**. Molecules of crystal water are omitted for clarity. Symmetry transformations used to generate equivalent atoms: (i) -*x* + 1, -*y* + 1, -*z* + 1; (ii) *x* + 1, *y*, *z*; (iii) -*x*, -*y* + 1, -*z* + 1.

**Table 3**
Hydrogen bonds and van der Waals interaction (Å and °) for HRQNO.

| | D-H•••A | *d*(D-H) | *d*(H•••A) | *d*(D•••A) | <(DHA) |
|---|---|---|---|---|---|
| 8-HQNO | C1-H1•••O2$^{i}$ | 0.93 | 2.39 | 3.320(2) | 178.9 |
| | O1-H1O1•••O2 | 1.05 | 1.48 | 2.4784(16) | 155.8 |
| HdClQNO | C1-H1•••O2$^{i}$ | 0.93 | 2.38 | 3.165(4) | 142.3 |
| | O1-H1O1•••O2 | 1.07 | 1.43 | 2.458(3) | 159.1 |
| HdBrQNOa | C1-H1•••O2$^{i}$ | 0.93 | 2.39 | 3.295(4) | 164.4 |
| | O1-H1O1•••O2 | 0.84(4) | 1.65(4) | 2.465(3) | 163(4) |
| HdBrQNOb | C1-H1•••O2$^{i}$ | 0.93 | 2.36 | 3.239(4) | 158.5 |
| | O1-H1O1•••O2 | 0.94 | 1.60 | 2.465(3) | 150.7 |
| HIQNO | C1-H1•••O2$^{i}$ | 0.93 | 2.25 | 3.178(15) | 174.0 |
| | O1-H1O1•••O2 | 0.79 | 1.74 | 2.463(15) | 152.1 |

Due to the extremely small crystal of **5**, its diffraction power was low and thus the data collection was very long and the crystal was iced. Therefore, the quality of the obtained data is low and we are able to present only a rough but undisputable model for the structure of **5**, which crystallizes in a monoclinic space group $P2_1/n$. Cu1 atom lies on a symmetry center and is coordinated at normal distances (Table 4) by two pairs of hydroxyl and N-oxide oxygen atoms from two centrosymmetrically related deprotonated dBrQNO ligands. These are chelate coordinated and occupy the equatorial plane of a tetragonal bipyramid around the Cu1 atom. Due to the Jahn-Teller effect, the axial positions of the bipyramid are at much longer distances (Table 4) and are occupied by bridging N-oxide O1$^{ii}$ and O1$^{iii}$ atoms from two neighboring complex species (ii = *x* + 1, *y*, *z*; iii = -*x*, -*y* + 1, -*z* + 1). Thus, a chain along the *a* axis is formed (Fig. 3). We suppose that compounds $Cu(dClQNO)_2(CHCl_3)$ (**3**) and $Cu(dClQNO)_2(H_2O)$ (**4**) with deprotonated N-oxides have the same type of the structure with chelate bonded N-oxides, while in the compounds $CuCl_2(8\text{-}HQNO)_2(H_2O)$ (**1**), $CuCl_2(HdClQNO)_2(H_2O)_2$ (**2**) and $CuCl_2(HIQNO)_2(H_2O)_4$ (**6**), in which the hydroxyl groups remain protonated, N-oxides are bonded terminally *via* the N-oxide O atom as in the published Co(II) and Mn(II) complexes [22–24].

**Table 4**
Selected bond distances and angles for 5.

| Bond | *d* [Å] | Angle | [°] |
|---|---|---|---|
| Cu1-O1 | 1.95(2) | O1-Cu1-O1$^{i}$ | 180.00 |
| Cu1-O2 | 1.86(2) | O2-Cu1-O1 | 89.6(9) |
| Cu1-O1$^{ii}$ | 2.74(2) | O2-Cu1-O1$^{i}$ | 90.4(9) |
| N1-O1 | 1.32(3) | O2-Cu1-O1$^{iii}$ | 90.2(7) |
| C8-O2 | 1.34(5) | O2-Cu1-O1$^{ii}$ | 89.8(7) |
| C5-Br5 | 1.89(3) | O1$^{i}$-Cu1-O1$^{iii}$ | 107.7(9) |
| C7-Br7 | 1.91(2) | O1-Cu1-O1$^{iii}$ | 72.3(9) |

Symmetry transformations used to generate equivalent atoms: (i) -*x* + 1, -*y* + 1, -*z* + 1; (ii) *x* + 1, *y*, *z*; (iii) -*x*, -*y* + 1, -*z* + 1.

The chains are stabilized by $\pi$–$\pi$ interactions between phenyl and pyridine rings of dBrQNO from neighbouring complex species. Distances between centroids of these rings ($Cg_{ph}$ and $Cg_{py}$, respec-

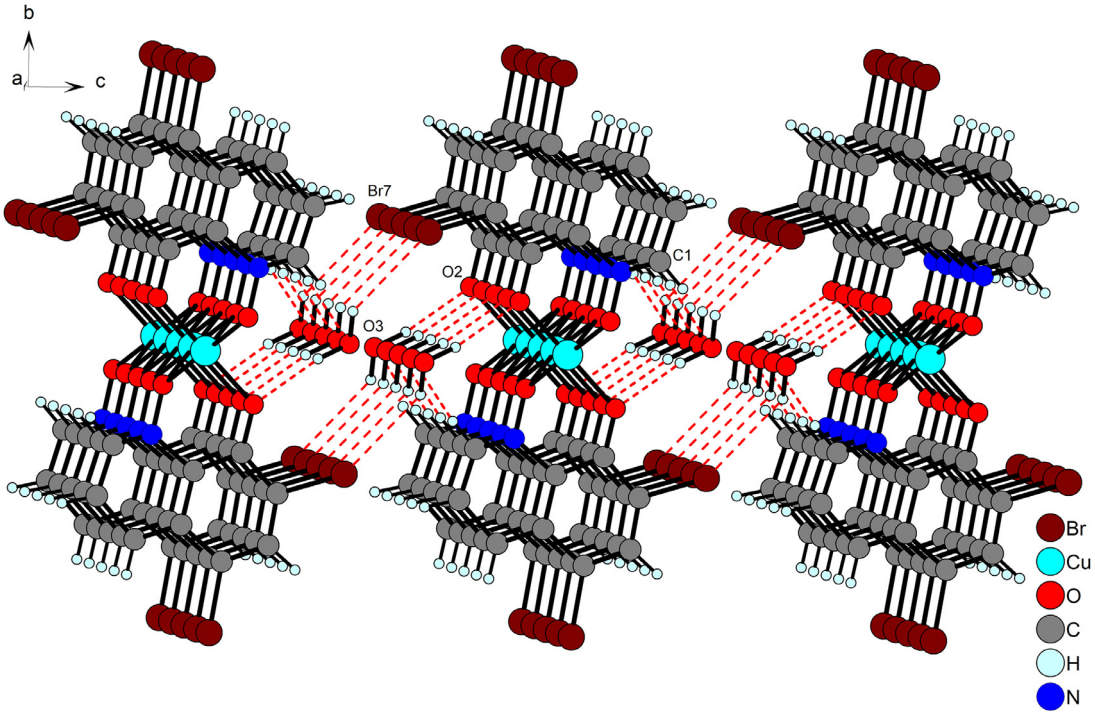



**Fig. 4.** System of hydrogen bonds (red dashed lines) in **5** forming a sheet in the *ac* plane viewed along the chains parallel with the *a* axis.

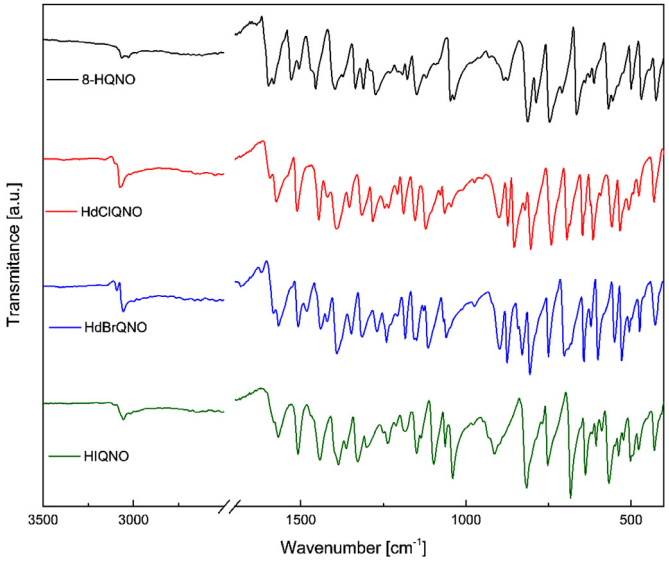



**Fig. 5.** Infrared spectra of the prepared N-oxides.

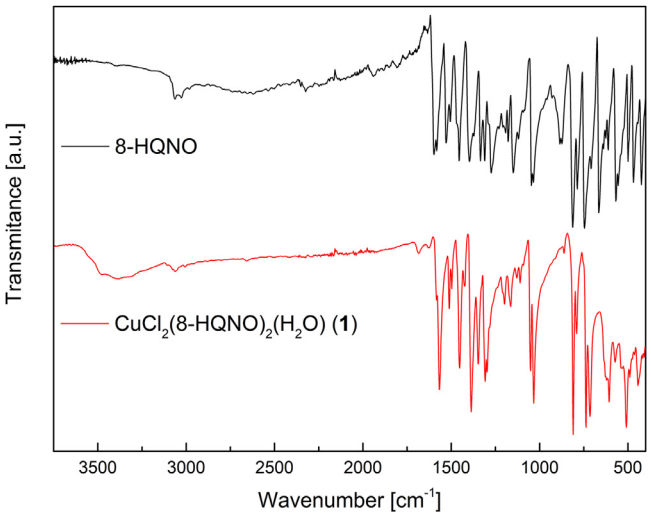



**Fig. 6.** IR spectra of 8-HQNO and complex 1.

**Table 5**
Cg•••Cg distances and angles (Å, °) characterizing $\pi$–$\pi$ interactions in **5**.

| Cg(I)•••Cg(J) | Cg•••Cg | $\alpha$ [a] | $\beta$ | $\gamma$ |
|---|---|---|---|---|
| $Cg_{py}$•••$Cg_{py}^{iv}$ | 3.81(1) | 0 | 25.6 | 25.6 |
| $Cg_{py}$•••$Cg_{ph}^{iv}$ | 3.57(1) | 2 | 16.2 | 17.5 |
| $Cg_{ph}$•••$Cg_{ph}^{iv}$ | 3.81(1) | 0 | 26.4 | 26.4 |

**Table 6**
Hydrogen bonds (Å and °) for 5.

| D-H...A | d(D-H) | d(H...A) | d(D...A) | <(DHA) |
|---|---|---|---|---|
| C1-H1•••O3$^{iii}$ | 0.95 | 2.28 | 3.20(4) | 163.3 |
| O3-H1O3•••O2 | 0.95 | 2.03 | 2.94(3) | 160.1 |
| O3-H2O3•••Br7$^{ii}$ | 0.97 | 2.86 | 3.64(3) | 137.2 |

Symmetry transformations used to generate equivalent atoms: (ii) $x+1, y, z$; (iii) $-x, -y+1, -z+1$.

tively) as well as further characteristics of $\pi$–$\pi$ interactions are given in the Table 5.

Symmetry transformation used to generate equivalent atoms: (iv) $x-1, y, z$.

[a] $\alpha$ is the dihedral angle between planes I and J. $\beta$ is the angle between Cg(I)•••Cg(J) vector and normal to plane I. $\gamma$ is the angle between Cg(I)•••Cg(J) vector and normal to plane J.

Molecules of a crystal water (O3 atoms) are outside of the chain and are involved in a hydrogen bond system (Table 6) which connects the chains into a sheet parallel with the *ac* plane (Fig. 4). In

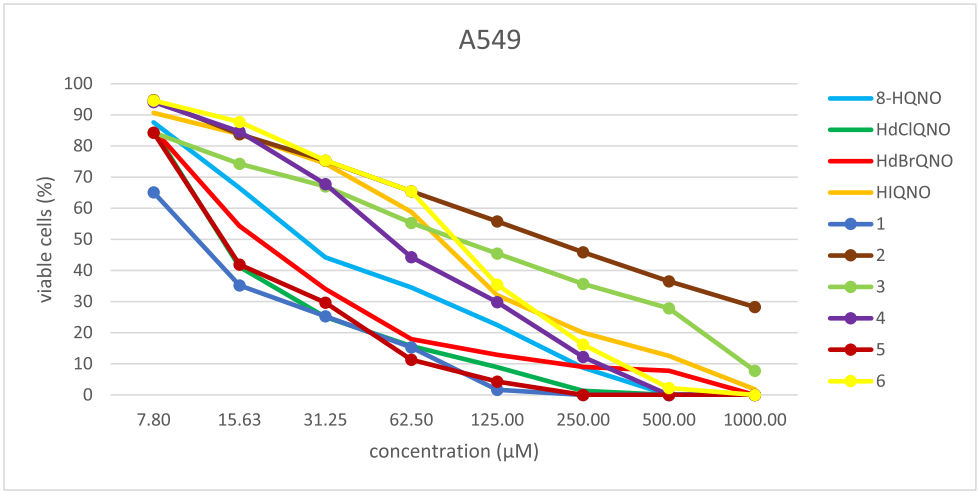



**Fig. 7.** Representative graphs of A549 cell survival after 72 h cell growth in the presence of 10 tested compounds. A549 cells were cultured with different doses of tested compounds ranging from 7.8 to 1000 $\mu$M. Cell viability was determined by MTT assay. Each point represents a mean value and standard deviation of 3 experiments with 3 replicates per dose.

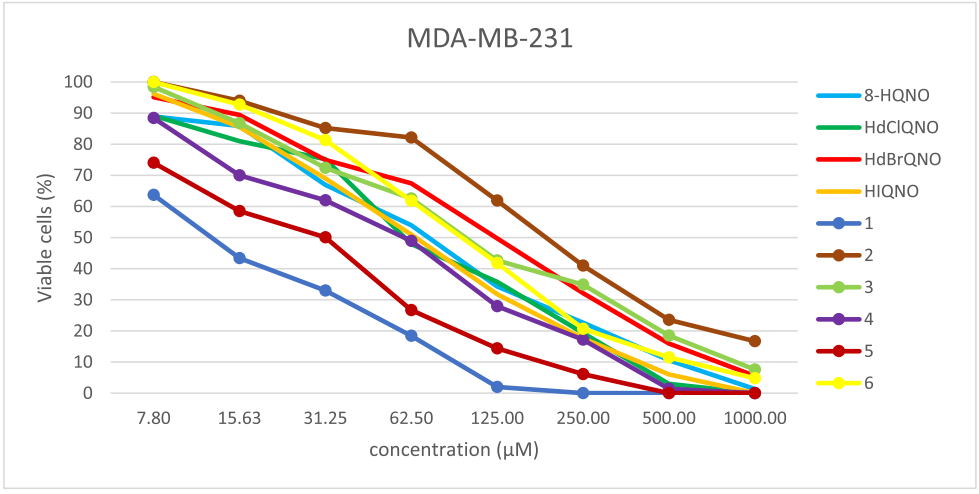



**Fig. 8.** Graph showing MDA-MB-231 viability after 72 h exposition to the different concentrations (7.8–1000 $\mu$M) of 10 compounds. Cell viability was determined by MTT assay. Each point represents a mean value and standard deviation of 3 experiments with 3 replicates per dose.

the sheet, the Cu atoms are separated by the lengths of the unit cell edges *a* and *c*.

### 3.3. Infrared spectroscopy

Infrared spectra of N-oxides HRQNO are shown in Fig. 5. Several characteristic regions can be identified in the IR spectrum of 8-HQNO (previously described in [13,26,37]) and in the spectra of other three prepared HRQNO. Characteristic $\nu$(C-H) vibrations, also observed in 8-HQ and its halogen derivatives [8], span the 3170–3000 cm$^{-1}$ region. Similarly, the ring vibrations of HRQNO correspond to ones observed for 8-HQ derivatives. The $\nu$(N-O) vibrations in HRQNO were observed in the range of 1355–1300 cm$^{-1}$ and 1260–1220 cm$^{-1}$.

Fig. 6 compares the IR spectrum of the complex **1** with the spectrum of 8-HQNO. The most significant differences are observed in the range of 3700–2800 cm$^{-1}$. Although, there is a hydroxyl group in the structure of 8-HQNO, only $\nu$(C-H) vibrations could be observed in this range. This might be explained by the formation of a strong intramolecular hydrogen bond involving oxygen atom of the N-O group as the acceptor, which is responsible for the significant shift of the $\nu$(O-H) band. In the work of Dziembowska et al. [13], the authors observed corresponding $\nu$(O-H) vibrations at 2330 cm$^{-1}$. Although, there is a small band observed at 2324 cm$^{-1}$ in the spectrum of 8-HQNO, this band is nearly hidden in the background noise. However, a very broad band centred at about 2621 cm$^{-1}$ is observed in the spectrum of 8-HQNO, which could correspond to $\nu$(O-H) vibrations. In the spectrum of **1**, there is a wide band assigned to the water $\nu$(O-H) vibrations between 3625 and 3200 cm$^{-1}$. Two bands assigned to $\nu$(N-O) vibrations at 1334 and 1310 cm$^{-1}$ in 8-HQNO are shifted to lower wavenumbers in the complex **1**, 1307 and 1297 cm$^{-1}$, which is due to the binding of the oxygen atom to the central copper atom.

Similar features can be observed in the IR spectra of individual ligands and corresponding copper complexes (Figs. S9 – S11). Generally, two bands of $\nu$(N-O) vibrations are observed at lower wavenumbers in all copper complexes comparing to the respective ligands. The $\nu$(O-H) vibrations are undoubtedly observed in the range of 3600–3190 cm$^{-1}$ for complexes **2, 4** – **6** comprising water molecules. In the spectrum of **3**, wide band of the $\nu$(C-H)$_{al}$ vibrations at 2983–2797 cm$^{-1}$ confirm the presence of chloroform in this complex.

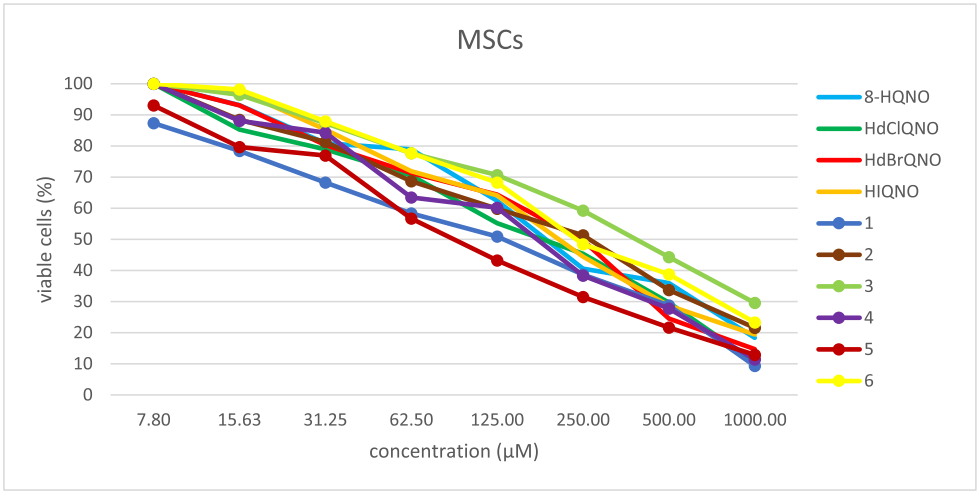



**Fig. 9.** Representative graphs of MSCs survival after 72 h cell growth in the presence of 10 tested compounds. MSCs were cultured with different doses of tested compounds ranging from 7.8 to 1000 $\mu$M. Cell viability was determined by MTT assay. Each point represents a mean value and standard deviation of 3 experiments with 3 replicates per dose.

### 3.4. Antiproliferative activity

The evaluation of cancer cell viability reveals that all tested compounds were cytotoxic against human lung carcinoma epithelial cells (Fig. 7), human colon carcinoma cells (Fig. S12), and human breast cancer cells (Fig. 8). According to these results, the tumor cell viability decreases with increasing the concentration of the tested compounds. Importantly, the compounds showed a reasonable to high cytotoxicity against cancer cells (Figs. 7, 8 and Fig. S12) compared to MSCs (Fig. 9).

Among all tested complexes, **1** was the most cytotoxic against tumor cells. At concentration of 250 $\mu$M, complex **1** induced cell death in all A549, HCT116 and MDA-MB-231 cells. High cytotoxicity of complex **1** was also observed at the lowest tested concentrations (7.8 $\mu$M and 15.625 $\mu$M) (Figs. 7, 8 and Fig. S12). Importantly, at concentration of 15.625 $\mu$M, which could, by our opinion, used in animal studies, complex **1** induced cell death of about 65% of A549 cells (Fig. 7) and 57% of MDA-MB-231 cells (Fig. 8). Importantly, at the same concentration, complex **1** was significantly less cytotoxic to MSCs (Fig. 9). Complex **1** at concentration of 15.625 $\mu$M induced cell death in about 20% of MSCs (Fig. 9), indicating its selective tumorotoxicity.

## 4. Conclusion

In the present work, we optimized syntheses of three N-oxides, 8-quinolinol N-oxide (8-HQNO), 5,7-dichloro-8-quinolinol N-oxide (HdClQNO) and 5,7-dibromo-8-quinolinol N-oxide (HdBrQNO), and prepared new 7-iodo-8-quinolinol N-oxide (HIQNO). All four N-oxides were used as ligands in syntheses of six copper(II) complexes with the following formulae: $CuCl_2(8\text{-}HQNO)_2(H_2O)$ (**1**), $CuCl_2(HdClQNO)_2(H_2O)_2$ (**2**), $Cu(dClQNO)_2(CHCl_3)$ (**3**), $Cu(dClQNO)_2(H_2O)$ (**4**), $\{[Cu(dBrQNO)_2]\bullet 2H_2O\}_n$ (**5**), and $CuCl_2(HIQNO)_2(H_2O)_4$ (**6**). Composition of the complexes, as well as their ligands, was confirmed by necessary physico-chemical methods, including NMR spectroscopy and single crystal X-ray diffraction analysis in the case of N-oxides and **5**. The structures of N-oxides are formed by planar molecules held together through hydrogen bonds involving oxygen atom of N-oxide group as acceptor. It was also proved that HdBrQNO exists in two polymorphic modifications. We also described the first crystal structure of a Cu(II) complex with N-oxide ligand derived from 8-HQNO, complex **5**. The Cu(II) atom lies on a symmetry center and is coordinated by four oxygen atoms of two pairs of hydroxyl and N-oxide oxygen atoms from centrosymmetrically related deprotonated dBrQNO ligands. These coordinate in a chelating mode occupying the equatorial plane of a tetragonal bipyramid around the Cu(II) atom, while the axial positions are filled by bridging N-oxide oxygen atoms from two neighboring complex species at much longer distances. Thus, a chain along the *a* axis is formed, which is stabilized by $\pi$–$\pi$ interactions between phenyl and pyridine rings of dBrQNO from neighbouring complex species. Molecules of a crystalline water are involved in a hydrogen bond system, which connects the chains into a sheet parallel with the *ac* plane. Antiproliferative activity of the complexes and N-oxides was tested by MTT assay. All tested compounds showed moderate and high cytotoxicity against rapidly proliferating cancer cells. Among tested cancer cell lines, A549 and MDA-MB-231 cells were the most sensitive to the tested complexes. The highest cytotoxicity against both tumor cell lines showed complex **1**, which, at concentration which could be tested in animal models, induced cell death in more than 50% of cancer cells and in 20% of MSCs. Therefore, we believe that complex **1** could be considered as a good candidate for future pharmacological investigation in the field of lung and breast cancer research.

## Author contributions

Conceptualization, P.B. and I.P.;
Data curation, P.B., V.V. and I.P.;
Formal analysis, V.V. and I.P.;
Funding acquisition, V.V and I.P.;
Investigation, A.L., P.B., A.I., M.V., M.L. and M.H.;
Project administration, V.V. and I.P.;
Resources, P.B., V.V. and I.P;
Supervision, P.B., V.V. and I.P.;
Visualization, A.L., A.I. and M.H.;
Writing—original draft, A.L., P.B., V.V. and I.P.;
Writing—review and editing, V.V. and I.P.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

CCDC 2085729-2085734 contain the supplementary crystallographic data for XRQNO and **5**. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk.

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## Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.molstruc.2021.131144.

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