Mixed Valence Copper(I,II) Binuclear Complexes with Unexpected Structure: Synthesis, Biological Properties and Anticancer Activity

ARTICLE *in* JOURNAL OF MEDICINAL CHEMISTRY · JUNE 2014

Impact Factor: 5.45 · DOI: 10.1021/jm500154f · Source: PubMed

18 AUTHORS, INCLUDING:

Maria Zvereva
Lomonosov Moscow State University
46 PUBLICATIONS **235** CITATIONS
SEE PROFILE

Maria P Rubtsova
Lomonosov Moscow State University
17 PUBLICATIONS **170** CITATIONS
SEE PROFILE

Elena Beloglazkina
Lomonosov Moscow State University
138 PUBLICATIONS **389** CITATIONS
SEE PROFILE

Nikolai S Zefirov
Tufts Medical Center
1,134 PUBLICATIONS **6,719** CITATIONS
SEE PROFILE

Journal of Medicinal Chemistry

Brief Article

pubs.acs.org/jmc

Mixed Valence Copper(I,II) Binuclear Complexes with Unexpected Structure: Synthesis, Biological Properties and Anticancer Activity

Alexander G. Majouga,*[,†,⊥] Maria I. Zvereva,*[,†,‡] Maria P. Rubtsova,[†,‡] Dmitry A. Skvortsov,[†] Andrei V. Mironov,[†] Dulat M. Azhibek,[†,#] Olga O. Krasnovskaya,[†] Vasily M. Gerasimov,[†] Anna V. Udina,[†] Nikolay I. Vorozhtsov,[†] Elena K. Beloglazkina,[†] Leonid Agron,[†] Larisa V. Mikhina,[∥] Alla V. Tretyakova,[∥] Nikolay V. Zyk,[†] Nikolay S. Zefirov,[†] Alexander V. Kabanov,[†,§] and Olga A. Dontsova[†,‡]

[†]Department of Chemistry, Lomonosov Moscow State University, Leninskie Gory, 1/3, 119991 Moscow, Russian Federation

[‡]Belozersky Institute, Lomonosov Moscow State University, Leninskie Gory, 1/40, 119992 Moscow, Russian Federation

[§]Center for Nanotechnology in Drug Delivery and Division of Molecular Pharmaceutics, Eshelman School of PharmacyUniversity of North Carolina at Chapel Hill, , Genetic Medicine Building, room 1094, Campus Box 7362, Chapel Hill, North Carolina 27599-7362, United States

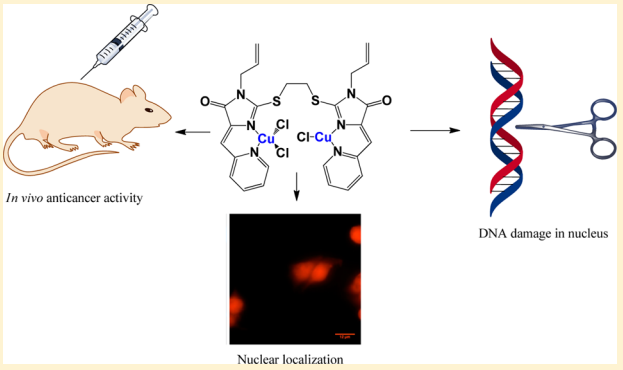
[∥]Federal State-Financed Institution "Research Center for Toxicology and Hygienic Regulation of Biopreparations", Federal Medico-Biological Agency, 102A, Lenin Str., Serpukhov, Moscow Region142253, Russian Federation

[⊥]National University of Science and Technology MISiS, Leninsky Ave, 4, 119049 Moscow, Russian Federation

[#]Skolkovo Institute of Science and Technology, Ul. Novaya, d. 100, Skolkovo 143025, Russian Federation

Supporting Information

ABSTRACT: We have synthesized and characterized a panel of new binuclear mixed valence Cu(I,II) complexes containing substituted 2-alkylthio-5-arylmethylene-4*H*-imidazolin-4-ones with unusual structure. These complexes are shown to be cytotoxic for various cell lines. We have found that these compounds did not intercalate DNA, inhibited number of polymerases (telomerase predominantly), accumulated in the cell nucleus, and caused DNA degradation. Preliminary studies revealed that lead compound inhibited human breast adenocarcinoma growth in mice model.



INTRODUCTION

Metal-based anticancer drugs play an important role in chemotherapy. The discovery of cisplatin resulted in tremendous growth in research associated with inorganic metal complexes for cancer therapy.[1] Therefore, in recent years, there has been a rapid expansion in research and development of novel metal-based anticancer drugs to improve clinical effectiveness, to reduce general toxicity, and to broaden the spectrum of activity. The variety of metal ion functions in biology has stimulated the development of new metallodrugs other than Pt drugs with the aim of obtaining compounds with alternative mechanisms of action. Among non-Pt compounds, copper complexes are potentially attractive. It has been established that the properties of copper-coordinated compounds are largely determined by the nature of ligands and donor atoms bound to the metal ion. Cytotoxic activity of copper-containing complexes is based either on induction of cell apoptosis or enzyme inhibition.[2,3] Among the diversity of copper complexes, most researcher's attention is attracted by copper bis(thiosemicarbazone) complexes[4] and casiopeinas,[5,6] copper-based coordinated complexes with generic structure of $[Cu(N-N)(O-N)]^+$ or $[Cu(N-N)(O-O)]^+$]. Copper bis-(thiosemicarbazone) complexes are used in the application of radioactive copper isotopes in radiopharmaceuticals. The use of coordination compounds as an imaging agent are currently in clinical trials.[7,8] It was shown that these complexes exhibit anticancer activity by inhibition of DNA and RNA synthesis and disruption of ATP production, and surprisingly none of them function through ROS generation.[4] In contrast to the first type of complexes, the generation of ROS is a major factor of the high toxicity and anticancer activity of casiopeinas.[9,10]

In an attempt to receive more insight and determine the action mechanism and antitumor activity of copper complexes, we have synthesized and characterized new binuclear mixed valence Cu(I,II) complexes containing substituted 2-alkylthio-5-arylmethylene-4*H*-imidazolin-4-ones. These complexes were shown to be cytotoxic for various cell lines. We have shown that

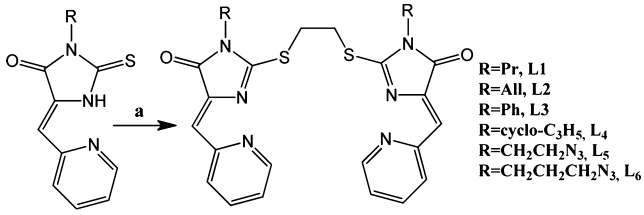
Received: January 20, 2014
Published: June 20, 2014

dx.doi.org/10.1021/jm500154f | J. Med. Chem. 2014, 57, 6252−6258

in experiments in vitro, these compounds inhibit human telomerase, HIV reverse transcriptase, T7 RNA polymerase, calf DNA polymeraseβ, *Taq* DNA polymerase to a different extent and do not intercalate DNA and do not cause significant DNA cleavage. However, these complexes accumulate in the nucleus within the cell and cause rapid chromosomal DNA degradation which results in the cell death. Preliminary studies revealed that lead compound inhibits human breast adenocarcinoma growth in mice model.

As it was previously described, initial 5-arylmethylene substituted thiohydantoines were prepared by Knoevenagel condensation reaction between heteroaromatic aldehydes and the thiohydantoin.[11] The key step in the formation of the thiohydantoin backbone was achieved by a S-alkylation reactions in the basic conditions (Scheme 1).

Scheme 1. Synthesis of 2-Alkylthio-5-arylmethylene-4*H*-imidazolin-4-one Ligands L1−L6[a]



Reagents and conditions: (a) $Br(CH_2)_2Br$, K_2CO_3, DMF, rt.

[a]Reagents and conditions: (a) $Br(CH_2)_2Br$, K_2CO_3, DMF, rt.

Presence of azido group allows us to modify complexes with fluorescent dyes via the well-known click-chemistry approaches.

The Cu(I,II) complexes were prepared by direct reaction of ligands dissolved in dichloromethane with $CuCl_2 \cdot 2H_2O$ in methanol. According to the X-ray data received during the study, absolutely unexpectedly, we revealed a novel type of mixed valence copper(I,II) complexes (Figure 1).

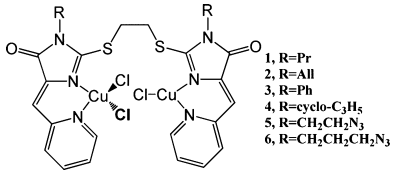


Figure 1. Copper complexes based on 2-alkylthio-5-arylmethylene-4*H*-imidazolin-4-ones.

The crystal structures of **2**, **4**, **5**, and **6** were determined by X-ray crystallography (see Figure 2 and Figure S1, TablesS1−4, Supporting Information). All synthesized complexes have the same geometry and metal ion surroundings (Figure 2). An organic ligand consists of two equivalent parts, each part is linked with copper atoms. Even though both parts of the molecule are identical (symmetrical), during the interaction with copper they become very different in structure. The main difference is in the coordination of copper atoms: one atom is three coordinated, while the other atom is four coordinated. The calculation of oxidation state of copper atoms by bond valence sum method[12] gives 2.00 for Cu(II) and 1.54 for Cu(I). The coordination of both copper atoms is highly asymmetric. The deformation of the copper coordination polyhedron is common, but usually, the deformation is not too strong. In the

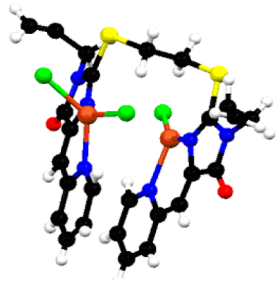


Figure 2. X-ray molecular structures of Cu(I,II) complex **2**. Solid thermal ellipsoids are reported at the 30% probability level. C (black), N (blue), Cl (green), O (red), S (yellow), Cu (brown).

titled compound, the triangle coordination of Cu(I) atom is usually almost flat, unequal angles, which is atypical for such type of copper coordination. The $Cl_{11}-Cu_1-N_{12}$ angle is 151.9°, while the other angles $Cl_{11}-Cu_1-N_{11}$ and $N_{11}-Cu_1-N_{12}$ are 109.8°and 98.0°, respectively.

We have studied electrochemical behavior of complexes **2**, **3**, and **4** by means of cyclic voltammetry and rotating disk electrode techniques. Under the same conditions, one-electron and two-electron reduction waves are observed during the reverse scan and assigned to the Cu^{2+} to Cu^{1+} and Cu^{1+} to Cu^0 (respectively), which are quasireversible in nature (see Table S5, Supporting Information).

The anticancer activities of **1**−**6** against the MCF-7 (human breast adenocarcinoma), SiHa (human cervical squamous cell carcinoma), and HEK293 (human embryonic kidney) cell lines have been tested with the help of a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[13] The comparison of these data with the widely used cisplatin (literature and our data) and doxorubicine under identical conditions by using MTT assay is shown in Table 1.

Table 1. Level of Cytotoxicity Measured in Vitro by Various Complexes

no.	IC_{50} (μM) MCF-7	IC_{50} (μM) SiHa	IC_{50} (μM) HEK293
2	3.7 ± 1.6	3.0 ± 0.2	2.5 ± 0.4
L2	15.9 ± 1.4	61.4 ± 25.5	>100
3	2.1 ± 0.8	2.2 ± 0.7	2.3 ± 0.9
L3	11.3 ± 2.6	>100	53.0 ± 4.4
4	7.4 ± 1.4	3.9 ± 2.3	25.3 ± 1.2
L4	>100	>100	>100
6	13.4 ± 3.8	8.5 ± 0.4	12.7 ± 3.7
L6	12.0 ± 10	54.8 ± 10.5	37.3 ± 15.9
Dox	2.1 ± 0.8	2.0 ± 0.8	1.1 ± 0.1
cisplatin	64.13 ± 3.9[a]		12.4 ± 3.9
	>30		

[a]Reference 14.

Coordination of copper ions was essential for cytotoxicity of novel compounds. Indeed, IC_{50} values for free ligands were much higher than the values for corresponding complexes (Table 1), for in cytotoxicity not only does the coordination of copper ions play an important role but it also does for the nature of ring substituents. The most active complexes (**2**, **3**) contained allyl or phenyl substituents. Complexes with aliphatic substituents, placed at the same positions in the ring (**4**, **5**, **6**), were less active. For further biological studies, complex **2** was chosen, because in comparison to complex **3**, it had a perfect activity and better solubility in biological media. The

dx.doi.org/10.1021/jm500154f | J. Med. Chem. 2014, 57, 6252−6258

cytotoxicity for the complex **2** was tested in a broadened panel of human cancer cell lines which included MDA-MB-231, HepG2, and PC3 cell lines that represent human breast adenocarcinoma, human hepatocellular carcinoma, and human prostate adenocarcinoma, respectively (see Table S6, Supporting Information). In all of the cases, complex **2** showed similar toxicity.

To test complex **2**'s influence on HEK293 cell morphology, they were treated with complex **2** (2.6 μM) for 3 h. Significant changes such as contraction, loss of adherence, and rounded shape were detected (see Figure S2, Supporting Information). Next, we have tested an uptake of the copper complex by the cell. Inductively coupled plasma mass spectrometry detected 10 times enrichment of Cu^{2+} in cells compared to the natural copper cell level after incubation with 3 μM of copper complex **2** (see Table S7, Supporting Information). Thus, after the cells were treated with complex **2**, copper ions accumulated inside of the cell. We used fluorescent microscopy to investigate whether the copper ions enter the cell as a part of copper containing complex **2**. Because complexes **1**−**4** have low fluorescence level and are insufficient for microscopy, we used complexes **5**−**6** bearing an azido group to conjugate them with diSulfo-Cy5 alkyne (Cyandye LLC) fluorophore using the click-chemistry approach[15] after the cells were fixed for microscopy. Series of fluorescent microscopy images (Figure 3) showed intracellular

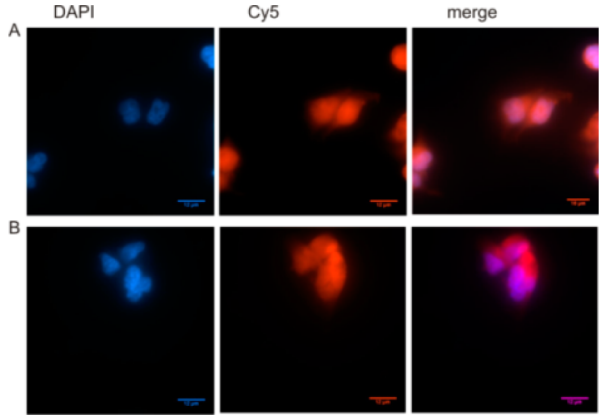


Figure 3. Cellular uptake properties of the complex **5**. (A) HEK293 cells after complex **5** uptake were stained with DAPI and conjugated with diSulfo-Cy5 alkyne. (B) HEK293 cells after ligand **L5** uptake were stained with DAPI and conjugated with diSulfo-Cy5 alkyne.

localization of complex **5** conjugate with its predominant presence in the nucleus. In contrast to complex **5**, localization of ligand **L5** marked with the same fluorescent dye under the same conditions was also detected inside the cell but spread over the entire cell volume.

To investigate changes in DNA integrity after complex accumulation in a cellular nucleus, we performed PI DNA staining followed by flow cytometry (Figure 4). We observed a significant decrease in the amount of PI-positive cells in the presence of complex **2** that provided evidence that the number of long DNA fragments capable for PI binding was reduced. The analysis of forward and side scattering distribution of these samples revealed the dramatic changes in morphology of cells after treatment with complex **2**. In contrast, **L2** did not affect HEK293 cell morphology and distribution of PI-positive cells. These data allow us to propose that complex **2** targets copper ions to the cellular nucleus that causes significant DNA damage.

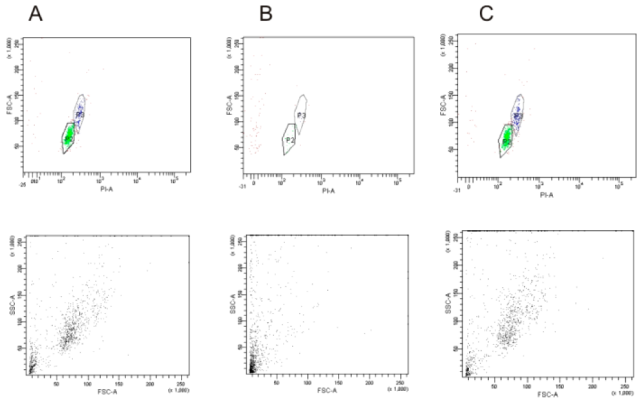


Figure 4. DNA damage occurs in HEK293 cells after complex **2** treatment according to flow cytometry analysis. HEK293 cells without treatment (A) and after treatment with complex **2** (B) and ligand **L2** (C) were analyzed by PI-staining followed by flow cytometry (upper panel). In the lower panel, forward and side scattering for the same samples are shown.

To prove that complex **2** causes significant DNA destruction, TUNEL,[16] which allows detection of DNA breaks, was performed. All cells demonstrated TUNEL signal after treatment with complex **2** (Figure5), thus confirming that

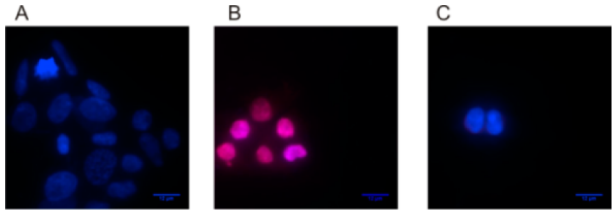


Figure 5. DNA damage occurs in HEK293 cells after complex **2** treatment according to TUNEL assay. HEK293 cells without treatment (A) and after treatment with 6.5 μM complex **2** (B) and 16 μM ligand **L2** (C) were analyzed by TUNEL assay. Nucleus stained with DAPI, and the red color represents TUNEL signal.

complex **2** causes DNA cleavage followed by massive DNA fragmentation by cellular nucleases that provide a free DNA 3′-end necessary for induction of TUNEL signal.

DNA damage can occur due to the formation of reactive oxygen species upon cell treatment with copper complexes. To investigate whether ROS are essential for copper complexes induced cell death, the ability of complex **2** to stimulate the appearance of intracellular ROS was assessed by flow cytometry with the fluorescent CM-H_2DCFDA probe[17] in HEK293 cells (see Figure S3, Supporting Information). ROS value increased after incubation with 2.6 μM of complex **2** and 16 μM of ligand **L2**. The level of ROS production was increased similarly for cells treated with complex **2** or ligand **L2** or H_2O_2in comparison with untreated HEK293 cells. Although similar levels of ROS production was detected both for complex **2** and ligand **L2**, DNA damage happened only in the case of complex **2** but not ligand **L2** (see Figure S3, Supporting Information). These data suggest that formation of ROS did not play an essential role in DNA fragmentation activity of the desired complexes.

We have shown that complex **2** causes DNA damage within the cell nucleus not due to ROS induction. To understand the mechanism of this process, we decided to test whether this complex can directly interact with DNA and cleave it.

dx.doi.org/10.1021/jm500154f | J. Med. Chem. 2014, 57, 6252−6258

There are several types of interaction between DNA and small molecules: formation of a covalent bond, intercalation, and the interaction with the bases or sugar−phosphate backbone.[18] To test whether complex **2** can bind DNA as an intercalating agent or minor groove binder, we used a fluorescence-based ethidium bromide displacement protocol.[4] If the substance competes with ethidium bromide for DNA binding and causes ethidium bromide dissociation from the preformed DNA adduct, then that results in ethidium bromide fluorescence emission quenching. We monitored such quenching from either ligand **L2** or corresponding copper complex **2**. Both substances did not cause detectable quenching, thus indicating that these compounds did not bind DNA (see Figure S4, Supporting Information). A Majority of substances interacting with DNA by different mechanisms affect duplex stability, and thus their binding could be detected by the influence on dsDNA oligonucleotide thermal denaturation. Melting curves for both DNA duplex and DNA−RNA heteroduplex did not show any difference in the presence or absence of complex **2** (see Figures S5, S6, Supporting Information) thus providing additional evidence that complex **2** does not interact with DNA duplex or DNA−RNA heteroduplex. Number of organic ligands and metal complexes can interact with noncanonical forms of DNA, like G-quadruplexes.[19−21] Complex **2** has its own fluorescence, sufficient for measuring interaction with G-quadruplexes. We studied complex **2** fluorescence in the presence of 0.2−4-fold excess of single-stranded or quadruplex DNA and did not observe any changes (see Figures S7, S8, Supporting Information), which proves the absence of the strong complex−DNA binding. We checked whether the complex **2** cleaves DNA in vitro. The conversion of supercoiled DNA into relaxed DNA as an indicator of the cuts/nicks in DNA induced by compound was used. For that purpose, pUC18 plasmid DNA was incubated with increasing concentrations of complex **2** as described[4] and resulting DNA molecules were analyzed by agarose gel electrophoresis. The results are shown in Figure 6. Accumulation of relaxed plasmid DNA form was observed starting from 10 μM complex **2**. It means that at this concentration, one phosphor−ester bond of DNA per 3500bp is cleaved.

Within the cell, the DNA was significantly destroyed into fragments after cell treatment with the complex, but in solution, we could detect only limited DNA degradation after longer incubation with higher concentration of the reagent. Such a difference allows us to hypothesize that there is a partner in the cell that can interact with the complex and stimulate DNA damage by release of copper ions at direct proximity to DNA followed by massive DNA degradation by nucleases. On that basis, we have decided to test if this reagent can interact with DNA binding proteins. For that purpose, we tested whether complex **2** could inhibit such important DNA operating enzymes as DNA or RNA polymerases. The effect of complex **2** on different polymerases under enzyme reaction conditions were tested by standard assays (see Figure 7, Figures S9−S15, Supporting Information). The strongest inhibitory effect was observed for human telomerase. It is important to mention that complex **2** was stable during an incubation for a long time in a buffer used for experimental procedures (see Figure S16, Supporting Information). Complex **2** inhibits HIV reverse transcriptase, which has similar catalytic core, at similar level (Figure 7).

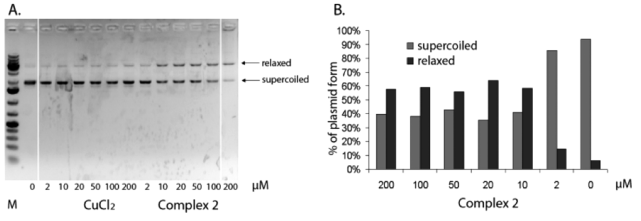


Figure 6. Test of DNA cleavage in vitro by complex **2**. (A) Agarose gel electrophoresis of pUC18 plasmid DNA treated with 0−200 μM complex **2** or 0−200 μM $CuCl_2$. "0": DNA incubated in the same buffer conditions without treatment. "M": "1 kb+" DNA marker. (B) Quantitative densitometry of A. Amounts of supercoiled and relaxed forms of pUC18 presented as a% of the total amount of plasmid depend on the concentration of complex **2**. Supercoiled form is represented by gray columns, the relaxed form - by dark bars.

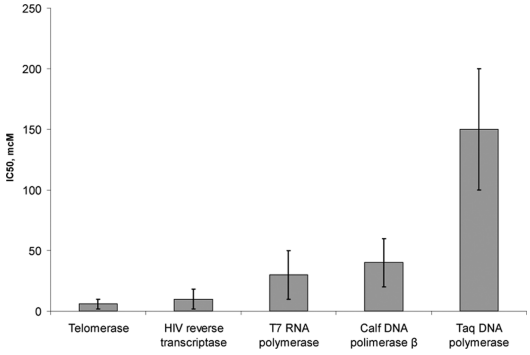


Figure 7. Telomerase (1), HIV reverse transcriptase (2), T7 RNA polymerase (3), calf thymus DNA polymerase β (4), and *Taq* DNA polymerase (5) inhibition data for complex **2**.

All other polymerases tested were also inhibited by complex **2** but with lower efficiency. However, these results showed that indeed the variety of DNA operating enzymes and possibly DNA binding proteins can bind complex **2** and may stimulate copper ion release near DNA in the cell nucleus. Such release can provide the appearance of short-living hydroxyl radicals at direct proximity to DNA that cause DNA damage.

It should be mentioned that we have detected significant level of telomerase inhibition by complex **2**. Telomerase is a very attractive molecular target for developing therapeutic anticancer agents due to difference in the performance of active enzyme in normal and cancerous tissues (for review, see ref 22). We used two independent approaches to investigate telomerase inhibition by complex **2**: TRAP assay[23] and direct telomerase assay[24] (see Figures S9, S10, S15, Supporting Information). Complex **2** inhibited telomerase with IC_{50} 6.5 ± 4 μM in TRAP assay, but ligand **L2** showed lower telomerase-inhibitory activity that proves that copper ions are essential for inhibition process. Complex **2** can possibly inhibit telomerase by binding to catalytic core or/and site-directed cleavage of telomerase RNA or telomerase elongation product by hydroxyl radicals upon copper ions release after complex interaction with the enzyme. The fact that complex **2** was inhibiting HIV reverse transcriptase to a similar extent suggests that this reagent can bind to conserved reverse transcriptase catalytic core. Despite the fact that complex **2** inhibits telomerase activity in vitro, we cannot exclude that complex **2** disrupts indirectly shelterin complex and induce telomere-dependent DNA damage similar to the situation observed for SWNTs.[25] Thus, in spite the fact

dx.doi.org/10.1021/jm500154f | J. Med. Chem. 2014, 57, 6252−6258

that complex **2** itself cannot be considered as a potential telomerase specific anticancer drug due to significant DNA damage in the cell nucleus, it provides a chemical platform for searching for telomerase inhibitors with different ions and/or substituents that may have telomerase inhibiting activity without DNA damaging due to copper release.

It should be mentioned that complex **2** inhibits different polymerases. All these enzymes share the possibility of interacting with nucleic acids. If we compare the structure of complex **2** with the structure of nucleic acid chain, we could notice that the position of aromatic rings and the distance between them resembles that in a DNA chain (see Figure S17, Supporting Information). That allows us to hypothesize that complex **2** can interact with the variety of DNA binding proteins and induce DNA damage by copper release and subsequent DNA degradation in the cell nucleus. Complex **2** is a mixed valence copper (I,II) complex. It is known that Cu(I) catalyzes the production of hydroxyl radical from endogenous hydrogen peroxide after release from complex that is highly damaging for cellular lipids, proteins, and especially for nucleic acids.[26,27]

Complex **2** was active in antiproliferative assay with different cancer cell cultures. To test whether this reagent can have antitumor activity in vivo we used mouse breast adenocarcinoma model. MTD for C57Bl mice for complex **2** in 10% DMSO was 24 mg/kg correspondingly. Five intraperitoneal injection of complex **2** at MTD to animals did not lead to deaths during the course and during the observation period; after 14 days, the mice body weight loss did not exceed 10%. Breast adenocarcinoma 755 was inoculated into mice lines C57BL/6 (female) and treatment began 48 h after vaccination. Complex **2** (14 and 12 mg/kg/d) was intraperitoneally injected with intervals of 24 h for 5 days. Statistical comparison was made between control and treated groups (10 mice in each group). A p-value <0.05 was considered to be statistically significant. Indicators of tumor growth inhibition for mice with a course of the test substance at a dose of 12 mg/kg was 46.1% on the seventh day after the end of treatment and 36.1% on the 14th day after the end of treatment. For a dose of 24 mg/kg, it was 73.5% on the seventh day after the treatment, and 59.5% on the 14th day after the end of treatment. These data show that the complex **2** demonstrates antitumor activity in vivo (see Table S8, Supporting Information).

CONCLUSIONS

As a result of our research, a number of novel nitrogen containing organic ligands were synthesized. It is interesting to note that for the first time formation of the mixed valence copper(I,II) complexes from Cu(II) precursor and imidazolin-4-ones was observed. The characterization has been based on single-crystal X-ray analysis, elemental analysis, and cyclic voltamperometry. Copper containing complexes were cytotoxic for a variety of cancer cell lines. In contrast to majority of other known metal-containing drugs, these compounds were shown to be accumulated at the cell nucleus and the presence of copper ions as a complex constituent was essential for both accumulation in the cell nucleus and rapid DNA fragmentation accompanied by DNA 3′-end generation as a result of nuclease cleavage. Copper containing complexes were able to inhibit number of polymerases that allowed us to hypothesize that interaction of copper containing complexes with the proteins in the vicinity of DNA may result in copper ions release that cause DNA damage followed by rapid DNA degradation by cellular nucleases. Experiments with mice models showed that complex **2** is less toxic than the well-known drug cisplatin[28] and have a pronounced antitumor effect. A novel class of metal complexes, based on a 2-thioxoimidazol-2-one heterocyclic corem could be utilized as a smart platform for construction of anticancer chemotherapy agents. Ability to penetrate the cell membrane, localize in cell nucleus, and inhibit polymerase opens access to development of novel noncopper metal complexes with less toxicity.

EXPERIMENTAL SECTION

Chemistry. All solvents and chemicals were used as purchased without further purification. The progress of all reactions was monitored on Silufol precoated silica gel plates (with fluorescence indicator UV254) using ethyl acetate/n-hexane as solvent system. Melting points (mp) were taken in open capillaries on a Stuart melting point apparatus SMP11 and are uncorrected. Proton (^{1}H) NMR spectra were recorded on a Bruker Avance 400 (400.13 MHz for ^{1}H) using $CDCl_3$ as solvent. Chemical shifts are given in parts per million (ppm) (δ relative to residual solvent peak for ^{1}H). Elemental analysis was performed on a vario MICRO cube elemental analyzer, Elementar Analysen Systeme GmbH, Hanau, Germany. IR spectra were recorded on a Varian 800 FT-IR Scimitar series. All compounds exhibited >95% purity according to elemental analysis (within ±0.4% of the calculated value).

Materials. Initial 2-thioxo-5-(pyridine-2-ylmethylene)-3,5-dihydro-4*H*-imidazole-4-ones were prepared previously by Beloglazkina.[28]

General Method for the Synthesis of L1−L6. Selected 2-thioxo-5-(pyridine-2-ylmethylene)-3,5-dihydro-4*H*-imidazole-4-on (2 equiv), α,ω-dibromoalkane (1 equiv), and solid potassium carbonate (3 equiv) were suspended in DMF (10 mL) at 0 °C. The mixture was stirred for 2 h at RT. Water (50 mL) was added, and the resulting precipitate was filtered and washed with water, ethanol, and ether.

*(5Z,5′Z)-1,2-Bis-[3-propyl-5-(pyridine-2-ylmethylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethane (**L1**).* Bright-yellow solid (82%); mp 152 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.69 (d, J = 8.0 Hz, 1H, Hα-Py), 8.65 (d, J = 4.7 Hz, 1H, $H_{\beta'}$-Py), 7.63 (td, J_1 = 7.4 Hz, J_2 = 2.0 Hz, 1H, H_{β}-Py), 7.19 (dd, J_1 = 7.5 Hz, J_2 = 0.9 Hz, 1H, Hγ-Py), 7.12 (s, 1H, CH═), 7.12 (s, 1H, CH═), 3.93 (s, 2H, SCH_2), 3.60 (t, J = 7.5 Hz, 2H, CH_2N), 1.72 (m, 2H, CH_2), 0.96 (t, J = 7.5 Hz, 3H, CH_3) ppm. IR (KBr): υ =1705 (C═O), 1675 (C═N), 1640 (C═C) cm^{-1}. Elemental analysis calculated (%) for $C_{26}H_{28}N_6O_2S_2$: C 59.98, H 5.42, N 16.14. Found: C 59.89, H 5.48, N 15.97.

*(5Z,5′Z)-1,2-Bis-[3-(propen-2-yl)-5-(pyridine-2-ylmethylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethanr (**L2**).* Bright-yellow solid (92%); mp 187 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.65 (m, 2H, H_{α}-Py + $H_{\beta'}$-Py), 7.62 (t, J = 7.5 Hz, 1H, H_{β}-Py), 7.19 (m, 1H, H_{γ}-Py), 7.14 (s, 1H, CH═), 7.12 (s, 1H, CH═), 5.82 (m, 1H, CH═), 5.23 (m., 2H, CH_2═), 4.23 (m, 2H, CH_2N), 3.89 (s, 2H, S-CH_2) ppm. IR (KBr): υ = 1720 (C═O), 1680 (C═N), 1640 (C═C)cm^{-1}. Elemental analysis calculated (%) for $C_{26}H_{26}N_6O_2S_2$: C 60.44, H 4.68, N 16.27. Found: C 60.14, H 4.48, N 16.03.

*(5Z,5′Z)-1,2-Bis-[3-phenyl-5-(pyridine-2-ylmethylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethane (**L3**).* Yellow solid (73%); mp 259 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, J = 7.9 Hz, 1H, H_{α}-Py), 8.66 (d, J = 4.0 Hz, 1H, $H_{\beta'}$-Py), 7.81 (td, J_1 = 7.3 Hz, J_2 = 2.3 Hz,1H, H_{β}-Py), 7.42 (m, 3H, H-Ph), 7.29 (m, 2H, H-Ph), 7.11 (td, J_1 = 7.5 Hz, J_2 = 1.0 Hz, 1H, H_{γ}-Py), 7.18 (s, 1H, CH═), 3.11 (t, J = 7.5 Hz, 2H, SCH_2) ppm. IR (KBr): υ = 1710 (C═O), 1670 (C═N), 1640 (C═C) cm^{-1}. Elemental analysis calculated (%) for $C_{32}H_{24}N_6S_2O_2$: C 65.31, H 4.08, N 14.29. Found: C 65.28, H 4.10, N 14.11.

*(5Z,5′Z)-1,2-Bis-[3-cyclopropyl-5-(pyridine-2-ylmethylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethane (**L4**).* Bright-yellow solid (59%); mp 200 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (m, 2H, $H_{\alpha'}H_{\beta'}$-Py), 7.67 (t, J = 7.00 Hz, 1H, H_{β}-Py), 7.22 (bs, 1H, H_{γ}-Py), 7.08 (s, 1H, CH═), 3.87 (s, 2H, CH_2S), 2.66 (m, 1H, $\underline{CH}(CH_2)_2$), 1.04 (m, 4H, $CH(\underline{CH_2})_2$). IR (KBr): υ = 1710 (C═O), 1670 (C═N), 1640 (C═C) cm^{-1}. Elemental analysis calculated (%) for

dx.doi.org/10.1021/jm500154f | J. Med. Chem. 2014, 57, 6252−6258

$C_{26}H_{26}N_6O_2S_2$: C 60.44, H 4.68, N 16.27. Found: C 60.23, H 4.57, N 16.01.

*(5Z,5′Z)-1,2-Bis-[3-(2-azidoethyl)-5-(pyridine-2-ylmethylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethane (**L5**).* Yellow solid (48%); mp 150 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.66 (m, 2H, H_α, $H_{\beta'}$-Py), 7.65 (td, J_1 = 7.63 Hz, J_2 = 1.76 Hz, 1H, H_γ-Py), 7.21 (m, 1H, H_β-Py), 7.15 (s, 1H, CH═), 3.95 (s, 2H, CH_2S), 3.83 (t, J = 5.87 Hz, 2H, $N\underline{CH_2}CH_2\ N_3$), 3.61 (t, J = 5.67 Hz, 2H, $NCH_2\underline{CH_2}N_3$). IR (KBr, cm^{-1}): υ = 2080 (N_3), 1680 (C═O), 1630 (C═C), 1600 (C═N). Elemental analysis calculated (%) for $C_{24}H_{22}N_{12}O_2S_2$: C 50.16, H 3.86, N 29.25. Found: C 50.37, H 3.90, N 29.07.

*Synthesis of (5Z,5′Z)-1,2-Bis-[3-(3-azidopropyl)-5-(pyridine-2-yl-methylene)-3,5-dihydro-4H-4-oxoimidazol-2-yl)-thio]-ethane (**L6**).* Yellow solid (51%); mp 145 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.77 (d, J = 5.48, 1H, H-$_\alpha$Py), 8.66 (d, J = 8.02, 1H, $H_{\beta'}$-Py), 7.80 (t, J = 6.46 Hz, 1H, H_γ-Py), 7.32 (t, J = 5.38 Hz, 1H, H_β-Py), 7.15 (s, 2H, CH═), 4.02 (s, 2H, CH_2S), 3.75 (t, J = 6.85 Hz, 2H, CH_2N_3), 3.43 (t, J = 6.26 Hz, 2H, NCH_2), 1.97 (m, 4H, $NCH_2\underline{CH_2}CH_2N_3$). IR (KBr, cm^{-1}): υ = 2092 (N_3), 1730 (C═O, C═N), 1660 (C═C). Elemental Analysis calculated (%) for $C_{26}H_{26}N_{12}O_2S_2$: C 51.81, H 4.35, N 27.89. Found: C 51.48, H 4.35, N 27.75.

General Method for the Synthesis of Complexes 1−6. To a solution of 0.068 mmol of selected ligand **L1**−**L6** in 2 mL of CH_2Cl_2 (placed in a glass tube with approximately 1 cm diameter) pure EtOH (1 mL) was slowly added in order to form a diphasic system. Then a solution of equivalent amount of $CuCl_2{\cdot}2H_2O$ (0.136 mmol) in 2−3 mL of EtOH was added down the tube side without mixing the ligand and salt solutions. Thereafter, the tube was covered and kept for crystal solid precipitation. The solid was filtered and dried in air.

1. Brown crystals (45%); mp> 340 °C. Elemental analysis calculated (%) for $C_{26}H_{28}N_6O_2S_2$ *$CuCl_2$*CuCl: C 41.41, H 3.74, N 11.14. Found C 41.30, H 3.77, N 11.05.

2. Brown crystals (40%); mp >340 °C. Elemental analysis calculated (%) for $C_{26}H_{24}N_6O_2S_2$ *$CuCl_2$*CuCl: C 41.63, H 3.23, N 11.15. Found: C 41.38, H 3.30, N, 10.97.

3. Brown crystals (55%); mp> 340 °C. Elemental analysis calculated (%) for $C_{32}H_{24}N_6O_2S_2$ *$CuCl_2$*CuCl: C 46.75, H 2.94, N 10.22. Found: C 46.62, H 2.99, N 10.27.

4. Brown crystals (38%); mp 200 °C. Elemental analysis calculated (%) for $C_{26}H_{24}N_6O_2S_2$ *$CuCl_2$*CuCl: C 41.63, H 3.23, N 11.15. Found: C 41.39, H 3.30, N 11.02.

5. Brown crystals (35%); mp> 340 °C. Elemental analysis calculated (%) for $C_{24}H_{22}N_{12}O_2S_2$ *$CuCl_2$*CuCl: C 35.67, H2.74, N 20.80. Found: C 35.73, H 2.56, N20.50.

6. Brown crystals (30%); mp> 340 °C. Elemental analysis calculated (%) for $C_{26}H_{26}N_{12}O_2S_2Cu_2Cl_3$: C 37.35, H 3.13, N 20.10. Found: C 37.24, H 3.29, N 20.02.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Synthesis details, structural data, biochemical in vitro and in vivo data, materials and methods, abbreviations. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

*Phone: +7 495 9394020. Fax: +7 495 9328846. E-mail: majouga@org.chem.msu.ru.

*E-mail: zvereva@genebee.msu.ru.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. A.G.M., M.I.Z., and M.P.R. authors contributed equally.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

The study was supported by Russian Foundation for Basic Research (12-03-33148, 12-04-00988-a, 14-04-01092-a), PNR 5.13, and the International Associated Laboratory LIA-NUCPROT (CNRS-Russia). We thank Cyandye LLC for providing diSulfo-Cy5 alkyne and the Rostock group. We thank Russian Ministry of Science and Education (11.G34.31.0004, K1-2014-022).

■ ABBREVIATIONS USED

DAPI, 2-(4-amidinophenyl)-1*H*-indole-6-carboxamidine); TUNEL, TdT-mediated dUTP nick end labeling; CM-H_2DCFDA, 5-(and-6)-chloromethyl-2′,7′-dichlorodihydro-fluorescein diacetate, acetyl ester; TRAP, telomeric repeat amplification protocol; PI, propidiumiodide; SWNTs, single-walled carbon nanotubes

■ REFERENCES

(1) Jung, Y.; Lippard, S. J. Direct cellular responses to platinum-induced DNA damage. *Chem. Rev.* **2007**, *107*, 1387−1407.

(2) Iakovidis, I.; Delimaris, I.; Piperakis, S. M. Copper and its complexes in medicine: a biochemical approach. *Mol. Biol. Int.* **2011**, ID594529.

(3) Marzano, C.; Pellei, M.; Tisato, F.; Santini, C. Copper complexes as anticancer agents. *Anticancer Agents Med. Chem.* **2009**, *9*, 185−211.

(4) Palanimuthu, D.; Shinde, S. V.; Somasundaram, K.; Samuelson, A. G. In vitro and in vivo anticancer activity of copper bis-(thiosemicarbazone) complexes. *J. Med. Chem.* **2013**, *56*, 722−734.

(5) Ruiz-Azuara, L.; Bravo-Gomez, M. E. Copper compounds in cancer chemotherapy. *Curr. Med. Chem.* **2010**, *17*, 3606−3615.

(6) De Vizcaya-Ruiz, A.; Rivero-Muller, A.; Ruiz-Ramirez, L.; Kass, G. E.; Kelland, L. R.; Orr, R. M.; Dobrota, M. Induction of apoptosis by a novel copper-based anticancer compound, casiopeinaII, in L1210 murine leukaemia and CH1 human ovarian carcinoma cells. *Toxicol. in Vitro* **2000**, *14*, 1−5.

(7) Fujibayashi, Y.; Taniuchi, H.; Yonekura, Y.; Ohtani, H.; Konishi, J.; Yokoyama, A. Copper-62-ATSM: a new hypoxia imaging agent with high membrane permeability and low redox potential. *J. Nucl. Med.* **1997**, *38*, 1155−1160.

(8) Buncic, G.; Hickey, J. L.; Schieber, C.; White, J. M.; Crouch, P. J.; White, A. R.; Xiao, Z.; Wedd, A. G.; Donnelly, P. S. Water-soluble bis(thiosemicarbazonato)copper(II) Complexes. *Aust. J. Chem.* **2011**, *64*, 244−252.

(9) Alemon-Medina, R.; Munoz-Sanchez, J. L.; Ruiz-Azuara, L.; Gracia-Mora, I. Casiopeína IIgly induced cytotoxicity to HeLa cells depletes the levels of reduced glutathione and is prevented by dimethyl sulfoxide. *Toxicol. in Vitro* **2008**, *22*, 710−715.

(10) Trejo-Solis, C.; Palencia, G.; Zuniga, S.; Rodriguez-Ropon, A.; Osorio-Rico, L.; Luvia, S. T.; Gracia-Mora, I.; Marquez-Rosado, L.; Sanchez, A.; Moreno-Garcia, M. E.; Cruz, A.; Bravo-Gomez, M. E.; Ruiz-Ramirez, L.; Rodriguez-Enriquez, S.; Sotelo, J. CasIIgly induces apoptosis in glioma C6 cells in vitro and in vivo through caspase-dependent and caspase-independent mechanisms. *Neoplasia* **2005**, *7*, 563−574.

(11) Khodair, A. I. Glycosylation of 2-thiohydantoin derivatives. Synthesis of some novel S-alkylated and S-glucosylated hydantoins. *Carbohydr. Res.* **2001**, *331*, 445−453.

(12) Brown, I. D.; Altermatt, D. Bond-valence parameters obtained from a systematic analysis of the inorganic crystal structure database. *Acta Crystallogr., Sect. B: Struct. Biol.* **1985**, *41*, 244−247.

(13) Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

(14) Chakraborty, A.; Kumar, P.; Ghosh, K.; Roy, P. Evaluation of a Schiff base copper complex compound as potent anticancer molecule with multiple targets of action. *Eur. J. Pharmacol.* **2010**, *647*, 1−12.

(15) Kolb, H. C.; Finn, M. G.; Sharpless, K. B. Click chemistry: diverse chemical function from a few good reactions. *Angew. Chem., Int. Ed. Engl.* **2001**, *40*, 2004−2021.
(16) Negoescu, A.; Lorimier, P.; Labat-Moleur, F.; Drouet, C.; Robert, C.; Guillermet, C.; Brambilla, C.; Brambilla, E. In situ apoptotic cell labeling by the TUNEL method: improvement and evaluation on cell preparations. *J. Histochem. Cytochem.* **1996**, *44*, 959−968.
(17) Eruslanov, E.; Kusmartsev, S. Identification of ROS using oxidized DCFDA and flow-cytometry. In *Advanced Protocols in Oxidative Stress II*; Armstrong, D., Ed.; Humana Press: Totowa, NJ, 2010; Vol. *594*, pp 57−72.
(18) Barone, G.; Terenzi, A.; Lauria, A.; Almerico, A. M.; Leal, J. M.; Busto, N.; García, B. DNA-binding of nickel(II), copper(II) and zinc(II) complexes: structure−affinity relationships. *Coord. Chem. Rev.* **2013**, *257*, 2848−2862.
(19) Palchaudhuri, R.; Hergenrother, P. J. DNA as a target for anticancer compounds: methods to determine the mode of binding and the mechanism of action. *Curr. Opin. Biotechnol.* **2007**, *18*, 497−503.
(20) Artese, A.; Costa, G.; Distinto, S.; Moraca, F.; Ortuso, F.; Parrotta, L.; Alcaro, S. Toward the design of new DNA G-quadruplex ligands through rational analysis of polymorphism and binding data. *Eur. J. Med. Chem.* **2013**, *68*, 139−149.
(21) Wang, J.; Chen, Y.; Ren, J.; Zhao, C.; Qu, X. G-Quadruplex binding enantiomers show chiral selective interactions with human telomere. *Nucleic Acids Res.* **2014**, *42*, 3792−802.
(22) Zvereva, M. I.; Shcherbakova, D. M.; Dontsova, O. A. Telomerase: structure, functions, and activity regulation. *Biochemistry (Moscow)* **2010**, *75*, 1563−1583.
(23) Kim, N. W.; Piatyszek, M. A.; Prowse, K. R.; Harley, C. B.; West, M. D.; Ho, P. L.; Coviello, G. M.; Wright, W. E.; Weinrich, S. L.; Shay, J. W. Specific association of human telomerase activity with immortal cells and cancer. *Science* **1994**, *266*, 2011−2015.
(24) Rha, S. Y.; Izbicka, E.; Lawrence, R.; Davidson, K.; Sun, D.; Moyer, M. P.; Roodman, G. D.; Hurley, L.; Von Hoff, D. Effect of telomere and telomerase interactive agents on human tumor and normal cell lines. *Clin. Cancer Res.* **2000**, *6*, 987−993.
(25) Chen, Y.; Qu, K.; Zhao, C.; Wu, L.; Ren, J.; Wang, J.; Qu, X. Insights into the biomedical effects of carboxylated single-wall carbon nanotubes on telomerase and telomeres. *Nature Commun.* **2012**, *3*, 1074.
(26) Valko, M.; Morris, H.; Cronin, M. T. Metals, toxicity and oxidative stress. *Curr. Med. Chem.* **2005**, *12*, 1161−208.
(27) Leite, E. A.; Lana, A. M.; Junior, A. D.; Coelho, L. G.; De Oliveira, M. C. Acute toxicity study of cisplatin loaded long-circulating and pH-sensitive liposomes administered in mice. *J. Biomed. Nanotechnol.* **2012**, *8*, 229−239.
(28) Majouga, A. G.; Beloglazkina, E. K.; Vatsadze, S. Z.; Frolova, N. A.; Zyk, N. V. Synthesis of isomeric 3-phenyl-5-(pyridylmethylene)-2-thiohydantoins and their S-methylated derivatives. Molecular and crystal structures of (5*Z*)-3-phenyl-5-(pyridin-2-ylmethylene)-2-thiohydantoin and (5*Z*)-2-methylthio-3-phenyl-5-(pyridin-2-ylmethylene)-3,5-dihydro-4*H*-imidazol-4-one. *Russ. Chem. Bull.* **2004**, *53*, 2850−2855.