Highly water-soluble cyclopentadienyl and indenyl molybdenum(II) complexes: second generation of molybdenum-based cytotoxic agents.

Ondřej Mrózek,[a] Lucie Šebestová,[b] Jaromír Vinklárek,*[a] Martina Řezáčová,[c] Aleš Eisner,[b] Zdeňka Růžičková,[a] and Jan Honzíček[d]

Abstract: A series of the cyclopentadienyl and indenyl molybdenum compounds bearing alkyl ammonium functions [(η^5-Cp')Mo(CO)$_2$(^{N,N}L)][BF$_4$]$_2$ were synthesized and characterized by the analytical and spectroscopic methods. Structures of [{η^5-$C_5H_4CH_2CH_2NH(CH_2)_5$}Mo(CO)$_2$(4,7-Ph$_2$-phen)][BF$_4$]$_2$ and [(η^5-$C_9H_6CH_2CH_2NHMe_2$)Mo(CO)$_2$(phen)][BF$_4$]$_2$ were determined by the X-ray crystallography. All synthesized compounds exhibit increased activity against human leukemia cell lines MOLT-4 and HL-60. They are about one order of magnitude more active than cisplatin. This study has proven that modification of the outer coordination sphere of molybdenum complexes has a strong impact on their activity and may be successfully used for a design of novel highly cytotoxic active species.

Introduction

Despite our constantly deepening knowledge about pathobiochemical mechanisms of various haematological malignancies, many leukemias remain incurable with very bad survival prognosis. Although targeted therapy, such as tyrosin kinase inhibitors or specific monoclonal antibodies, was a great revolution for the therapy of some types of leukemias, others rely fully on classical chemotherapy.[1] For example, the core of treatment regimen of acute myeloid leukemia (variations on a theme of cytosine arabinoside combined with an anthracycline or anthracenedione) remains nearly unchanged for 40 years, and the prognosis remains poor, mainly in the elderly patients.

[a] O. Mrózek, J. Vinklárek, Z. Růžičková
Department of General and Inorganic Chemistry,
Faculty of Chemical Technology, University of Pardubice
Studentská 573, 532 10 Pardubice, Czech Republic
E-mail: jaromir.vinklarek@upce.cz
[b] L. Šebestová, A. Eisner
Department of Analytical Chemistry
Faculty of Chemical Technology, University of Pardubice
Studentská 573, 532 10 Pardubice, Czech Republic
[c] M. Řezáčová
Department of Medical Biochemistry, Faculty of Medicine in Hradec Králové, Charles University in Prague
Šimkova 870, 500 01 Hradec Králové, Czech Republic
[d] J. Honzíček
Institute of Chemistry and Technology of Macromolecular Materials
Faculty of Chemical Technology, University of Pardubice
Studentská 573, 532 10 Pardubice, Czech Republic

Supporting information for this article is given via a link at the end of the document.

Therefore, novel cytostatics are constantly researched, which would be associated with less undesirable side-effects while maintaining potent antitumor activity.[2] Transition metal complexes and organometallic compounds attract considerable attraction of biochemists and farmacochemists since cytostatic properties of cisplatin (*cis*-[PtCl$_2$(NH$_3$)$_2$]; **DDP**) were discovered by Rosenberg.[3] In past decades, they scrutinized various transition metal compounds and found many structural patterns with enhanced activity toward cisplatin resistant tumor cells.[4] Nevertheless, quest for new highly active species with reduced side-effects is still ongoing. Our investigation follows the fundamental work of Romão *et al.*, who established the allyl [(η^3-C_3H_5)Mo(CO)$_2$L$_2$Br], cyclopentadienyl and indenyl molybdenum compounds [(η^5-Cp')Mo(CO)$_2$L$_2$][BF$_4$] (Cp': Cp = C_5H_5, Ind = C_9H_7), where L$_2$ is *N,N*-, *S,S*- or *P,P*-chelating ligand, as new class of highly cytotoxic species against several tumor cell lines.[5] Following studies have extended the series of cytotoxic active compounds [6] and brought an early insight into mechanism of their action.[7] The cytotoxicity of the molybdenum compounds seems to correlate mainly with nature of coordinated chelating ligand while the substitution in the π-coordinated ligand play only minor role.[8, 9] High activity was observed for complexes baring 1,10-phentanthroline (phen) and its 4,7-diphenyl derivative (4,7-Ph$_2$-phen). However, main drawback of these complexes lay in their poor solubility in water that embarrasses their pharmaceutical application.

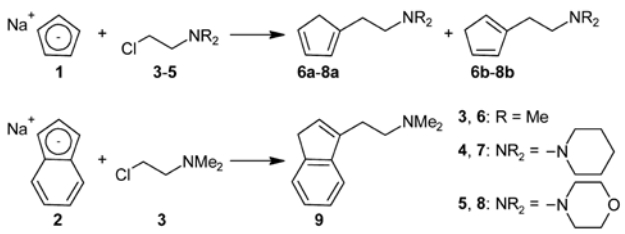
The aim of this study is to modify the outer coordination sphere of molybdenum(II) compounds in the attempt to improve their water solubility. The substitution in cyclopentadienyl ligand with hydrophilic functions seems to be very suitable approach for this purpose since the coordination sphere of central metal stays unchanged. This work describes the assembly of species bearing ammonium salt of *tert.* amines in cyclopentadienyl and indenyl ligand. Cytotoxic properties of these derivatives were examined *in vitro* on two human leukemia cell lines.

Results and Discussion

Synthesis of allyl molybdenum precursors

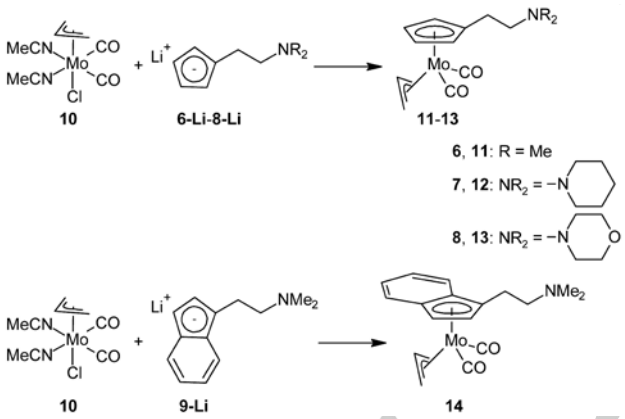
Tert. amine-functionalized cyclopentadienes, $C_5H_5CH_2CH_2NR_2$ (**6**: R = Me; **7**: NR$_2$ = N(CH$_2$)$_5$; **8**: NR$_2$ = N(CH$_2$)$_4$O), and indene, $C_9H_7CH_2CH_2NMe_2$ (**9**), were synthesized using reaction of appropriate alkyl chloride (**3**–**5**) with sodium cyclopentadienide (**1**) and sodium indenide (**2**), respectively (Scheme 1). This synthetic strategy was used previously several times [10-13] but the use of freshly distilled free amines (**3**–**5**) enhances the yields

that enables to synthesize reasonable quantities of these ligands and facilitates the exploration of corresponding coordination chemistry.



Scheme 1. Synthesis of substituted cyclopentadienes **6**–**8** and indene **9**.

Lithium cyclopentadienides **6-Li**–**8-Li**, prepared by deprotonation of cyclopentadienes **6**–**8** with *n*-BuLi, react with chloride complex [(η^3-C_3H_5)Mo(CO)$_2$(NCMe)$_2$Cl] (**10**) to give cyclopentadienyl complexes [(η^3-C_3H_5)(η^5-$C_5H_4CH_2CH_2NR_2$)Mo(CO)$_2$] (**11**: R = Me; **12**: NR_2 = $N(CH_2)_5$; **13**: NR_2 = $N(CH_2)_4O$), see Scheme 2. Indenyl complex [(η^3-C_3H_5)(η^5-$C_9H_6CH_2CH_2NMe_2$)Mo(CO)$_2$] (**14**) was prepared accordingly starting from indene **9**.



Scheme 2. Synthesis of molybdenum compounds **11**–**14**.

Infrared spectra of compounds **11**–**14** show two CO stretching bands in the range typical for terminal carbonyl ligands, see Table 1. Higher wavenumbers observed for indenyl derivative **14** (ν_a = 1933 cm^{-1}, ν_s = 1852 cm^{-1}) reflect lower electron density on central metals that is caused weaker donor properties of the indenyl ligand. ^{1}H NMR spectra show two sets of signals for allyl ligand that reveals presence of two conformations of this ligand in solution. At room temperature, conformer with allyl ligand eclipsing OC–Mo–CO moiety (*exo*) predominates in all compounds under the study. This observation is in line with literature data reported for unsubstituted analogues.[14] Compounds **11**–**13** show two broadened signals at ~5.15 ppm (2 × 2H) those were assigned to protons of η^5-coordinated cyclopentadienyl ring. In compound **14**, protons from C_5 ring of η^5-coordinated indenyl ligand appear as two sets of doublets at ~5.8 ppm and ~5.6 ppm [$^3J(^1H,^1H)$ = 2.7 Hz], and were assigned to given conformers. The pattern of ^{1}H NMR spectra further prove that pendant amine arm in compounds **11**–**14** is not coordinated to molybdenum that satisfies the 18e rule.

Table 1. Infrared data of molybdenum compounds **11**–**14**.[a]

	ν_a(CO)	ν_s(CO)		ν_a(CO)	ν_s(CO)
11	1920	1827	**13**	1930	1839
12	1929	1844	**14**	1933	1852

[a] Wavenumbers are given in cm^{-1}.

Crystal structure of the compound **12** was determined by the X-ray diffraction analysis (see Figure 1). The molecule has a distorted tetrahedral structure with η^3-allyl, η^5-cyclopentadienyl and two carbonyl ligands around molybdenum in formal oxidation state II. Allyl ligand adopts *exo*-conformation. The geometric parameters, describing the coordination sphere of molybdenum, are given in caption of Figure 1. Large bond angle Cg(allyl)–Mo–Cg(Cp) [128.3(1)°] and small bond angle C(CO)–Mo–C(CO) [80.2(1)°] represent the largest deviations from the ideal tetrahedron. Nevertheless, these values are in line with the data reported for similar complexes bearing a substituent in the cyclopentadienyl ring.[15]

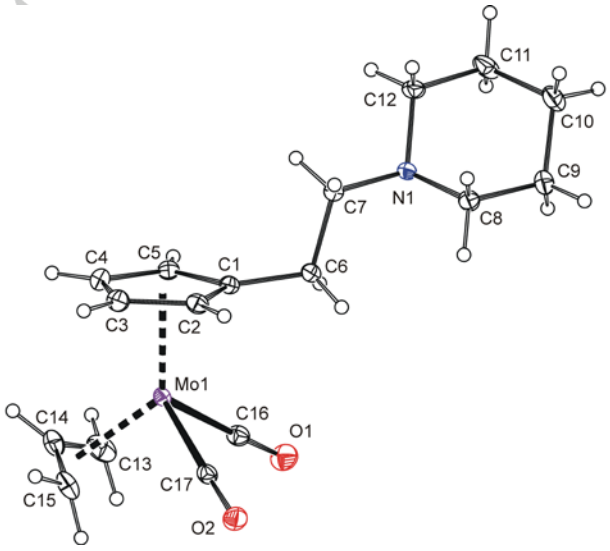
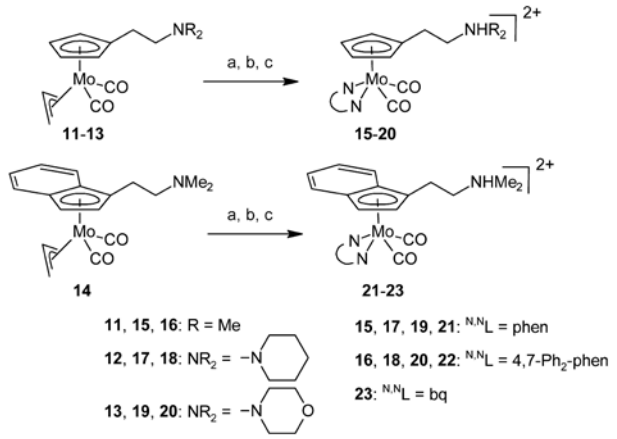


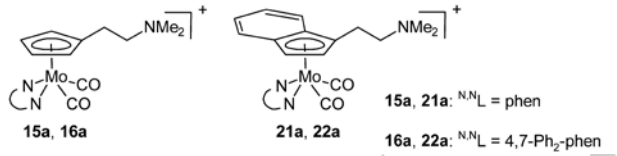
Figure 1. ORTEP drawing of [(η^3-C_3H_5){η^5-$C_5H_4CH_2CH_2N(CH_2)_5$}Mo(CO)$_2$] (**12**). The labeling scheme for all non-hydrogen atoms is shown. Thermal ellipsoids are drawn at the 30% probability level. Selected bond lengths (Å) and angles (°): Mo1–Cg(C1-C5) 2.019(1), Mo1–Cg(C13-C15) 2.047(2), Mo1–C16 1.943(2), Mo1–C17 1.950(2), Cg(C1-C5)–Mo1–Cg(C13-C15) 128.3(1), C16–Mo1–C17 80.2(1).

Synthesis of complexes with *N,N*-chelating ligands

The molybdenum complexes bearing *N,N*-chelating ligands **15**–**23** were prepared according to Scheme 3 following a route developed for the analogues without function group in the Cp ring.[8, 16] Treatment of the starting complexes **11**–**14** with HBF_4, in presence of a coordinating solvent, leads to protonation of allyl ligand. Following ligand-exchange gives unstable acetonitrile intermediates. These species were not isolated but only washed to remove the excess of the acid and the boron-based impurities. The treatment with *N,N*-chelating ligands followed with a protonation of the pendant arm gives dicationic complexes **15**–**23**.



Scheme 3. Synthesis of molybdenum compounds bearing *N,N*-chelating ligands. a) $HBF_4{\cdot}Et_2O$ (2 eq.)/MeCN, b) $^{N,N}L/CH_2Cl_2$ c) $HBF_4{\cdot}Et_2O$ (1 eq.)/MeCN.



Scheme 4. Recognized monocationic intermediates.

One could expect that the acidification after the treatment with *N,N*-chelating ligand is unnecessary since the pendant arm should be protonated already in the first reaction step. However, our experiments on $Me_2NCH_2CH_2$-functionalized compounds **11** and **14** reveal the appearance of the products without protonated arms (**15a**, **16a**, **21a** and **22a**), see Scheme 4, when the last reaction step was omitted. These species were recognized by ^1H NMR spectroscopy due to the absence of spin-spin interaction between protons of ammonium group and protons from the neighboring methyl [$^3J(^1H,^1H)$ ~ 5.2 Hz] and methylene groups [$^3J(^1H,^1H)$ ~ 5.5 Hz]. Remaining patterns of the NMR spectra were virtually the same. We suggest that the appearance of the monocationic compounds **15a**, **16a**, **21a** and **22a** could be clarified by coordination of the pendant arm in acetonitrile intermediates but these species were not isolated and satisfactorily characterized owing to low stability.

The infrared spectra of compounds **15**–**23** show two CO stretching bands at ~1960 cm^{-1} (ν_a) and ~1890 cm^{-1} (ν_s), see Table 2. The considerably higher values of carbonyl ligand stretching frequencies, when compared to the neutral precursors **11**–**14**, is due to a decreasing back donation from molybdenum to carbonyl ligands in the cationic complexes. The NMR spectroscopic measurements reveal that cyclopentadienyl complexes **15**–**20** have C_s-symmetric structure. The protons of η^5-coordinated cyclopentadienyl ligands show typical AA'BB' type pattern, in which the signals appear as two pseudotriplets at ~5.9 ppm and ~5.8 ppm [$^3J(^1H,^1H)$ ~ $^4J(^1H,^1H)$ ~ 2.2 Hz]. Lower overall symmetry of indenyl complexes **21**–**23** results in a more complex pattern of the NMR spectra. Nevertheless, η^5-coordination mode of the indenyl ligand was evidenced from signal of proton H^2 that appears at higher field (~5.7 ppm) than remaining protons of indenyl framework (H^{3-7}).[17]

Table 2. Infrared data of complexes bearing *N,N*-chelating ligands.[a]

	ν_a(CO)	ν_s(CO)		ν_a(CO)	ν_s(CO)
15	1968	1887	**20**	1971	1900
16	1967	1887	**21**	1963	1888
17	1974	1894	**22**	1966	1891
18	1971	1903	**23**	1951	1878
19	1959	1902			

[a] Wavenumbers are given in cm^{-1}.

The assembly of complex cations **15**–**23** was further supported by ESI mass spectrometry. These compounds show peaks of $[M - H]^+$ in positive-ion mode. The absence of parent dicationic species $[M]^{2+}$ is not surprising since deprotonation of ammonium group from the pendant arm is obvious acid-basic reaction. Nevertheless, some dications (**15**–**18**, **21**–**23**) were stabilized by acetonitrile as evidenced from observed intensive peaks of $[M + MeCN]^{2+}$.

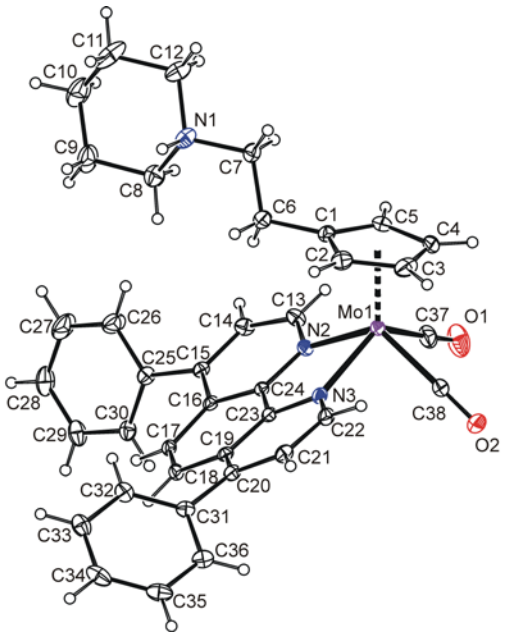


Figure 2. ORTEP drawing of dication [{η^5-$C_5H_4CH_2CH_2NH(CH_2)_5$}$Mo(CO)_2$(4,7-Ph_2-phen)]$^{2+}$ present in crystal structure of **18**. The labeling scheme for all non-hydrogen atoms is shown. Thermal ellipsoids are drawn at the 30% probability level. Selected bond lengths (Å) and angles (°): Mo1–Cg(C1-C5) 1.993(2), Mo1–C37 1.976(4), Mo1–C38 1.975(3), Mo1–N2 2.178(2), Mo1–N3 2.179(3), C37–Mo1–C38 75.7(2), N2–Mo1–N3 73.3(1).

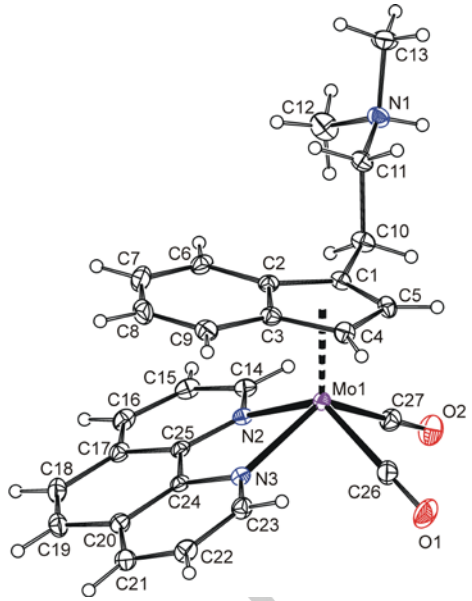


Figure 3. ORTEP drawing of dication [(η^5-$C_9H_6CH_2CH_2NHMe_2$)$Mo(CO)_2$(phen)]$^{2+}$ present in crystal structure of **21·phen**. The labeling scheme for all non-hydrogen atoms is shown. Thermal ellipsoids are drawn at the 30% probability level. Selected bond lengths (Å) and angles (°): Mo1–Cg(C1-C5) 1.992(1), Mo1–C26 1.967(2), Mo1–C27 1.973(2), Mo1–N2 2.200(2), Mo1–N3 2.199(2), C26–Mo1–C27 76.8(1), N2–Mo1–N3 73.5(1).

The solid state structures of the compounds **18** and **21·phen** were determined by X-ray diffraction analysis. The dicationic complex species have a square-pyramidal coordination sphere of molybdenum(II) with η^5-coordinated cyclopentadienyl (**18**) or indenyl ligand (**21·phen**) in the apical position. The basal plane is occupied with two carbonyl ligands in *cis*-configuration and the nitrogen donor atoms of *N,N*-chelating ligand. The bond lengths and the bond angles, describing the environment of molybdenum, are very similar to related 2,2'-bipyridine complexes [(η^5-$C_5H_4CH_2C_6H_4OMe$)$Mo(CO)_2$(bpy)][BF_4], [{η^5-$C_5H_4CH_2C_6H_2(OMe)_3$}$Mo(CO)_2$(bpy)][BF_4], [(η^5-Ind)$Mo(CO)_2$(bpy)][BF_4].[8] It reveals that modification of cyclopentadienyl and indenyl ligands with alkyl ammonium substituents has a little effect on the inner coordination.

The cation of **21** and phenanthroline are connected through intermolecular hydrogen bonds between hydrogen atom of ammonium group and nitrogen atoms of phenanthroline molecule. The distances between nitrogen atoms were found to be 3.012(3) Å and 2.909(3) Å for N1···N4 and N1···N5, respectively.

Cytotoxicity of chelate complexes

Using procedures described previously,[18] molybdenum compounds **15**–**23** were tested for their cytotoxic effects on human leukemia cell lines MOLT-4 and HL-60. The half maximal inhibitory concentration (IC_{50}) values are listed in Table 3. The cytotoxicity curves showing the effect on the viability are given in the Supporting Information (Figs. S1–S9).

Table 3. Cytotoxicity data of complexes bearing *N,N*-chelating ligands.[a]

	MOLT-4	HL-60		MOLT-4	HL-60
15	2.4 ± 0.1	6.9 ± 0.7	**20**	2.2 ± 0.1	2.3 ± 0.3
16	6.6 ± 0.6	8.3 ± 0.5	**21**	2.0 ± 0.1	6.1 ± 0.9
17	3.7 ± 0.7	6.1 ± 0.5	**22**	1.4 ± 0.1	1.5 ± 0.2
18	1.2 ± 0.1	3.4 ± 0.2	**23**	1.2 ± 0.1	2.6 ± 0.3
19	4.6 ± 0.5	5.4 ± 0.4	**DDP** [b]	15.8 ± 1.9	11.3 ± 2.5

[a] IC_{50} values are given in µmol/l. [b] Data published elsewhere.[19]

All the compounds under the study (**15**–**23**) are highly active against both cell lines. Their IC_{50} values are much lower than reported for **DDP**, see Table 3. The considerable increase of the cytotoxicity is a result modification of the cyclopentadienyl compounds since the unsubstituted parent, [(η^5-Cp)$Mo(CO)_2$(phen)][BF_4], is only medium active against leukemic cells MOLT-4 (IC_{50} = 19.9 ± 0.7 µmol/l;[8] *cf.* with **15**–**20** in Table 3). The parent indenyl compound, [(η^5-Ind)$Mo(CO)_2$(phen)][BF_4], is more effective [IC_{50} (MOLT-4) = 4.9 ± 0.7 µmol/l] [8] but even here the modification leads to the species with about 2.5 times higher cytotoxicity (see **21**–**23** in Table 3). Very similar cytotoxic behavior of the species under

the study suggests that the increased hydrophilicity plays a very important role in the transport of the active species into the diseased cell that seem to be crucial for effective cytotoxic action of the molybdenum compounds. The exchange of 1,10-phenanthroline with larger π-systems (*e.g.* 4,7-Ph_2-phen or bq) has negligible effect on activity that contrasts with the previous studies on the species without polar function groups in the cyclopentadienyl or indenyl ligand.[8, 9] It is probably a result of much lower effect of the chelating ligand on physical properties of the complexes species when highly the polar function group is attached in the cyclopentadienyl or indenyl ligand.

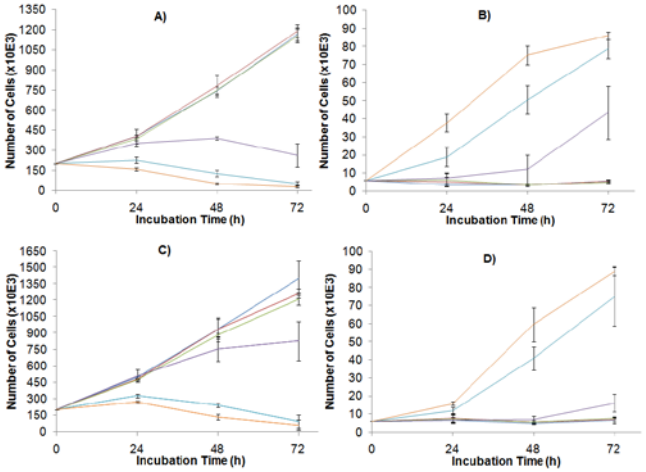


Figure 4. The effect of the complex **20** on the proliferation and viability of leukemia cells during 3 days after the treatment with tested compound. Concentration of **20**: 1 µmol/l (brown line), 2 µmol/l (green line), 3 µmol/l (purple line), 5 µmol/l (blue line), 7 µmol/l (orange line), control (dark blue line). A) The proliferation of the MOLT-4 cells, B) the viability of the MOLT-4 cells, C) the proliferation of the HL-60 cells, D) the viability of the HL-60 cells. The results are taken from three independent experiments.

Highly efficient compound **20** was subject of deeper biological investigation to reveal more information about the mechanism of the cytotoxic effect. The mechanism was studied using the flow cytometry, spectral methods and the Western blotting in combination with the SDS-PAGE and the immunochemical detection on the cell lines MOLT-4 and HL-60. Obtained IC_{50} values were used to determine the optimal concentrations for the follow-up experiments.

The flow cytometry measurements reveal that the low concentrations of the complex **20** (1 and 2 µmol/l) have negligible effect on the cells MOLT-4 during the whole experiment; see Figs. 4 A and B. A considerable cytotoxicity was observed for concentration 3 µmol/l after 48 h incubation with compound **20**, which is accompanied with increased percentage of dead cells. The increasing concentration of the complex **20** and prolonged incubation time lead to increase in the cytotoxicity. At concentrations 5 and 7 µmol/l, the viability decreases shortly after the treatment by the compound **20**.

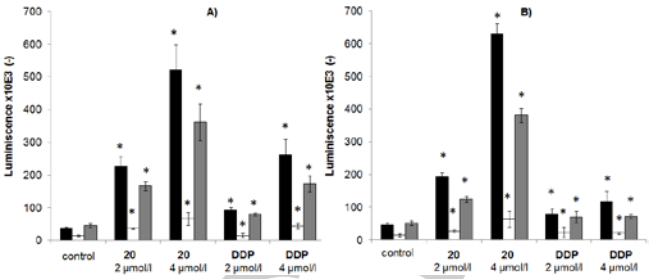


Figure 5. The activity of caspases 3/7 (black), caspases 8 (white) and caspases 9 (grey) determined 24 h after application of **20** and **DDP** (2 and 4 µmol/l) on the leukemia cells. A) Cell line MOLT-4, B) cell line HL-60. * Significantly different from control ($P < 0.05$). The results were taken from three independent experiments.

Figs. 4 C and D exhibit the cytostatic behavior of the compound **20** on the leukemia cells HL-60. The effect on viability and proliferation is similar as described for the cell line MOLT-4. Hence, the proliferation is inhibited at the concentration 3 µmol/l and the number of dead cells increases during the time as well. Application of the higher concentrations (5 and 7 µmol/l) leads to considerable decrease in proliferation and viability shortly after the application. In accordance with the WST-1 assay, the complex **20** exhibits higher cytotoxic effect toward the MOLT-4 than toward HL-60 cells.

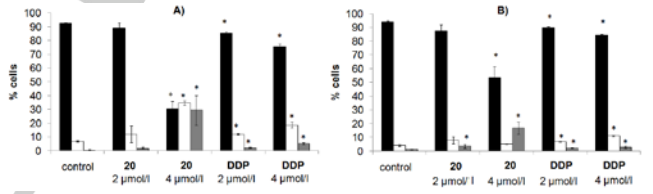


Figure 6. The effect of **20** and **DDP** on apoptosis induction in leukemia cells 24 h after the treatment with tested compound determined by flow cytometry. The percentage of viable (black), late-apoptotic (white) and late-apoptotic cells (grey) is shown. A) Cell line MOLT-4, B) cell line HL-60. * Significantly different from control ($P < 0.05$). The results were taken from three independent experiments.

Measuring of activity of the caspases 3/7, 8 and 9 confirmed that the apoptotic process of the cells MOLT-4 (Fig. 5 A) as well as HL-60 (Fig. 5 B) is induced by treatment with the complex **20**. The application of the tested compound (2 and 4 µmol/l) on these leukemia cells caused the significant dose dependent increase in the activity of the effector caspase 3/7 and the initiator caspases 8 and 9. This behavior seems to be similar to **DDP**, which mechanism of action has been described in literature.[20,21] After the purine bases to the DNA are damaged by **DDP**, the inner pathway activation of apoptosis in tumor cell is induced. Beside the inner induction (mitochondrial pathway) the FasL mRNA expression is induced. This one starts extrinsic pathway of apoptosis. The antitumor effect of the **DDP** is also described by increased oxidative stress and calcium efflux from mitochondria.[20,21] Our measurements reveal that cytotoxic effect of the complex **20** is more powerful than observed for **DDP**.

Furthermore, apoptotic process is predominantly induced via mitochondrial-dependent apoptosis-inducing pathway (caspase 9) and followed by activation of effector caspases 3 and 7 on the both cell lines.

The apoptosis was further cross verified by the flow cytometry detection using the Annexin V and PI staining on the MOLT-4 (Fig. 6 A) and HL-60 cells (Fig. 6 B). On the cell line MOLT-4, no significant change in number of apoptotic cells occurs after 24 h incubation with the complex **20** at concentration level 2 µmol/l. The noticeable decrease in the amount of viable cells appears at concentration 4 µmol/l, where a considerable growth of early-apoptotic and late-apoptotic cells was observed. The **DDP** induces the significant apoptotic changes of the cells MOLT-4 already at concentration 2 µmol/l after 24 h incubation. However, the increase of concentration up to 4 µmol/l does not cause further considerable apoptotic changes. The experiments on the cell line HL-60 bring similar result as described for MOLT-4 cells. Hence, **DDP** seems to be more active than compound **20** at concentration level 2 µmol/l. Nevertheless, at concentration 4 µmol/l, the compound **20** induces stronger apoptotic effect than **DDP**. The comparison of the apoptotic induction verified that the HL-60 cell line is more resistant toward the treatment with the complex **20** that the MOLT-4.

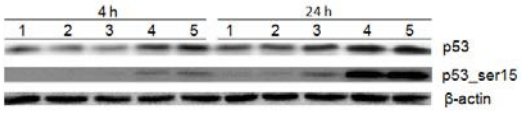


Figure 7. Induction and activation of the p53 in the MOLT-4 cells exposed to tested compounds for 4 and 24 h. 1) Control, 2) 2 µmol/l of **20**, 3) 4 µmol/l of **20**, 4) 2 µmol/l of **DDP**, 5) 4 µmol/l of **DDP**. p53_ser15: p53 phosphorylated at the serine 15. To confirm equal protein loading membranes were reincubated with β-actin. Representative results of one of three independent experiments are shown.

Follow-up experiments reveal that response of the MOLT-4 cells on the treatment with the complex **20** and **DDP** includes changes in expression of the p53 protein and its form phosphorylated at the serine 15 (p53_ser15); see Fig. 7. The quantity of a β-actin was monitored as a verification of the equal protein loading. The noticeable increase of the p53 level occurs 4 h after the treatment with the compound **20** and it rises with the concentration of tested compound. The level of p53 remains elevated during the whole experiment (24 h). Additionally, it was proved that the p53 protein was phosphorylated at the serine 15. This modification is responsible for stability and accumulation of the p53 protein in the cell. The expression of the p53_ser15 increases with the rising concentration of the complex **20** and with the length of the experiment. The p53 protein is a transcription factor involved in response to DNA damage and in subsequent induction of DNA repair, cell cycle arrest and apoptosis. Its level in the cell is regulated through posttranslational modifications interfering with p53 degradation and translocation. The phosphorylation of ser15 is crucial function of p53. Hence, it inhibits the interaction with ubiquitin ligase Mdm2 and protects the protein p53 from ligation to ubiquitin, stabilizes it [21] and extends its half-life.[22]

Although the complex **20** causes the significant changes of the p53 protein on the MOLT-4 cells, the cell cycle stays unchanged for both leukemia cells under the study (MOLT-4 and HL-60), see Fig. 8. The increase in expression of the p53 protein and his phosphorylated form p53_ser15 was monitored on the **DDP** as well. However, the relevant changes in the cell cycle were not observed using the same concentration of the **DDP** as in case of complex **20** (2 and 4 µmol/l).

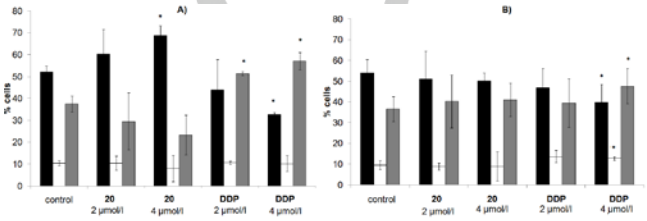


Figure 8. The cell cycle analysis of the apoptosis induction in the leukemia cells 24 h after the treatment with the **20** and **DDP** determined by flow cytometry with propidium iodide. The percentage of cells in G1 (black), G2 (white) and S phase (grey) is shown. The results were taken from three independent experiments. A) Cell line MOLT-4, B) cell line HL-60. * Significantly different from control ($P < 0.05$).

Conclusions

Here reported complexes **15**–**23** represent a second generation of molybdenum-based cytotoxic agents with increased water solubility. The synthetic part of this study describes the pathway toward functionalized molybdenum compounds together with detailed analytical and spectroscopic characterization. Structures of one key intermediate (**12**) and two final products (**18** and **21**) were elucidated by single crystal X-ray diffraction analysis.

The functionalization of the outer coordination sphere of molybdenum(II) compounds with ammonium functions has strong impact on cytotoxicity. All compounds, under the study, are about one order magnitude more active against leukemia cells MOLT-4 and HL-60 than **DDP**. The effect of the highly efficient compound **20** on the leukemia cells was studied in detail. It was revealed that this molybdenum(II) compound induces apoptotic process in cell lines MOLT-4 and HL-60. The apoptosis is activated via the inner apoptotic pathway by the caspase 9 activation. The complex **20** causes an up-regulation of the p53 protein and its phosphorylated form on the serine 15. This complex seems to be more effective than **DDP** and it should undergo further scrutiny as a potential cytostatic agent.

Experimental Section

Methods and materials. All operations were performed under nitrogen using conventional Schlenk-line techniques. The solvents were purified and dried by standard methods.[23] Starting materials were available

commercially or prepared according to literature procedures: [(η^3-C_3H_5)Mo(CO)$_2$(NCMe)$_2$Cl] (**10**).[14]

Measurements. Infrared spectra were recorded in the 4000–400 cm^{-1} region with a Nicolet Magna 6700 FTIR spectrometer using a diamond smart orbit ATR. ^{1}H NMR spectra were measured at 300 K on a Bruker 400 Avance spectrometer. Chemical shifts are given in ppm relative to the external standard (TMS). Deuterated solvents were used as obtained (Acros Organics) without further purification. Mass spectrometry was performed with a quadruple mass spectrometer (LCMS 2010, Shimadzu, Japan). The sample was injected into the mass spectrometer with infusion mode at a constant flow rate of 10 µl/min. Electrospray ionization-mass spectrometry (ESI-MS) was used for the identification of analyzed samples.

Synthesis of ClCH$_2$CH$_2$NMe$_2$ (3). Potassium hydroxide (15 g, 0.27 mol) was dissolved in distilled water (150 ml) and treated with [ClCH$_2$CH$_2$NHMe$_2$]Cl (18.7 g, 0.13 mol). The mixture was extracted with pentane (4 × 75 ml). The combined organic phases were dried with magnesium sulfate. Solvent was removed by distillation at normal pressure. Product was vacuum distilled at 40°C (60 Torr). Yield: 7.6 g (71 mmol; 54%). Colorless liquid. Analytical and spectroscopic data are in line with those published elsewhere.[24]

Synthesis of ClCH$_2$CH$_2$N(CH$_2$)$_5$ (4). Potassium hydroxide (15 g, 0.27 mol) was dissolved in distilled water (150 ml) and treated with [ClCH$_2$CH$_2$NH(CH$_2$)$_5$]Cl (24.0 g, 0.13 mol). The mixture was extracted with hexane (4 × 75 ml). The combined organic phases were dried with magnesium sulfate. Solvent was removed by distillation at normal pressure. Product was vacuum distilled at 65°C (15 Torr). Yield: 8.8 g (60 mmol; 46%). Colorless liquid. Analytical and spectroscopic data are in line with those published elsewhere.[24]

Synthesis of ClCH$_2$CH$_2$N(CH$_2$)$_4$O (5). The reaction was carried out as was described for compound **4**, but with [ClCH$_2$CH$_2$NH(CH$_2$)$_4$O]Cl (24.2 g, 0.13 mol). Product was vacuum distilled at 80°C (15 Torr). Yield: 11.5 g (77 mmol; 59%). Colorless liquid. Analytical and spectroscopic data are in line with those published elsewhere.[24]

Synthesis of C$_5$H$_5$CH$_2$CH$_2$NMe$_2$ (6). Sodium cyclopentadienide (**1**; 4.4 g; 50 mmol) was dissolved in THF (150 ml) and cooled at 0°C. ClCH$_2$CH$_2$NMe$_2$ (**3**; 4.95 g, 46 mmol) was added dropwise and the reaction mixture was stirred at room temperature overnight. The mixture was poured into distilled water (75 ml) and crude product was extracted with CH_2Cl_2 and dried over magnesium sulfate. The volatiles were vacuum evaporated and product was vacuum distilled at 70°C (20 Torr). Yield: 3.1 g (23 mmol; 49%). Pale yellow liquid. Analytical and spectroscopic data are in line with those published elsewhere.[12]

Synthesis of C$_5$H$_5$CH$_2$CH$_2$N(CH$_2$)$_5$ (7). The reaction was carried out as was described for compound **7**, but with NaCp (**1**; 4.4 g; 50 mmol), ClCH$_2$CH$_2$N(CH$_2$)$_5$ (**4**; 6.8 g, 46 mmol). Product was vacuum distilled at 60°C (15 Torr). Yield: 4.3 g (24 mmol; 53%). Pale yellow liquid. Analytical and spectroscopic data are in line with those published elsewhere.[11]

Synthesis of C$_5$H$_5$CH$_2$CH$_2$N(CH$_2$)$_4$O (8). The reaction was carried out as was described for compound **7**, but with NaCp (**1**; 4.4 g; 50 mmol), ClCH$_2$CH$_2$N(CH$_2$)$_4$O (**5**; 6.9 g, 46 mmol). Product was vacuum distilled at 60°C (15 Torr). Yield: 3.7 g (21 mmol; 45%). Pale yellow liquid. Analytical and spectroscopic data are in line with those published elsewhere.[13]

Synthesis of C$_9$H$_7$CH$_2$CH$_2$NMe$_2$ (9). The reaction was carried out as was described for compound **7**, but with sodium indenide (**2**; 6.9 g; 50 mmol), ClCH$_2$CH$_2$NMe$_2$ (**3**; 4.95 g, 46 mmol). Product was vacuum distilled at 75°C (12 Torr). Yield: 5.5 g (29 mmol; 64%). Pale yellow liquid. Analytical and spectroscopic data are in line with those published elsewhere.[11]

Synthesis of [(η^3-C$_3$H$_5$)(η^5-C$_5$H$_4$CH$_2$CH$_2$NMe$_2$)Mo(CO)$_2$] (11). C$_5$H$_5$CH$_2$CH$_2$NMe$_2$ (**6**; 0.69 g, 5 mmol) was diluted with 30 ml of THF, cooled at 0°C and treated dropwise with 3.1 ml of *n*-BuLi (1.6 mol l^{-1}). The reaction mixture was stirred for two hours at room temperature and then added dropwise to THF solution of [(η^3-C$_3$H$_5$)Mo(CO)$_2$(NCMe)$_2$Cl] (**10**; 1.55 g, 5 mmol) precooled to –80°C. The reaction mixture was stirred at room temperature overnight and then vacuum evaporated to dryness. The solid residue was extracted with hexane. The vacuum evaporation gives yellow viscous liquid. The product was purified by recrystallization from mixture hexane-ether at –80 °C and then vacuum dried. Yield: 0.78 g (2.4 mmol, 47%). Yellow viscous oil. Calcd for C$_{14}$H$_{19}$MoNO$_2$: C, 51.07; H, 5.82; N, 4.25. Found: C, 51.24; H, 5.60; N, 4.51. ^{1}H NMR (CDCl$_3$; 400 MHz; δ ppm; 4:1 mixture of **11a** (*exo*-C$_3$H$_5$) and **11b** (*endo*-C$_3$H$_5$)): 5.16 (br, 2H of **a** and 2H of **b**, C$_5H_4$), 5.14 (br, 2H of **a** and 2H of **b**, C$_5H_4$), 3.86 (tt, ^3J(^{1}H,^{1}H) = 10.8 Hz, ^3J(^{1}H,^{1}H) = 7.0 Hz, 1H of **a**, *meso* of C$_3H_5$), 3.62 (br, 1H of **b**, *meso* of C$_3H_5$), 2.79 (d, ^3J(^{1}H,^{1}H) = 4.8 Hz, 2H of **b**, *syn* of C$_3H_5$), 2.70 (d, ^3J(^{1}H,^{1}H) = 7.0 Hz, 2H of **a**, *syn* of C$_3H_5$), 2.45–2.35 (m, 4H of **a** and 4H of **b**, CH_2), 2.26 (s, 6H of **a** and 6H of **b**, CH_3), 1.59 (d, ^3J(^{1}H,^{1}H) = 10.6 Hz, 2H of **b**, *anti* of C$_3H_5$), 0.93 (d, ^3J(^{1}H,^{1}H) = 10.8 Hz, 2H of **a**, *anti* of C$_3H_5$). IR(ATR; cm^{-1}): 1920 vs [ν_a(CO)], 1827 vs [ν_s(CO)].

Synthesis of [(η^3-C$_3$H$_5$){η^5-C$_5$H$_4$CH$_2$CH$_2$N(CH$_2$)$_5$}Mo(CO)$_2$] (12). The reaction was carried out as was described for compound **11**, but with C$_5$H$_5$CH$_2$CH$_2$N(CH$_2$)$_5$ (**7**; 0.89 g, 5 mmol) and [(η^3-C$_3$H$_5$)Mo(CO)$_2$(NCMe)$_2$Cl] (**10**; 1.55 g, 5 mmol). Yield: 0.71 g (1.9 mmol, 38%). Yellow powder. Calcd for C$_{17}$H$_{23}$MoNO$_2$: C, 55.29; H, 6.28; N, 3.79. Found: C, 55.42; H, 6.23; N, 3.86. ^{1}H NMR (CDCl$_3$; 400 MHz; δ ppm; 4:1 mixture of **12a** (*exo*-C$_3$H$_5$) and **12b** (*endo*-C$_3$H$_5$)): 5.15 (br, 2H of **a** and 2H of **b**, C$_5H_4$), 5.13 (br, 2H of **a** and 2H of **b**, C$_5H_4$), 3.86 (tt, ^3J(^{1}H,^{1}H) = 10.7 Hz, ^3J(^{1}H,^{1}H) = 7.0 Hz, 1H of **a**, *meso* of C$_3H_5$), 3.62 (br, 1H of **b**, *meso* of C$_3H_5$), 2.79 (d, ^3J(^{1}H,^{1}H) = 5.0 Hz, 2H of **b**, *syn* of C$_3H_5$), 2.69 (d, ^3J(^{1}H,^{1}H) = 7.0 Hz, 2H of **a**, *syn* of C$_3H_5$), 2.45–2.35 (m, 8H of **a** and 8H of **b**, CH_2), 1.58 (m, 4H of **a** and 4H of **b**, CH_2), 1.42 (m, 2H of **a** and 2H of **b**, CH_2), 0.92 (d, ^3J(^{1}H,^{1}H) = 10.7 Hz, 2H of **a**, *anti* of C$_3H_5$). IR(ATR; cm^{-1}): 1929 vs [ν_a(CO)], 1844 vs [ν_s(CO)]. Single crystals suitable for X-ray diffraction analysis were prepared by slow evaporation hexane solution under inert atmosphere.

Synthesis of [(η^3-C$_3$H$_5$){η^5-C$_5$H$_4$CH$_2$CH$_2$N(CH$_2$)$_4$O}Mo(CO)$_2$] (13). The reaction was carried out as was described for compound **11**, but with C$_5$H$_5$CH$_2$CH$_2$N(CH$_2$)$_4$O (**8**; 0.90 g, 5 mmol) and [(η^3-C$_3$H$_5$)Mo(CO)$_2$(NCMe)$_2$Cl] (**10**; 1.55 g, 5 mmol). Yield: 0.73 g (2.0 mmol, 39%). Yellow powder. Calcd for C$_{16}$H$_{21}$MoNO$_3$: C, 51.76; H, 5.70; N, 3.77. Found: C, 51.65; H, 5.57; N, 3.92. ^{1}H NMR (CDCl$_3$; 400 MHz; δ ppm; 4:1 mixture of **13a** (*exo*-C$_3$H$_5$) and **13b** (*endo*-C$_3$H$_5$)): 5.18 (br, 2H of **a** and 2H of **b**, C$_5H_4$), 5.12 (br, 2H of **a** and 2H of **b**, C$_5H_4$), 3.88 (tt, ^3J(^{1}H,^{1}H) = 10.7 Hz, ^3J(^{1}H,^{1}H) = 6.9 Hz, 1H of **a**, *meso* of C$_3H_5$), 3.72 (m, 4H of **a** and 4H of **b**, CH_2), 3.62 (br, 1H of **b**, *meso* of C$_3H_5$), 2.78 (br, 2H of **b**, *syn* of C$_3H_5$), 2.71 (d, ^3J(^{1}H,^{1}H) = 6.9 Hz, 2H of **a**, *syn* of C$_3H_5$), 2.53–2.39 (m, 8H of **a** and 8H of **b**, CH_2), 1.60 (d, ^3J(^{1}H,^{1}H) = 10.1 Hz, 2H of **b**, *anti* of C$_3H_5$), 0.92 (d, ^3J(^{1}H,^{1}H) = 10.7 Hz, 2H of **a**, *anti* of C$_3H_5$). IR(ATR; cm^{-1}): 1930 vs [ν_a(CO)], 1839 vs [ν_s(CO)].

Synthesis of [(η^3-C$_3$H$_5$)(η^5-C$_9$H$_6$CH$_2$CH$_2$NMe$_2$)Mo(CO)$_2$] (14). The reaction was carried out as was described for compound **11**, but with C$_9$H$_7$CH$_2$CH$_2$NMe$_2$ (**9**; 0.94 g, 5 mmol) and [(η^3-

C_3H_5)Mo(CO)$_2$(NCMe)$_2$Cl] (**10**; 1.55 g, 5 mmol). Yield: 0.64 g (1.7 mmol, 34%). Yellow powder. Calcd for $C_{18}H_{21}MoNO_2$: C, 57.00; H, 5.58; N, 3.69. Found: C, 56.88; H, 5.71; N, 3.63. ^{1}H NMR ($CDCl_3$; 400 MHz; δ ppm; 3.3:1 mixture of **13a** (*exo*-C_3H_5) and **13b** (*endo*-C_3H_5)): 7.46–6.89 (m, 4H of **a** and 4H of **b**, C_9H_6), 5.85 (d, $^3J(^1H,^1H)$ = 2.7 Hz, 1H of **a**, C_9H_6), 5.78 (d, $^3J(^1H,^1H)$ = 2.7 Hz, 1H of **b**, C_9H_6), 5.66 (d, $^3J(^1H,^1H)$ = 2.7 Hz, 1H of **a**, C_9H_6), 5.59 (d, $^3J(^1H,^1H)$ = 2.7 Hz, 1H of **b**, C_9H_6), 3.37 (d, $^3J(^1H,^1H)$ = 6.4 Hz, 2H of **b**, *syn* of C_3H_5), 3.29 (m, 1H of **b**, *meso* of C_3H_5), 3.00–2.55 (m, 4H of **a** and 4H of **b**, CH_2), 2.39 (s, 6H of **b**, CH_3), 2.33 (s, 6H of **a**, CH_3), 2.21 (d, $^3J(^1H,^1H)$ = 7.4 Hz, 1H of **a**, *syn* of C_3H_5), 2.18 (d, $^3J(^1H,^1H)$ = 7.4 Hz, 1H of **a**, *syn* of C_3H_5), 0.92 (d, $^3J(^1H,^1H)$ = 11.2 Hz, 1H of **a**, *anti* of C_3H_5), 0.84 (d, $^3J(^1H,^1H)$ = 11.2 Hz, 1H of **a**, *anti* of C_3H_5), 0.17 (tt, $^3J(^1H,^1H)$ = 11.2 Hz, $^3J(^1H,^1H)$ = 7.4 Hz, 1H of **a**, *meso* of C_3H_5), –0.81 (d, $^3J(^1H,^1H)$ = 10.7 Hz, 1H of **b**, *anti* of C_3H_5), –0.85 (d, $^3J(^1H,^1H)$ = 10.7 Hz, 1H of **b**, *anti* of C_3H_5). IR(ATR; cm^{-1}): 1933 vs [ν_a(CO)], 1852 vs [ν_s(CO)].

Synthesis of [(η^5-$C_5H_4CH_2CH_2NHMe_2$)Mo(CO)$_2$(phen)][BF_4]$_2$ (15). [(η^3-C_3H_5)(η^5-$C_5H_4CH_2CH_2NMe_2$)Mo(CO)$_2$] (**11**; 0.23 g, 0.70 mmol) was dissolved in CH_2Cl_2 (5 ml), cooled at 0°C, treated with acetonitrile (1 ml) and then with HBF_4·Et_2O (192 µl, 1.40 mmol). The reaction mixture was stirred for 4 h at room temperature and the solvent was vacuum evaporated. The intermediate was washed several times with ether, vacuum dried, dissolved in CH_2Cl_2 (15 ml) and treated with 1,10-phenanthroline (126 mg, 0.70 mmol). The solution was stirred at room temperature overnight, vacuum evaporated, washed with ether (2 × 10 ml), CH_2Cl_2 (2 × 5 ml), dissolved in MeCN and treated with HBF_4·Et_2O (96 µl, 0.70 mmol). The reaction mixture was stirred for 1 h at room temperature and solvent was vacuum evaporated. The crude product was washed with ether (2 × 10 ml), CH_2Cl_2 (2 × 5 ml), twice recrystallized from the mixture MeCN/Et_2O and vacuum dried. Yield: 0.26 g (4.0 mmol, 58%). Red powder. Calcd for $C_{23}H_{23}B_2F_8MoN_3O_2$: C, 42.96; H, 3.61; N, 6.54. Found: C, 42.82; H, 3.48; N, 6.49. Positive-ion MS (MeCN): *m*/*z* = 256 (100%) [M + MeCN]$^{2+}$, 470 [M – H]$^+$. ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.41 (dd, $^3J(^1H,^1H)$ = 5.4 Hz, $^4J(^1H,^1H)$ = 1.3 Hz, 2H, $C_{12}H_8N_2$, H^{2,9}), 8.80 (dd, $^3J(^1H,^1H)$ = 8.1 Hz, $^4J(^1H,^1H)$ = 1.3 Hz, 2H, $C_{12}H_8N_2$, H^{4,7}), 8.23 (s, 2H, $C_{12}H_8N_2$, H^{5,6}), 7.98 (dd, $^3J(^1H,^1H)$ = 8.1 Hz, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_{12}H_8N_2$, H^{3,8}), 6.85 (br, 1H, N*H*), 5.84 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 5.75 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 3.09 (td, $^3J(^1H,^1H)$ = 8.1 Hz, $^3J(^1H,^1H)$ = 5.7 Hz, 2H, CH_2CH_2NH), 2.69 (d, $^3J(^1H,^1H)$ = 5.3 Hz, 6H, CH_3), 2.05 (t, $^3J(^1H,^1H)$ = 8.1 Hz, 2H, CH_2CH$_2$NH). IR(ATR; cm^{-1}): 1968 vs [ν_a(CO)], 1887 vs [ν_s(CO)], 1030 vs-br [ν_a(BF)].

Synthesis of [(η^5-$C_5H_4CH_2CH_2NHMe_2$)Mo(CO)$_2$(4,7-Ph_2-phen)][BF_4]$_2$ (16). [(η^3-C_3H_5)(η^5-$C_5H_4CH_2CH_2NMe_2$)Mo(CO)$_2$] (**11**; 0.23 g, 0.70 mmol) was dissolved in CH_2Cl_2 (5 ml), cooled at 0°C, treated with acetonitrile (1 ml) and then with HBF_4·Et_2O (192 µl, 1.40 mmol). The reaction mixture was stirred for 4 h at room temperature and the solvent was vacuum evaporated. The intermediate was washed several times with ether, vacuum dried, dissolved in CH_2Cl_2 (15 ml) and treated with 4,7-diphenyl-1,10-phenanthroline (226 mg, 0.70 mmol). The solution was stirred at room temperature overnight, vacuum evaporated, washed with ether (2 × 10 ml), dissolved in CH_2Cl_2 and treated with HBF_4·Et_2O (96 µl, 0.70 mmol). The reaction mixture was stirred for 1 h at room temperature and solvent was vacuum evaporated. The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture MeCN/Et_2O and vacuum dried. Yield: 0.29 g (0.36 mmol, 52%). Red powder. Calcd for $C_{35}H_{31}B_2F_8MoN_3O_2$: C, 52.87; H, 3.93; N, 5.28. Found: C, 53.01; H, 3.95; N, 5.32. Positive-ion MS (MeCN): *m*/*z* = 332 (100%) [M + MeCN]$^{2+}$, 622 [M – H]$^+$. ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.44 (d, $^3J(^1H,^1H)$ = 5.7 Hz, 2H, $C_{12}H_6N_2$, H^{2,9}), 8.15 (s, 2H, $C_{12}H_6N_2$, H^{5,6}), 7.94 (d, $^3J(^1H,^1H)$ = 5.7 Hz, 2H, $C_{12}H_6N_2$, H^{3,8}), 7.67 (m, 10H, C_6H_5), 6.95 (br, 1H, N*H*) 5.90 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 5.80 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 3.15 (td, $^3J(^1H,^1H)$ = 8.0 Hz, $^3J(^1H,^1H)$ = 5.6 Hz, 2H, CH_2CH_2NH), 2.72 (d, $^3J(^1H,^1H)$ = 5.2 Hz, 6H, CH_3), 2.11 (t, $^3J(^1H,^1H)$ = 8.0 Hz, 2H, CH_2CH$_2$NH). IR(ATR; cm^{-1}): 1967 vs [ν_a(CO)], 1887 vs [ν_s(CO)], 1030 vs-br [ν_a(BF)].

Synthesis of [{η^5-$C_5H_4CH_2CH_2NH(CH_2)_5$}Mo(CO)$_2$(phen)][BF_4]$_2$ (17). The reaction was carried out as was described for compound **16**, but with [(η^3-C_3H_5){η^5-$C_5H_4CH_2CH_2N(CH_2)_5$}Mo(CO)$_2$] (**12**; 0.26 g, 0.70 mmol) and 1,10-phenanthroline (126 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture MeCN/Et_2O and vacuum dried. Yield: 0.29 g (0.42 mmol, 61%). Red powder. Calcd for $C_{26}H_{27}B_2F_8MoN_3O_2$: C, 45.72; H, 3.98; N, 6.15. Found: C, 45.78; H, 4.06; N, 6.21. Positive-ion MS (MeCN): *m*/*z* = 276 (100%) [M + MeCN]$^{2+}$, 510 [M – H]$^+$. ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.41 (dd, $^3J(^1H,^1H)$ = 5.4 Hz, $^4J(^1H,^1H)$ = 1.3 Hz, 2H, $C_{12}H_8N_2$, H^{2,9}), 8.80 (dd, $^3J(^1H,^1H)$ = 8.2 Hz, $^4J(^1H,^1H)$ = 1.3 Hz, 2H, $C_{12}H_8N_2$, H^{4,7}), 8.23 (s, 2H, $C_{12}H_8N_2$, H^{5,6}), 7.98 (dd, $^3J(^1H,^1H)$ = 8.2 Hz, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_{12}H_8N_2$, H^{3,8}), 6.70 (br, 1H, N*H*), 5.84 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 5.75 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 3.29 (m, 2H, CH_2), 3.03 (td, $^3J(^1H,^1H)$ = 8.2 Hz, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_5H_4CH_2CH_2$NH), 2.73 (m, 2H, CH_2), 2.08 (t, $^3J(^1H,^1H)$ = 8.2 Hz, 2H, $C_5H_4CH_2CH_2$NH), 1.86–1.32 (m, 6H, CH_2). IR(ATR; cm^{-1}): 1974 vs [ν_a(CO)], 1894 vs [ν_s(CO)], 1025 vs-br [ν_a(BF)].

Synthesis of [{η^5-$C_5H_4CH_2CH_2NH(CH_2)_5$}Mo(CO)$_2$(4,7-Ph_2-phen)][BF_4]$_2$ (18). The reaction was carried out as was described for compound **16**, but with [(η^3-C_3H_5){η^5-$C_5H_4CH_2CH_2N(CH_2)_5$}Mo(CO)$_2$] (**12**; 0.26 g, 0.70 mmol) and 4,7-diphenyl-1,10-phenanthroline (226 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture CH_2Cl_2/Et_2O and vacuum dried. Yield: 0.32 g (0.38 mmol, 54%). Red powder. Calcd for $C_{38}H_{35}B_2F_8MoN_3O_2$: C, 54.64; H, 4.22; N, 5.03. Found: C, 54.52; H, 4.17; N, 5.14. Positive-ion MS (MeCN): *m*/*z* = 352 (100%) [M + MeCN]$^{2+}$, 662 [M – H]$^+$. ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.45 (d, $^3J(^1H,^1H)$ = 5.7 Hz, 2H, $C_{12}H_6N_2$, H^{2,9}), 8.16 (s, 2H, $C_{12}H_6N_2$, H^{5,6}), 7.95 (d, $^3J(^1H,^1H)$ = 5.7 Hz, 2H, $C_{12}H_6N_2$, H^{3,8}), 7.67 (m, 10H, C_6H_5), 6.65 (br, 1H, N*H*), 5.91 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 5.81 (t, $^3J(^1H,^1H)$ = 2.2 Hz, 2H, C_5H_4), 3.32 (m, 2H, CH_2), 3.08 (td, $^3J(^1H,^1H)$ = 8.2 Hz, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_5H_4CH_2CH_2$NH), 2.76 (m, 2H, CH_2), 2.15 (t, $^3J(^1H,^1H)$ = 8.2 Hz, 2H, $C_5H_4CH_2CH_2$NH), 1.87–1.33 (m, 6H, CH_2). IR(ATR; cm^{-1}): 1971 vs [ν_a(CO)], 1903 vs [ν_s(CO)], 1050 vs-br [ν_a(BF)]. Single crystals of **18** suitable for X-ray diffraction analysis were prepared by overlayering of acetonitrile solution with ether.

Synthesis of [{η^5-$C_5H_4CH_2CH_2NH(CH_2)_4O$}Mo(CO)$_2$(phen)][$BF_4$]$_2$ (19). The reaction was carried out as was described for compound **16**, but with [(η^3-C_3H_5){η^5-$C_5H_4CH_2CH_2N(CH_2)_4O$}Mo(CO)$_2$] (**13**; 0.26 g, 0.70 mmol) and 1,10-phenanthroline (126 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture MeCN/Et_2O and vacuum dried. Yield: 0.16 g (0.23 mmol, 33%). Red powder. Calcd for $C_{25}H_{25}B_2F_8MoN_3O_3$: C, 43.83; H, 3.68; N, 6.13. Found: C, 43.91; H, 3.61; N, 6.10. Positive-ion MS (MeCN): *m*/*z* = 512 [M – H]$^+$ (100%). ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.41 (d, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_{12}H_8N_2$, H^{2,9}), 8.80 (d, $^3J(^1H,^1H)$ = 8.2 Hz, 2H, $C_{12}H_8N_2$, H^{4,7}), 8.22 (s, 2H, $C_{12}H_8N_2$, H^{5,6}), 7.99 (dd, $^3J(^1H,^1H)$ = 8.2 Hz, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_{12}H_8N_2$, H^{3,8}), 7.30 (br, 1H, N*H*), 5.85 (t, $^3J(^1H,^1H)$ = 2.1 Hz, 2H, C_5H_4), 5.75 (t, $^3J(^1H,^1H)$ = 2.1 Hz, 2H, C_5H_4), 3.95 (m, 2H, CH_2), 3.64 (m, 2H, CH_2), 3.25 (m, 2H, CH_2), 3.13 (td, $^3J(^1H,^1H)$ = 8.2 Hz, $^3J(^1H,^1H)$ = 5.4 Hz, 2H, $C_5H_4CH_2CH_2$NH), 2.94 (m, 2H, CH_2), 2.09 (t, $^3J(^1H,^1H)$ = 8.2 Hz, 2H, $C_5H_4CH_2CH_2$NH). IR(ATR; cm^{-1}): 1959 vs [ν_a(CO)], 1902 vs [ν_s(CO)], 1030 vs-br [ν_a(BF)].

Synthesis of [{η^5-$C_5H_4CH_2CH_2NH(CH_2)_4O$}Mo(CO)$_2$(4,7-$Ph_2$-phen)][$BF_4$]$_2$ (20). The reaction was carried out as was described for

compound **16**, but with [(η^3-C_3H_5){η^5-$C_5H_4CH_2CH_2N(CH_2)_4O$}Mo(CO)$_2$] (**13**; 0.26 g, 0.70 mmol) and 4,7-diphenyl-1,10-phenanthroline (226 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture CH_2Cl_2/Et_2O and vacuum dried. Yield: 0.18 g (0.21 mmol, 31%). Red powder. Calcd for $C_{37}H_{33}B_2F_8MoN_3O_3$: C, 53.08; H, 3.97; N, 5.02. Found: C, 53.17; H, 3.91; N, 4.95. Positive-ion MS (MeCN): *m*/*z* = 664 [M – H]$^+$ (100%). ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.45 (d, ^3J(^{1}H,^{1}H) = 5.6 Hz, 2H, $C_{12}H_6N_2$, H^{2,9}), 8.15 (s, 2H, $C_{12}H_6N_2$, H^{5,6}), 7.95 (d, ^3J(^{1}H,^{1}H) = 5.6 Hz, 2H, $C_{12}H_6N_2$, H^{3,8}), 7.67 (m, 10H, C_6H_5), 7.30 (br, 1H, N*H*), 5.93 (t, ^3J(^{1}H,^{1}H) = 2.2 Hz, 2H, C_5H_4), 5.82 (t, ^3J(^{1}H,^{1}H) = 2.2 Hz, 2H, C_5H_4), 3.96 (m, 2H, CH_2), 3.65 (m, 2H, CH_2), 3.29 (m, 2H, CH_2), 3.19 (td, ^3J(^{1}H,^{1}H) = 8.2 Hz, ^3J(^{1}H,^{1}H) = 5.4 Hz, 2H, $C_5H_4CH_2CH_2$NH), 2.98 (m, 2H, CH_2), 2.16 (t, ^3J(^{1}H,^{1}H) = 8.2 Hz, 2H, $C_5H_4CH_2CH_2$NH). IR(ATR; cm^{-1}): 1971 vs [ν_a(CO)], 1900 vs [ν_s(CO)], 1050 vs-br [ν_a(BF)].

Synthesis of [(η^5-$C_9H_6CH_2CH_2NHMe_2$)Mo(CO)$_2$(phen)][BF_4]$_2$ (21). The reaction was carried out as was described for compound **16**, but with [(η^3-C_3H_5)(η^5-$C_9H_6CH_2CH_2NMe_2$)Mo(CO)$_2$] (**14**; 0.27 g, 0.70 mmol) and 1,10-phenanthroline (126 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture CH_2Cl_2/Et_2O and vacuum dried. Yield: 0.30 g (0.43 mmol, 62%). Red powder. Calcd for $C_{27}H_{25}B_2F_8MoN_3O_2$: C, 46.79; H, 3.64; N, 6.06. Found: C, 46.63; H, 3.69; N, 5.96. Positive-ion MS (MeCN): *m*/*z* = 281 (100%) [M + MeCN]$^{2+}$, 520 [M – H]$^+$. ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.71 (dd, ^3J(^{1}H,^{1}H) = 5.4 Hz, ^4J(^{1}H,^{1}H) = 1.3 Hz, 1H, $C_{12}H_8N_2$, H^{2,9}), 9.54 (dd, ^3J(^{1}H,^{1}H) = 5.4 Hz, ^4J(^{1}H,^{1}H) = 1.3 Hz, 1H, $C_{12}H_8N_2$, H^{2,9}), 8.70 (m, 2H, $C_{12}H_8N_2$, H^{4,7}), 8.10 (s, 2H, $C_{12}H_8N_2$, H^{5,6}), 7.98 (m, 2H, $C_{12}H_8N_2$, H^{3,8}), 7.30 (br, 1H, N*H*), 6.99 (dd, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^7), 6.79 (dt, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^4), 6.62 (dd, ^3J(^{1}H,^{1}H) = 2.8 Hz, ^4J(^{1}H,^{1}H) = 0.8 Hz, 1H, C_9H_6, H^3), 6.41 (ddd, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^3J(^{1}H,^{1}H) = 6.8 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^{5,6}), 6.32 (ddd, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^3J(^{1}H,^{1}H) = 6.8 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^{5,6}), 5.65 (d, ^3J(^{1}H,^{1}H) = 2.8 Hz, 1H, C_9H_6, H^2), 3.62 m (1H, CH_2), 3.35 m (2H, CH_2), 3.15 m (1H, CH_2), 2.96 (d, ^3J(^{1}H,^{1}H) = 5.2 Hz, 3H, CH_3), 2.93 (d, ^3J(^{1}H,^{1}H) = 5.2 Hz, 3H, CH_3). IR(ATR; cm^{-1}): 1963 vs [ν_a(CO)], 1888 vs [ν_s(CO)], 1030 vs-br [ν_a(BF)]. Single crystals of **21·phen** suitable for X-ray diffraction analysis were prepared by overlayering of acetonitrile solution of the crude product with ether.

Synthesis of [(η^5-$C_9H_6CH_2CH_2NHMe_2$)Mo(CO)$_2$(4,7-Ph_2-phen)][BF_4]$_2$ (22). The reaction was carried out as was described for compound **16**, but with [(η^3-C_3H_5)(η^5-$C_9H_6CH_2CH_2NMe_2$)Mo(CO)$_2$] (**14**; 0.27 g, 0.70 mmol) and 4,7-diphenyl-1,10-phenanthroline (226 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture MeCN/Et_2O and vacuum dried. Yield: 0.33 g (0.39 mmol, 56%). Red powder. Calcd for $C_{39}H_{33}B_2F_8MoN_3O_2$: C, 55.42; H, 3.94; N, 4.97. Found: C, 55.60; H, 3.89; N, 4.91. Positive-ion MS (MeCN): *m*/*z* = 357 [M + MeCN]$^{2+}$, 672 [M – H]$^+$ (100%). ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.75 (d, ^3J(^{1}H,^{1}H) = 5.6 Hz, 1H, $C_{12}H_6N_2$, H^{2,9}), 9.57 (d, ^3J(^{1}H,^{1}H) = 5.6 Hz, 1H, $C_{12}H_6N_2$, H^{2,9}), 8.03 (s, 2H, $C_{12}H_6N_2$, H^{5,6}), 7.93 (d, ^3J(^{1}H,^{1}H) = 5.6 Hz, 2H, $C_{12}H_6N_2$, H^{3,8}), 7.65 (m, 10H, C_6H_5), 7.33 (br, 1H, N*H*), 7.06 (dd, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^7), 6.88 (dt, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^4), 6.67 (dd, ^3J(^{1}H,^{1}H) = 2.8 Hz, ^4J(^{1}H,^{1}H) = 0.8 Hz, 1H, C_9H_6, H^3), 6.53 (ddd, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^3J(^{1}H,^{1}H) = 6.8 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^{5,6}), 6.44 (ddd, ^3J(^{1}H,^{1}H) = 8.5 Hz, ^3J(^{1}H,^{1}H) = 6.8 Hz, ^4J(^{1}H,^{1}H) = 0.9 Hz, 1H, C_9H_6, H^{5,6}), 5.70 (d, ^3J(^{1}H,^{1}H) = 2.8 Hz, 1H, C_9H_6, H^2), 3.66 m (1H, CH_2), 3.38 m (2H, CH_2), 3.16 m (1H, CH_2), 2.98 (d, ^3J(^{1}H,^{1}H) = 5.2 Hz, 3H, CH_3), 2.94 (d, ^3J(^{1}H,^{1}H) = 5.2 Hz, 3H, CH_3). IR(ATR; cm^{-1}): 1966 vs [ν_a(CO)], 1891 vs [ν_s(CO)], 1055 vs-br [ν_a(BF)].

Synthesis of [(η^5-$C_9H_6CH_2CH_2NHMe_2$)Mo(CO)$_2$(bq)][BF_4]$_2$ (23). The reaction was carried out as was described for compound **16**, but with [(η^3-C_3H_5)(η^5-$C_9H_6CH_2CH_2NMe_2$)Mo(CO)$_2$] (**14**; 0.27 g, 0.70 mmol) and 2,2'-biquinoline (179 mg, 0.70 mmol). The crude product was washed with ether (2 × 10 ml), twice recrystallized from the mixture MeCN/Et_2O and vacuum dried. Yield: 0.28 g (0.36 mmol, 52%). Red powder. Calcd for $C_{33}H_{29}B_2F_8MoN_3O_2$: C, 51.53; H, 3.80; N, 5.46. Found: C, 51.69; H, 3.74; N, 5.24. Positive-ion MS (MeCN): *m*/*z* = 319 (100%) [M + MeCN]$^{2+}$, 596 [M – H]$^+$. ^{1}H NMR (CD_3CN; 400 MHz; δ ppm): 9.51 (d, ^3J(^{1}H,^{1}H) = 8.9 Hz, 1H, $C_{18}H_{12}N_2$), 8.84 (d, ^3J(^{1}H,^{1}H) = 8.9 Hz, 1H, $C_{18}H_{12}N_2$), 8.77 (d, ^3J(^{1}H,^{1}H) = 8.9 Hz, 1H, $C_{18}H_{12}N_2$), 8.68 (d, ^3J(^{1}H,^{1}H) = 8.9 Hz, 1H, $C_{18}H_{12}N_2$), 8.55 (d, ^3J(^{1}H,^{1}H) = 8.9 Hz, 1H, $C_{18}H_{12}N_2$), 8.51 (d, ^3J(^{1}H,^{1}H) = 8.9 Hz, 1H, $C_{18}H_{12}N_2$), 8.36–8.22 (m, 4H, $C_{18}H_{12}N_2$), 8.06–8.00 (m, 2H, $C_{18}H_{12}N_2$), 7.24 (br, 1H, N*H*), 6.86 (d, ^3J(^{1}H,^{1}H) = 2.9 Hz, 1H, C_9H_6, H^3), 6.62–6.48 (m, 3H, C_9H_6, H$^{4–7}$), 6.30 (d, ^3J(^{1}H,^{1}H) = 8.7 Hz, 1H, C_9H_6, H^{4,7}), 5.85 (d, ^3J(^{1}H,^{1}H) = 2.9 Hz, 1H, C_9H_6, H^2), 3.60–3.25 (m, 4H, CH_2), 2.97 (d, ^3J(^{1}H,^{1}H) = 5.1 Hz, 3H, CH_3), 2.92 (d, ^3J(^{1}H,^{1}H) = 5.1 Hz, 3H, CH_3). IR(ATR; cm^{-1}): 1951 vs [ν_a(CO)], 1878 vs [ν_s(CO)], 1030 vs-br [ν_a(BF)].

X-ray crystallography The X-ray data for the crystals of the compounds **12**, **18** and **21**·phen were obtained at 150 K using an Oxford Cryostream low-temperature device on a Nonius KappaCCD diffractometer with Mo Kα radiation (λ = 0.71073 Å) and a graphite monochromator. Data reductions were performed with DENZO-SMN.[25] The absorption was corrected by integration methods.[26] Structures were solved by direct methods (Sir92) [27] and refined by full-matrix least squares based on F^2 (SHELXL97).[28] Hydrogen atoms were mostly localized on a difference Fourier map. However, to ensure uniformity of the treatment of the crystal, all hydrogen atoms were recalculated into idealized positions (riding model) and assigned temperature factors U_{iso}(H) = 1.2[U_{eq}(pivot atom)] or 1.5U_{eq} for the methyl moiety with C−H = 0.96, 0.97, and 0.93 Å for methyl, methylene, and hydrogen atoms in aromatic rings or the allyl moiety, respectively.

Biological studies. The studies were performed on the human T-lymphocytic leukemia cells MOLT-4 obtained from the American Type Culture Collection (USA) and human promyelocytic leukemia cells HL-60 obtained from the European Collection of Cell Cultures (Porton Down, Salisbury, Great Britain). The cells were cultured in Iscove's modified Dulbecco's medium (IMDM) supplemented with a 20% fetal calf serum and 0.05% L-glutamine (all Sigma-Aldrich, USA) in a humidified incubator at 37 °C and a controlled 5% CO_2 atmosphere. The cell lines in the maximal range of up to 20 passages have been used for this study. Cytotoxicity of the compounds was evaluated by the WST-1 cell viability test (Roche, Germany) according to manufacturer's instructions as described previously.[18] Briefly, the leukemia cells were seeded in 96-wells plate, incubated in solutions of the compounds for 24 h, then washed and incubated for 180 min in WST-1 solution The absorbance at 440 nm corresponding to cell viability was measured using multiplate reader (Tecan Infinite 200). The inhibitory effect of the compound **20** was analyzed through measurement of cell proliferation and viability using propidium iodide (Sigma-Aldrich, USA) and a flow cytometer with Coulter volume. The concentrations 1, 2, 3, 5, 7 μmol/l of the complex **20** were selected to determine cell proliferation and viability after incubation of MOLT-4 and HL-60 cells for 24, 48 and 72 h. Activity of the caspses 3/7, 8 and 9 were evaluated for **20** and **DDP** using the Caspase-Glo Assays (Promega, USA) 24 h after the treatment of the cells MOLT-4 and HL-60. For determination of apoptosis, the Apoptest-FITC kit (Dako-Cytomation, Denmark) was used. The incubation time for the tests of **20** and **DDP** on the cell lines MOLT-4 and HL-60 was 24 h. The quantity of the proteins p53 and p53-ser15 was determined by electrophoresis and western blotting using immunochemical detection 4 and 24 h after the treatment of the cells MOLT-4 by the compounds **20** and **DDP**. The cell cycle was analyzed using flow cytometry on the cell lines MOLT-4 and the HL-60 with **20** and **DDP**. The cells were washed with cold PBS and fixed with

70% ethanol. For detection of DNA, the cells were incubated for 5 minutes at room temperature in a buffer (192 ml 0.2 M Na_2HPO_4 + 8 ml of 0.1 M citric acid, pH = 7.8) and then labeled with propidium iodide in Vindelov's solution for 1 h at 37°C. The DNA content was determined by the flow cytometer DakoCyAn (Beckman Coulter, USA). Data were analyzed using the Multicycle AV software (Phoenix Flow systems, USA). All reagents were obtained from Sigma-Aldrich, USA. Concentrations of the tested complexes (**20** and **DDP**) were 2 and 4 µmol/l and non-treated cells, called control, were evaluated together with the treated cells for all experiments. Measurements were done according to a standard protocol (out cell cycle).[29]

Acknowledgements

This work was supported by Ministry of Education of the Czech Republic (Project No. SG350004) and Charles University in Prague (program PRVOUK P37/01).

Keywords: molybdenum • cyclopentadienyl • indenyl • cytotoxicity • leukemia

[1] D. Hoelzer, *Hematology Am. Soc. Hematol. Educ. Program.* **2011**, 243-249.

[2] G. J. Roboz, *Hematology Am. Soc. Hematol. Educ. Program.* **2011**, 43-50.

[3] B. Rosenberg, L. van Camp, T. Krigas, *Nature* **1965**, *205*, 698-699; B. Rosenberg, L. van Camp, J. E. Trosko, V. H. Mansour, *Nature* **1969**, *222*, 385-386.

[4] K. D. Mjos, C. Orvig, *Chem. Rev.* **2014**, *114*, 4540-4563; Z. Liu, P. J. Sadler, *Acc. Chem. Res.* **2014**, *47*, 1174-1185; G. Süss-Fink, *Dalton Trans.* **2009**, *39*, 1673-1688; W. Liu, R. Gust, *Chem. Soc. Rev.* **2013**, *42*, 755-773; C. Santini, M. Pellei, V. Gandin, M. Porchia, F. Tisato, C. Marzano, *Chem. Rev.* **2014**, *114*, 815-862; K. Strohfeldt, M. Tacke, *Chem. Soc. Rev.* **2008**, *37*, 1174-1187; H. K. Liu, P. J. Sadler, *Acc. Chem. Res.* **2011**, *44*, 349-359; A. Gautier, F. Cisnetti, *Metallomics* **2012**, *4*, 23-32; N. P. E. Barry, P. J. Sadler, *Chem. Soc. Rev.* **2012**, *41*, 3264-3279; A. F. A. Peacock, P. J. Sadler, *Chem. Asian J.* **2008**, *3*, 1890-1899; O. J. Stacey, S. J. A. Pope, *RSC Adv.* **2013**, *3*, 25550-25564; S. K. Singh, D. S. Pandey, *RSC Adv.* **2014**, *4*, 1819-1840; N. Cutillas, G. S. Yellol, C. de Haro, C. Vicente, V. Rodríguez, J. Ruiz, *Coord. Chem. Rev.* **2013**, *257*, 2784-2797; J. S. Butler, P. J. Sadler, *Curr. Opin. Chem. Biol.* **2013**, *17*, 175-188; N. Muhammad, Z. Guo, *Curr. Opin. Chem. Biol.* **2014**, *19*, 144-153; S. H. van Rijt, P. J. Sadler, *Drug Discov. Today* **2009**, *14*, 1089-1097.

[5] M. R. P. Norton de Matos, C. C. Romão, C. C. L. Pereira, S. S. Rodrigues, M. Mora, M. J. P. Silva, P. M. Alves, C. A. Reis, **2005**, International Patent WO/2005/087783.

[6] M. S. Saraiva, S. Quintal, F. M. C. Portugal, T. A. Lopes, V. Félix, J. M. F. Nogueira, M. Meireles, M. G. B. Drew, M. J. Calhorda, *J. Organomet. Chem.* **2008**, *693*, 3411-3418; H. Pfeiffer, M. Dragoun, A. Prokop, U. Schatzschneider, *Z. Anorg. Allg. Chem.* **2013**, *639*, 1568-1576; C. C. L. Pereira, C. V. Diogo, A. Burgeiro, P. J. Oliveira, M. P. M. Marques, S. S. Braga, F. A. A. Paz, M. Pillinger, I. S. Gonçalves, *Organometallics* **2008**, *27*, 4948-4956.

[7] D. Bandarra, M. Lopes, T. Lopes, J. Almeida, M. S. Saraiva, M. Vasconcellos-Dias, C. D. Nunes, V. Félix, P. Brandão, P. D. Vaz, M. Meireles, M. J. Calhorda, *J. Inorg. Biochem.* **2010**, *104*, 1171-1177; T. Turki, T. Guerfel, F. Bouachir, *Polyhedron* **2009**, *28*, 569-573.

[8] J. Honzíček, J. Vinklárek, Z. Padělková, L. Šebestová, K. Foltánová, M. Řezáčová, *J. Organomet. Chem.* **2012**, *716*, 258-268.

[9] J. Honzíček, J. Vinklárek, M. Erben, Z. Padělková, L. Šebestová, M. Řezáčová, *J. Organomet. Chem.* **2014**, *749*, 387-393.

[10] P. Jutzi, T. Redeker, B. Neumann, H. G. Stammler, *Organometallics* **1996**, *15*, 4153-4161; C. Qian, H. Li, J. Sun, W. Nie, *J. Organomet. Chem.* **1999**, *585*, 59.

[11] M. S. Blais, J. C. W. Chien, M. D. Rausch, *Organometallics* **1998**, *17*, 3775-3783.

[12] P. W. Causey, M. C. Baird, S. P. C. Cole, *Organometallics* **2004**, *23*, 4486-4494.

[13] G. D. Potter, M. C. Baird, S. P. C. Cole, *J. Organomet. Chem.* **2007**, *692*, 3508-3518.

[14] J. W. Faller, C. C. Chen, M. J. Mattina, A. Jakubowski, *J. Organomet. Chem.* **1973**, *52*, 361-386.

[15] P. Neves, C. C. L. Pereira, F. A. A. Paz, S. Gago, M. Pillinger, C. M. Silva, A. A. Valente, C. C. Romão, I. S. Gonçalves, *J. Organomet. Chem.* **2010**, *695*, 2311-2319; J. Honzíček, A. Mukhopadhyay, T. S. Silva, M. J. Romão, C. C. Romão, *Organometallics* **2009**, *28*, 2871-2879; D. R. van Staveren, T. Weyhermüller, N. Metzler-Nolte, *Organometallics* **2000**, *19*, 3730-3735; W. E. Vanarsdale, J. K. Kochi, *J. Organomet. Chem.* **1986**, *317*, 215-232.

[16] J. Markham, K. Menard, A. Cutler, *Inorg. Chem.* **1985**, *24*, 1581-1587.

[17] M. J. Calhorda, C. A. Gamelas, I. S. Gonçalves, E. Herdtweck, C. C. Romão, L. F. Veiros, *Organometallics* **1998**, *17*, 2597-2611; J. Honzíček, J. Vinklárek, M. Erben, J. Lodinský, L. Dostál, Z. Padělková, *Organometallics* **2013**, *32*, 3502-3511; J. Lodinský, J. Vinklárek, L. Dostál, Z. Růžičková, J. Honzíček, *RSC Adv.* **2015**, *5*, 27140-27153.

[18] J. Honzíček, I. Klepalová, J. Vinklárek, Z. Padělková, I. Císařová, P. Šiman, M. Řezáčová, *Inorg. Chim. Acta* **2011**, *373*, 1-7.

[19] J. Vinklárek, H. Hurychová, J. Honzíček, L. Šebestová, Z. Padělková, M. Řezáčová, *Eur. J. Inorg. Chem.* **2013**, 2665-2672.

[20] V. M. Gonzales, M. A. Fuertes, C. Alonso, J. M. Perez, *Mol. Pharm*. **2001**, *59*, 657-663; G. Chu, *J. Biol. Chem.* **1994**, *269,* 787-790; K. A. Tacka, J. C. Dabrowiak, J. Goodisman, H. S. Penefsky, A. K. Souid, *Chem. Reas. Toxicol*. **2004**, *17*, 1102-1111; S. Dasari, P. B. Tchounwou*, Eur. J. Pharmacol*. **2014**, *740*, 364-378; T. Ozben, *J. Pharm. Sci.* **2007**, *9*, 2181-2196; J. Rivadeneira, A. L. Di Virgilio, D. A. Barrio, C. I. Muglia, L. Bruzzone, S. B. Etcheverry, *Med. Chem.* **2010**, *6*, 9-23.

[21] F. Toledo, G. M. Wahl, *Int. J. Biochem. Cell Biol.* **2007**, *39*, 1467-1482.

[22] S. Y. Shieh, M. Ikeda, Y. Taya, C. Prives, *Cell* **1997**, *91*, 325-334.

[23] W. L. F. Armarego, D. D. Perrin, in *Purification of Laboratory Chemicals*, Butterworth-Heinemann, Oxford, **1996**.

[24] P. W. Hickmott, S. Wood, P. Murray-Rust, *J. Chem. Soc., Perkin Trans. 1* **1985**, 2033-2038.

[25] Z. Otwinowski, W. Minor, *Methods Enzymol.* **1997**, *276*, 307-326.

[26] P. Coppens, in *Crystallographic Computing* (Eds.: F. R. Ahmed, S. R. Hall, C. P. Huber), Munksgaard, Copenhagen, **1970**, pp. 255-270.

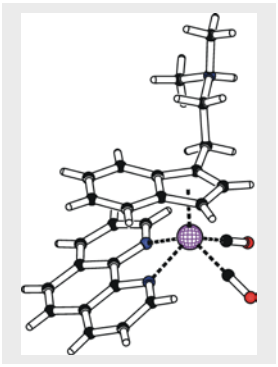
[27] A. Altomare, G. Cascarano, C. Giacovazzo, A. Guagliardi, M. C. Burla, G. Polidori, M. Camalli, *J. Appl. Cryst.* **1994**, *27*, 435-436.

[28] G. M. Sheldrick, in *SHELXL97*, University of Göttingen, Germany, **2008**.

[29] L. Šebestová, R. Havelek, M. Řezáčová, J. Honzíček, Z. Kročová, J. Vinklárek, *Chem-Biol Interact.* **2015**, *242*, 61-70.

FULL PAPER

A series of new highly cytotoxic active agents was synthesized. Their activity was established on leukemia cells MOLT-4 and HL-60.



O. Mrózek, J. Vinklárek, L. Šebestová, M. Řezáčová, A. Eisner, Z. Růžičková, J. Honzíček*

Page No. – Page No.

Highly water-soluble cyclopentadienyl and indenyl molybdenum(II) complexes: second generation of molybdenum-based cytotoxic agents.