European Journal of Medicinal Chemistry 45 (2010) 4169–4174

Contents lists available at ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

Synthesis and cytotoxic activities of group 3 metal complexes having monoanionic tridentate ligands

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ARTICLE INFO

Article history:
Received 19 February 2010
Received in revised form
14 May 2010
Accepted 7 June 2010
Available online 12 June 2010

Keywords:
Metal complexes
Cytotoxic activity
C6
HEK-293
WHEI-164

ABSTRACT

Complexes of scandium, yttrium, samarium and neodymium bearing monoanionic tridentate ancillary ligands have been synthesized and characterized. The cytotoxic activities of novel compounds, as well as that of similar compounds previously reported have been evaluated on rat glioma (C6), murine fibrosarcoma (WHEI-164) and human embryonic kidney (HEK-293) cell lines. Scandium complex with quinolinephenoxyamine (NNHO) ligand showed very interesting activity against C6 cell line.

1. Introduction

The design of new metal complexes as anticancer agents has received much attention in recent years.

After the discovery of cis-platin, the first inorganic cancer chemotherapeutic agent, there has been considerable exploration of other transition metal complexes. Among the metals studied to date, which comprise much of periodic table, lanthanides complexes showed significant biological activity [1].

Their biologic properties, primarily based on their similarity to calcium, have been the basis for research into potential therapeutic applications [2,3]. Lanthanide compounds, for instance cerium as metal center, have been used for the treatment of cancer or as antiemetics [4]. In addition, lanthanum carbonate (Fosrenol) as a phosphate binder, has used for the treatment of hyperphosphatemia [5].

One group of texaphyrins lanthanide complexes have progressed into clinical trials. The texaphyrins are penta-azo, Schiff base macrocycles that resemble porphyrins in being fully aromatic, are monoanionic ligands containing five, rather than four, coordinating nitrogen atoms in the central core and form colored complexes [6,7].

Also a series of cerium (III) bipyridyl, phenanthroline and related complexes has been reported with *in vitro* activity against cell line. Recently, the lanthanum complex of derivate phenanthroline (KP772) has shown to exert potent activity against a wide range of tumor cell line *in vitro* and a colon carcinoma xenograft model *in vivo* with properties comparable to cis-platin and methotrexate [8].

Previously, Rogers and coworkers showed that the scandium (III) and indium (III) complexes of enterochelin act as antimetabolites of the ferric complexes in both *Klebsiella pneumoniae* and *Escherichia coli* [9,10].

More recently, it has been reported the synthesis, characterization and cytotoxic activity of Acenocoumarol samarium (III) and gadolinium(III) complexes against melanoma B16 and fibrosarcoma L929 [11].

Complexes of rare earth metal as cerium (III), lanthanum (III) and neodymium (III) having coumarins as ligand, were examined on different cell lines [12]. Coumarines are an important group of organic compounds that show wide variety of biological activity as antitumor and antiproliferative effects [13]. The lanthanide

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doi:10.1016/j.ejmech.2010.06.008

complexes with coumarins exhibited superior activity in various tumor models as compared to the corresponding inorganic salts.

Thus, the complexation of lanthanides with heterocyclic ligands having more than one donor atom, is of great interest in the chemistry of coordination compounds, as these complexes can act either as neutral molecules or as deprotonated anions. Moreover, the complexation can lead to mono- or poly-dentate ligands [12]. However, still little data on the effects of cytotoxic complexes of scandium, yttrium and samarium are available in literature.

In the present study, we report the synthesis of new scandium (III), yttrium (III), samarium (III) and neodymium (III) chlorido or triflate complexes with tridentate monoanionic quinolinephenoxyamine (NNHO), quinolinephenoxyimine (NNO) and ansa-monocyclopentadienyl-imino-pyridine (NNCp) ancillary ligands (see Fig. 1), together with their characterization by elemental analysis, nuclear magnetic resonance (NMR), FT-IR and mass-spectroscopy. The cytotoxic activities of these novel lanthanide complexes and of similar compounds previously reported, depicted in Fig. 1, have been evaluated on rat glioma (C6), murine fibroscarcoma (WHEI-164) and human embryonic kidney (HEK-293) cell lines.

2. Results and discussion

2.1. Chemistry

The synthesis of the neutral ligands 2-*tert*-butyl-6-(quinolin-8-ylaminomethyl)phenol ($NNHO^H$), 2-*tert*-butyl-6-(quinolin-8-yliminomethyl)phenol (NNO^H) and (6-cyclopentadienylmethyl-pyridin-2-ylmethylene)-(2,6-diisopropyl-phenyl)-imine ($NNCp^H$) were carried out in good yields by applying the procedures reported in literature [14–16].

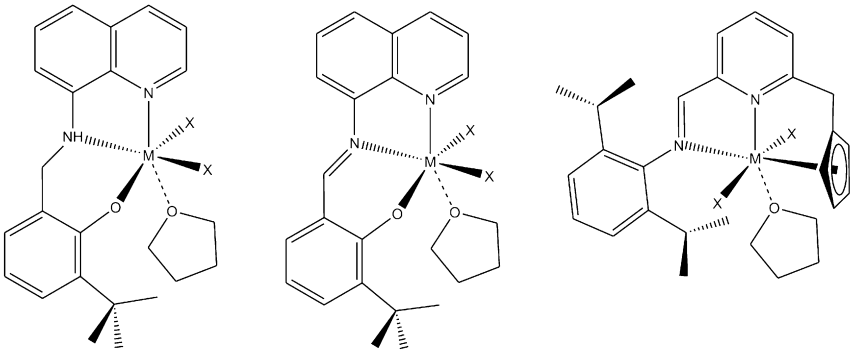
The scandium chlorido-complexes $ScCl_2$(NNHO)(THF) **1** and $ScCl_2$(NNO)(THF) **2** (see Fig. 1) were prepared in order to compare the cytotoxic behavior of two scandium complexes having two isoelectronic ligands with different basicity. $ScCl_2$(NNCp)(THF) **3** (see Fig. 1) was prepared for verify the effect of electrophilicity of the complex on the cytotoxicity, in fact **3** has a lower electrophilicity than complexes **1** and **2**, due to its ancillary ligand which have a higher number of electrons than ligand of complexes **1** and **2** (10 e^- for complex **3** *vs.* 6 e^- for complexes **1** and **2**). The scandium triflate complex $Sc(OTf)_2$(NNHO)(THF) **4**, where OTf = CF_3SO_3, was synthesized in order to evaluate the role of two different living ligands on cytotoxic effects. Finally, the yttrium, YCl_2(NNHO)(THF) **5**, YCl_2(NNO)(THF) **6**, neodymium $Nd(OTf)_2$(NNHO)(THF) **7** and samarium $Sm(OTf)_2$(NNO)(THF) **8** complexes were prepared in order to evaluate the differences due to metal center and to add new data on possible cytotoxic activity of rare earth elements.

The scandium complexes **1**, **2** and **3** were prepared as previously reported by us [14] following the same synthetic strategy, i.e. the deprotonation of the neutral ligands $NNHO^H$, NNO^H, $NNCp^H$ with a stoichiometric amount of potassium *tert*-butoxide, followed by the reaction of the anionic ancillary ligand with $ScCl_3$. Also the yttrium chlorido-complexes **5** and **6** and the trifluoromethanesulfonate complexes of Sc, Nd and Sm (**4**, **7**, and **8** respectively), have been synthesized in a similar way, by reacting the YCl_3 salt or the $LnOTf_3$ salts (Ln = Sc, Nd and Sm) with the previously deprotonated $NNHO^H$ or NNO^H ligands in THF (as an example in the Scheme 1 is reported the synthetic route for metal complex **4**). The synthesis of **6** was already present in the literature [17].

The new complexes were isolated in pure form and good yields and all the elemental analysis data were in agreement with the chemical formulas.

The ^1H NMR of the scandium triflate complex **4** shows the presence of the signals of the aromatic protons of the ancillary ligand in the range 8.95–6.38 ppm and of the *tert*-butyl group at 1.30 ppm. The assignments have been done on the basis of ^1H COSY and ^1H NOESY experiments. The N-bonded hydrogen atom is observable as a slightly broad signal at 6.56 ppm, while the methylene protons correspond to an AB spin system at room temperature ($\delta_A = 5.02$ ppm, $\delta_B = 4.39$ ppm, $^2J_{HH} = 12.5$ Hz). This last result indicates that the NH group is strongly coordinated to the metal center at 298 K. The ^1H NMR spectrum also shows the presence of a coordinated THF molecule. The $^{13}C\{^1H\}$ NMR spectrum confirms the proposed formulation, being present 15 signals for the aromatic carbon atoms in the range 160.5–117.7 ppm (nine singlets for the CH groups and six singlets for the not-H-bonded carbons). The ^{13}C NMR signals of the *tert*-butyl substituent, of the CH_2 bridge and of the THF molecule are comprised between 68.4 and 25.6 ppm.

The same coordination sphere of compound **4** is present in the paramagnetic neodymium complex **7**. Its ^1H NMR spectrum in CD_3CN shows 10 quite broad singlets attributable to the aromatic protons and the NH group between 21.87 and 4.23 ppm, while the CH_2 bridge corresponds to a broad signal at 10.9 ppm. The unpaired electrons of Nd(III) cause a relatively fast relaxation of all these



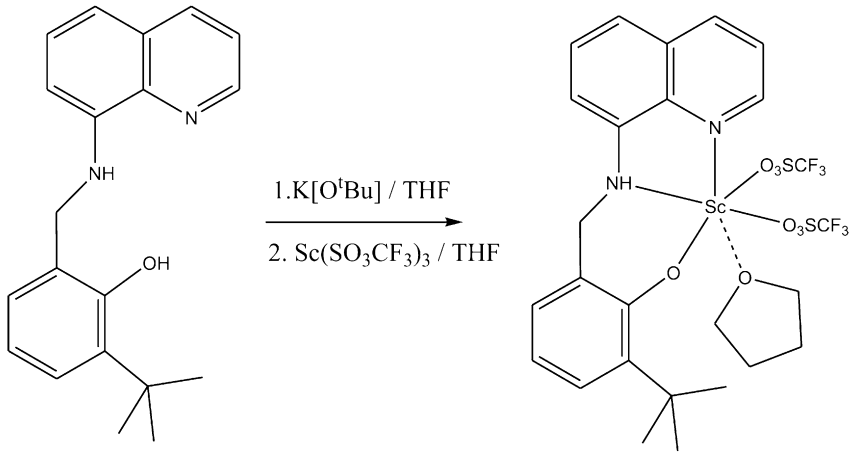
$ScCl_2$(NNHO)(THF) (1) $ScCl_2$(NNO)(THF) (2) $ScCl_2$(NNCp)(THF) (3)

$ScOTf_2$(NNHO)(THF) (4) YCl_2(NNO)(THF) (6)

YCl_2(NNHO)(THF) (5) $SmOTf_2$(NNO)(THF) (8)

$NdOTf_2$(NNHO(THF) (7)

Fig. 1. Sketches of the complexes considered for cytotoxic studies.



Scheme 1. Synthetic route for the preparation of metal complex **4**.

hydrogen atoms. In the aliphatic region the THF molecule and the *tert*-butyl substituent are observable, which result poorly affected by the paramagnetism of the metal center.

The ^{1}H NMR of the NNHO yttrium chlorido-complex **5** is very similar to that observed for the scandium complex **4**. All the aromatic protons, comprised in the range 9.53–6.17 ppm, have been assigned on the basis of COSY and NOESY experiments. The aliphatic region shows the signals of the *tert*-butyl substituent and of a THF molecule. The NH singlet falls at 5.78 ppm, while the CH_2 group corresponds to a broad singlet at 4.65 ppm, which becomes an AB spin system on cooling the CD_2Cl_2 solution.

The ^{1}H NMR and ^{13}C NMR signals of the samarium derivative Sm(OTf)$_2$(NNO)(THF) **8**, have been assigned on the basis of COSY, NOESY and HSQC experiments. The unpaired electrons of the metal center cause a negligible broadening of the NMR signals and only moderate paramagnetic shifts. The aromatic protons of the coordinated NNO ligand are comprised between 12.02 and 7.58 ppm in the ^{1}H NMR spectrum and between 204.6 and 148.7 ppm in the ^{13}C {^{1}H} NMR spectrum. The imine group corresponds to a ^{1}H NMR sharp signal at 8.79 ppm, which is correlated to a ^{13}C NMR singlet at 202.7 ppm. The aliphatic regions of the ^{1}H NMR and ^{13}C NMR spectra show the signals of the *tert*-butyl group, which result downfield shifted if compared to those observable for similar diamagnetic compounds [14–17].

Thus, NMR analysis ^{1}H and ^{13}C on the basis of COSY, NOESY and HSQC experiments have allowed a structure determination in solution very satisfactory. In Fig. 2 the ^{1}H and ^{13}C NMR spectra of complex **8** which are very significant, despite the Sm is paramagnetic, are reported as an example.

FT-IR spectra of complexes **4**, **5**, **7** and **8** confirm the coordination environment of the metals, in fact band attributable to N–H stretching for complexes **4**, **5** and **7** are at 3224, 3285 and 3234 cm^{-1}, respectively, whereas it is at 3300 cm^{-1} in the free ligand. C=N stretching for complex **8** is at 1543 cm^{-1}, whereas it is at 1670 cm^{-1} in the free ligand.

The mass spectra of the NNHO complexes **4**, **5** and **7** show clusters attributable to the molecular ion after the thermal loss of the THF molecule and the fragmentation of the *tert*-butyl group and sometimes of the NH group. The EI ionization caused also the fragmentation of the triflate groups of **4** and **7** and/or the break of the M–X bond. Similar fragmentations, affecting the $-C(CH_3)_3$ and $-SO_3CF_3$ groups, have been observed also for the samarium derivative **8**.

2.2. Pharmacology

The cytotoxic activities of synthesized lanthanide complexes have been evaluated on rat glioma (C6), murine fibroscarcoma (WHEI-164) and human embryonic kidney (HEK-293) cell lines. As reference drug we choose to use 6-mercaptopurine for the sole purpose of testing the susceptibility of our cell lines to cytotoxicity (Table 1).

On C6 cells, all tested compounds, except $ScCl_2$(NNCp)(THF) **3**, Sc(OTf)$_2$(NNHO)(THF) **4** and Sm(OTf)$_2$(NNO) **8** have shown a cytotoxic activity comparable to 6-mercaptopurine. Analysis of the three compounds of scandium used revealed that the complex **1** with the ligand NNHO is the most active. By comparison of complexes **1** and **4**, which have the same ancillary ligand, it was evident that **1** was much more active than **4**. This data was not surprising because the chlorides are better leaving groups compared to SO_3CF_3. Besides the two compounds of yttrium (**5** and **6**), our data showed that compound **5** with NNHO as ligand was more active than compound **6** with NNO as ligand. An interesting antiproliferative activity on C6 cells was exerted by compound of neodymium with NNHO (**7**), while compound of Sm (**8**) was not

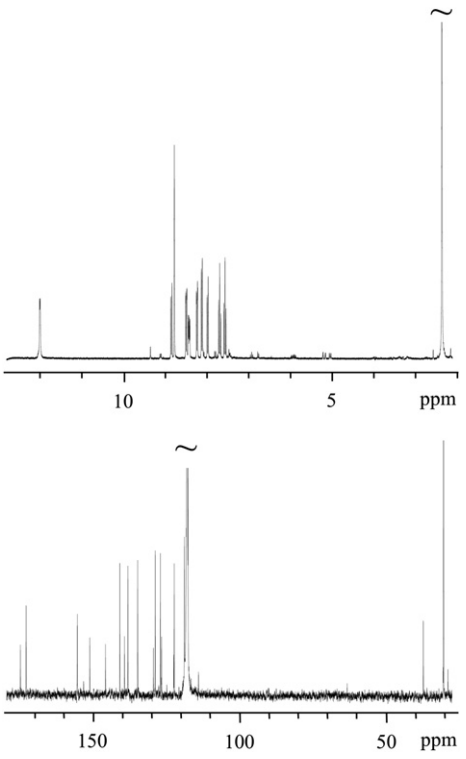


Fig. 2. ^{1}H (a) and ^{13}C (b) NMR spectra of complex **8**. TMS scale.

Table 1
Cytotoxic behavior of lanthanide complexes.

Compounds	Cell lines		
	WHEI-164	HEK-293	C6
1	>100	59.9	0.69
2	37	>100	15
3	54	>100	>100
4	14	93	>100
5	>100	>100	6.5
6	85	18	54
7	>100	36.8	4.7
8	95	21.5	>100
6-mercaptopurine	1.5	1.1	1.3

The cytotoxic activity of lanthanide was evaluated as IC_{50} (μM): the concentration of compound that affords cell growth by 50% as compared to control on the following cell lines: WHEI-164 murine fibrosarcoma cells, HEK-293: human embryonic kidney cells, C6: rat glioma cells. The 6-mercaptopurine was used as standard drug.

active. Our results showed that compound of scandium with NNHO as ligand (**1**) was the most active on C6.

On WHEI-164 cells, compounds of scandium, especially Sc $(OTf)_2$(NNHO)(THF) **4**, were the most active. On the contrary to the observation on C6 cells, the compound **4** showed a relative good activity, whereas the compound **1** was inactive, on WHEI-164 cell line. Finally, on HEK-293 cell line, the antiproliferative activities of yttrium (**6**), neodymium (**7**) and samarium (**8**) complexes were more evident than compounds of scandium (**1**–**4**).

3. Conclusions

The synthesis and characterization of new scandium (III), yttrium (III), samarium (III) and neodymium (III) chlorido or triflate complexes with tridentate monoanionic quinolinephenoxyamine (NNHO), quinolinephenoxyimine (NNO) and ansa-mono-cyclopentadienyl-imino-pyridine (NNCp) ancillary ligands has been reported, and their cytotoxic activity have been evaluated on rat glioma (C6), murine fibroscarcoma (WHEI-164) and human embryonic kidney (HEK-293) cell lines.

At moment it is not possible to make any hypothesis of a probable mechanism of action of tested compounds, since the aim of this work was addressed to check, in first instance, the cytotoxic activity. But from our result, it is possible to observe that a probable mechanism of action to reduce the cell growth should be different from mechanism operated by 6-mercaptopurine. In other words, the 6-mercaptopurine, as reported in literature, inhibits the cell growth by acting as purine antimetabolites, which prevent the biosynthesis, or utilization, of normal cellular metabolites for the synthesis of DNA/RNA. In fact, 6-mercaptopurine showed a comparable magnitude of inhibition in all three cell lines, due to the inhibition of the common pathway for DNA/RNA biosynthesis. In contrast, our compounds showed a magnitude of inhibition with high variability among cell lines. This observation could let us to speculate that probably compounds may act by targeting specific pathway most likely non-common to all three cell lines. For instance, the compound **1** resulted more active on C6 cell, even compared to 6-mercaptopurine, but much less active (about 87 times) on HEK-293 cells and almost ineffective on WHEI-164 cells. These data could suggest a mechanism of action on specific molecular pathway; to verify this hypothesis, further studies of molecular pharmacology are needed.

4. Experimental section

4.1. Spectroscopic measurements

The elemental analyses for C, H, N, Cl were performed according to standard microanalytical procedures. ^{1}H NMR, homodecoupled ^{1}H NMR, ^{1}H COSY, ^{1}H NOESY, HSQC and ^{13}C {^{1}H} NMR spectra were recorded at 298 K on a Bruker Avance 300 spectrometer operating at 300 MHz (^{1}H) and 75 MHz (^{13}C) and referred to internal tetramethylsilane. Mass spectra (E.I., 70 eV) were recorded on a Finnigan Trace GC-MS equipped with a probe controller for the sample direct inlet. Sample temperature was varied between 80 °C and 280 °C. The assignments were done by comparison between theoretical and experimental isotopic clusters and the most intense signals of each characterized cluster are reported. Fourier transform infrared (FT-IR) spectra were obtained at a resolution of 2.0 cm^{-1} with a Bruker-Vector 22 FT-IR spectrometer equipped with a deuterated triglycine sulphate (DTGS) detector and a Ge/KBr beam splitter. The frequency scale was internally calibrated to 0.01 cm^{-1} using a He–Ne reference laser. Thirty-two scans were signal-averaged to reduce spectral noise.

4.2. Chemistry

All manipulations were carried out under oxygen- and moisture-free atmosphere in an MBraun MB 200 glove-box. All the solvents were thoroughly deoxygenated and dehydrated under argon by refluxing over suitable drying agents, while NMR deuterated solvents (Euriso-Top products) were kept in the dark over molecular sieves. The anhydrous chloride $LnCl_3$ {Ln = Sc, Y} and triflate salts $Ln(OTf)_3$ {Ln = Sc, Nd, Sm; OTf = CF_3SO_3} (Strem, Aldrich) were used as received. Potassium *tert*-butoxide was purchased from Aldrich. The neutral ligands $NNHO^H$, NNO^H and $NNCp^H$ were prepared by following reported procedure [14–16]. The chlorido-complexes of scandium and yttrium $ScCl_2$(NNHO)(THF) **1**, $ScCl_2$(NNO)(THF) **2**, $ScCl_2$(NNCp)(THF) **3**, YCl_2(NNO)(THF) **6** were synthesized as reported in literature [14,17].

4.3. Synthesis of Sc(OTf)$_2$(NNHO)(THF) (*4*)

To a solution of neutral ligand $NNHO^H$ (0.460 g, 1.5 mmol) in THF (20 mL), a stoichiometric amount of potassium *tert*-butoxide (0.168 g, 1.5 mmol) was slowly added at room temperature. After 15 min the resulting solution was added drop wise to a solution of anhydrous scandium triflate $Sc(OTf)_3$ (0.738 g, 1.5 mmol) in THF (20 mL) and left for 4 h under stirring at room temperature. The solvent was evaporated at reduced pressure and the reaction mixture was extracted with dichloromethane (3 × 10 mL). The filtrate was concentrated to about 2 mL at reduced pressure and a yellow solid separated out by addition of *n*-hexane. The solid was filtered, washed with diethylether (3 × 5 mL) and dried in vacuum. Yield = 88% (0.951 g).

Elemental analysis of **4**: found (%): C 43.4, H 4.05, N 3.85. Calcd. for $C_{26}H_{29}F_6N_2O_8S_2Sc$ (%): C 43.3, H 4.06, N 3.89.

^{1}H NMR (CD_2Cl_2, 298 K): 8.95 (dd, 1H, $^3J_{HH}$ = 4.8 Hz, $^4J_{HH}$ = 1.3 Hz) *quinoline ring-H$_2$*; 8.41 (dd, 1H, $^3J_{HH}$ = 8.5 Hz, $^4J_{HH}$ = 1.3 Hz) *quinoline ring-H$_4$*; 8.05 (d, 1H, $^3J_{HH}$ = 7.6 Hz) *quinoline ring-H$_7$*; 7.86 (d, 1H, $^3J_{HH}$ = 7.6 Hz) *quinoline ring-H$_5$*; 7.76 (t, 1H, $^3J_{HH}$ = 7.6 Hz) *quinoline ring-H$_6$*; 7.62 (dd, 1H, $^3J_{HH}$ = 4.8 Hz, $^3J_{HH}$ = 8.5 Hz) *quinoline ring-H$_3$*; 6.96 (d, 1H, $^3J_{HH}$ = 8.1 Hz) *phenoxide ring-H$_3$*; 6.69 (d, 1H, $^3J_{HH}$ = 7.2 Hz) *phenoxide ring-H$_5$*; 6.56 (s, slightly br, 1H) *NH*; 6.38 (dd, 1H, $^3J_{HH}$ = 8.1 Hz, $^3J_{HH}$ = 7.2 Hz) *phenoxide ring-H$_4$*; 5.02 (d, 1H, $^2J_{HH}$ = 12.5 Hz) *CH*; 4.39 (dd, 1H, $^2J_{HH}$ = 12.5 Hz, $^3J_{NH-CH}$ = 3.5 Hz) *CH*; 3.79 (m, 4H) *THF*; 1.87 (m, 4H) *THF*; 1.30 (s, 9H) ^{t}*Bu*.

^{13}C {^{1}H} NMR (CD_2Cl_2, 298 K): 149.9, 140.6, 128.6, 128.3, 128.2, 127.6, 127.2, 122.1, 117.7 *H-bonded aromatic carbons*; 160.5, 143.7, 139.1, 136.2, 129.0, 123.2 *not-H-bonded aromatic carbons*; 68.4 *THF*; 56.4 *CH$_2$*; 34.4 ^{t}*Bu quaternary carbon*; 29.9 ^{t}*Bu*; 25.6 *THF*.

Mass spectrum: 483 [4-THF-CH_3-CF_3SO_3-H]$^+$; 351 [4-THF-CH_3-CF_3SO_3-CF_3SO_2]$^+$.

4.4. Synthesis of YCl_2(NNHO)(THF) (**5**)

To a solution of neutral ligand $NNHO^H$ (0.460 g, 1.5 mmol) in THF (20 mL), a stoichiometric amount of potassium *tert*-butoxide (0.168 g, 1.5 mmol) was slowly added at room temperature. After 15 min the resulting solution was added drop wise to a solution of anhydrous yttrium chloride YCl_3 (0.293 g, 1.5 mmol) in THF (20 mL) and left overnight under stirring at room temperature. The by-products were removed by centrifugation and the resulting clear yellow solution was concentrated to ca. 5 mL under reduced pressure. Slow addition of *n*-hexane caused the separation of a pale yellow solid, which was collected by filtration, washed with fresh *n*-hexane and dried under vacuum. Yield = 91% (0.733 g).

Elemental analysis of **5**: found (%): C 53.5, H 5.45, N 5.20. Calcd. for $C_{24}H_{29}Cl_2N_2O_2Y$ (%): C 53.7, H 5.44, N 5.21.

^{1}H NMR (CD_2Cl_2, 298 K): 9.53 (d, 1H, slightly br, $^3J_{HH} = 4.8$ Hz) *quinoline ring-H_2*; 8.24 (d, 1H, $^3J_{HH} = 7.8$ Hz) *quinoline ring-H_4*; 7.80 (d, 1H, slightly br, $^3J_{HH} = 8.0$ Hz) *quinoline ring-H_7*; 7.70 (d, 1H, $^3J_{HH} = 8.0$ Hz) *quinoline ring-H_5*; 7.58 (t, 1H, $^3J_{HH} = 8.0$ Hz) *quinoline ring-H_6*; 7.47 (dd, 1H, $^3J_{HH} = 4.8$ Hz, $^3J_{HH} = 7.8$ Hz) *quinoline ring-H_3*; 6.89 (d, 1H, $^3J_{HH} = 6.5$ Hz) *phenoxide ring-H_3*; 8.48 (d, 1H, slightly br, $^3J_{HH} = 6.5$ Hz) *phenoxide ring-H_5*; 6.17 (t, 1H, slightly br, $^3J_{HH} = 6.5$ Hz) *phenoxide ring-H_4*; 5.78 (s, 1H, slightly br) *NH*; 4.65 (s, br, 2H) *CH_2*; 4.13 (m, 4H) *THF*; 1.97 (m, 4H) *THF*; 1.34 (s, 9H) *^{t}Bu*. The CH_2 signal becomes a broad AB spin system at 213 K: $\delta_A = 4.82$ ppm, $\delta_B = 4.76$ ppm, $^2J_{HH} = 15$ Hz.

Mass spectrum: 414 [5-THF-CH_3-Cl]$^+$.

4.5. Synthesis of $Nd(OTf)_2$(NNHO)(THF) (**7**)

The complex was prepared as described for analogue **4** starting from $NNHO^H$ (0.460 g, 1.5 mmol) and anhydrous neodymium triflate $Nd(OTf)_3$ (0.887 g, 1.5 mmol). Yield = 77% (0.947 g).

Elemental analysis of **7**: found (%): C 38.0, H 3.55, N 3.40. Calcd. for $C_{26}H_{29}F_6N_2NdO_8S_2$ (%): C 38.1, H 3.57, N 3.42.

^{1}H NMR (CD_3CN, 298 K): 21.87 (s, br, 1H), 18.89 (s, br, 1H), 15.65 (s, br, 1H), 13.52 (s, very br, 1H), 8.81 (s, br, 1H), 8.27 (s, br, 1H), 7.26 (s, br, 1H), 6.97 (s, br, 1H), 4.59 (s, br, 1H), 4.23 (s, br, 1H) *aromatic protons + NH*; 10.9 (s, br, 2H) *CH_2*; 3.29 (m, br, 4H) *THF*; 1.79 (m, 4H) *THF*; 1.38 (s, br) *^{t}Bu*.

Mass spectrum: 629 [7-THF-CH_3-CF_3O-H-F]$^+$; 582 [7-THF-CH_3-SO_2CF_3-F]$^+$.

4.6. Synthesis of $Sm(OTf)_2$(NNO) (**8**)

The complex was synthesized following the procedure described for the complexes **4** and **7**, *i.e.* by reacting 1.5 mmol (0.457 g) of the ligand NNO^H with a stoichiometric amount of potassium *tert*-butoxide in 15 mL of THF and adding the resulting solution after 15 min to 1.5 mmol (0.898 g) of samarium triflate Sm $(OTf)_3$ dissolved in 20 mL of THF. The reaction mixture was left under stirring overnight at room temperature, then the solution was concentrated to *ca*. 10 mL under reduced pressure and centrifugated to removed the by-products. Slow addition of a 1:1 mixture of *n*-hexane/diethylether caused the separation of a bright yellow precipitate, which was filtered, washed with fresh *n*-hexane and dried under vacuum. Yield = 87% (0.981 g).

Elemental analysis of 8: found (%): C 35.0, H 2.55, N 3.70. Calcd. for $C_{22}H_{19}F_6N_2O_7S_2Sm$ (%): C 35.1, H 2.55, N 3.73.

^{1}H NMR (CD_3CN, 298 K): 12.02 (d, 1H, $^3J_{HH} = 4.4$ Hz) *quinoline ring-H_2*; 8.86 (d, 1H, $^3J_{HH} = 8.4$ Hz) *quinoline ring-H_4*; 8.79 (s, 1H) *imine-CH*; 8.51 (d, 1H, $^3J_{HH} = 7.8$ Hz) *phenoxide ring-H_5*; 8.44 (dd, 1H, $^3J_{HH} = 4.4$ Hz, $^3J_{HH} = 8.4$ Hz) *quinoline ring-H_3*; 8.25 (d, 1H, $^3J_{HH} = 7.8$ Hz, *phenoxide ring-H_3*); 8.13 (d, 1H, $^3J_{HH} = 7.7$ Hz) *quinoline ring-H_7*; 8.00 (d, 1H, $^3J_{HH} = 7.7$ Hz) *quinoline ring-H_5*; 7.71 (t, 1H, $^3J_{HH} = 7.7$ Hz) *quinoline ring-H_6*; 7.58 (t, 1H, $^3J_{HH} = 7.8$ Hz) *phenoxide ring-H_4*; 2.38 (s, 9H) *^{t}Bu*.

^{13}C {^{1}H} NMR (CD_3CN, 298 K): 173.1 *imine-C*; 155.7 *quinoline ring-C_2*; 141.3 *quinoline ring-C_4*; 138.5 *phenoxide ring-C_5*; 135.1 *phenoxide ring-C_3*; 129.1 *quinoline ring-C_6*; 127.4 *quinoline ring-C_5*; 122.7 *quinoline ring-C_3*; 119.2 *quinoline ring-C_7*; 118.0 *phenoxide ring-C_4*; 175.1, 151.4, 146.1, 139.6, 129.7, 126.9 *not-H-bonded aromatic carbons*; 37.7 *^{t}Bu quaternary carbon*; 30.7 *^{t}Bu*.

Mass spectrum: 605 [8-CH_3-CF_3SO_2]$^+$; 589 [8-CH_3-CF_3SO_3]$^+$.

4.7. Pharmacology

4.7.1. Cell lines and culture conditions

Murine fibrosarcoma cells (WEHI-164) were maintained and grown in adhesion on Petri dishes with DMEM supplemented with heat-inactivated FCS (10%), HEPES (25 mM), penicillin (100 U/mL) and streptomycin (100 μg/mL). Human embryonic kidney (HEK-293) and rat glioma (C6) cells were maintained and grown in adhesion on Petri dishes with DMEM supplemented with FCS (10%), HEPES (25 mM), penicillin (100 U/mL) and streptomycin (100 U/mL).

4.7.2. MTT assay for antiproliferative activity

C6, WEHI-164 and HEK-293 (3.5×10^4 cells/well) were plated on 96-well microtiter plates and allowed to adhere at 37 °C in a 5% CO_2 atmosphere for 2 h. Thereafter, the medium was replaced with fresh one (50 μL) and a 75 μL of 1:4 serial dilution of each tested compound was added, and then the cells incubated for further 72 h. 6-mercaptopurine was used as reference drug. Mitochondrial respiration, an indicator of cell viability, was assessed by the mitochondrial-dependent reduction of [3-(4,5-dimethylthiazol-2-yl)-2,5-phenyl-2*H*-tetrazolium bromide] (MTT) to formazan and cells viability was assessed accordingly to the method of Mosmann [18]. Briefly 5 μL of MTT (5 mg/mL) were added and the cells were incubated for an additional 3 h. Thereafter, cells were lysed and the dark blue crystals solubilised with 100 μL of a solution containing 50% (v:v) *N*, *N*-dimethylformamide, 20% (w:v) SDS with an adjusted pH of 4.5. The optical density (OD) of each well was measured with a microplate spectrophotometer (Titertek Multiskan MCC/340) equipped with a 620 nm filter. The viability of each cell line in response to treatment with tested compounds and 6-mercaptopurine was calculated as: % dead cells = 100 − (OD treated/OD control) × 100.

Acknowledgments

We wish to thank Italian Minister of University and Research for financial support of this work. The authors are grateful to Dr. I. Immediata and Dr. P. Oliva for helpful technical support.

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