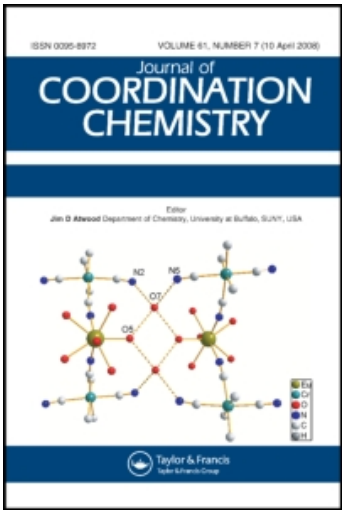



**Journal of Coordination Chemistry**
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713455674

# Synthesis, characterization and cytotoxic/cytostatic activity of Sm(III) and Gd(III) complexes

Irena Kostova[a]; Tsvetanka Stefanova[b]
[a] Faculty of Pharmacy, Department of Chemistry, Medical University, Sofia 1000, Bulgaria [b] Department of Immunology, Institute of Microbiology, Bulgarian Academy of Sciences, Sofia 1113, Bulgaria

**To cite this Article** Kostova, Irena and Stefanova, Tsvetanka(2009) 'Synthesis, characterization and cytotoxic/cytostatic activity of Sm(III) and Gd(III) complexes', Journal of Coordination Chemistry, 62: 19, 3187 — 3197
**To link to this Article: DOI:** 10.1080/00958970903019509
**URL:** http://dx.doi.org/10.1080/00958970903019509

*Journal of Coordination Chemistry*
Vol. 62, No. 19, 10 October 2009, 3187–3197

Taylor & Francis
Taylor & Francis Group

# Synthesis, characterization and cytotoxic/cytostatic activity of Sm(III) and Gd(III) complexes

IRENA KOSTOVA*† and TSVETANKA STEFANOVA‡

†Faculty of Pharmacy, Department of Chemistry,
Medical University, 2 Dunav St., Sofia 1000, Bulgaria
‡Department of Immunology, Institute of Microbiology,
Bulgarian Academy of Sciences, 26 Acad. G. Bonchev St., Sofia 1113, Bulgaria

(*Received 7 February 2009; in final form 19 March 2009*)



New Sm(III) and Gd(III) complexes of deprotonated 4-hydroxy-3[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one (Acenocoumarol) were synthesized and characterized using FT-IR, FT-Raman, NMR spectra, and elemental analyses. The vibrational study gave evidence for the coordination of ligand to lanthanide ions. The ligand and its lanthanide(III) complexes were tested for their cytotoxic/cytostatic activity against two tumor cell lines and peritoneal mouse macrophages. The Sm(III) and Gd(III) complexes exhibit good activity against melanoma B16 and fibrosarcoma L929 and are stronger inhibitors of tumor cell proliferation than the ligand. Besides their cytotoxicity to tumor cells, Acenocoumarol and its gadolinium(III) and samarium(III) complexes modulate NO generation in activated macrophages.

*Keywords*: Acenocoumarol; Sm(III); Gd(III); FT-Raman; FT-IR; Cytotoxic; Cytostatic activity

## 1. Introduction

Coumarins comprise an extended class of natural compounds found throughout the plant kingdom. Biological effects for natural and synthetic coumarins such as anticancer, antibacterial, and antimutagenic properties, scavenging of reactive oxygen species, inhibition of human platelet aggregation, and anti HIV-PR activity have been reported. Comprehensive reviews on the pharmacological activity of a large variety of coumarin derivatives have been reported recently, pointing out the importance of structure–activity relationship [1–4]. Various types of coumarin substitutions in their skeletal structure can influence biological activity. Therefore, a comprehensive structure–system–activity-relationship study of coumarins with special respect to carcinogenicity, mutagenicity, and cancer-preventing activity would be of high interest. Coumarin derivatives are also responsible for significant changes in the regulation of immune response, cell growth, and cell differentiation [5–9]. Current cancer therapies are highly toxic and often nonspecific. A potentially less toxic approach to treating cancer employs agents that modify cancer cell differentiation, termed differentiation therapy. This approach is based on the tacit

*Corresponding author. Email: irenakostova@yahoo.com


ISSN 0095-8972 print/ISSN 1029-0389 online 
DOI: 10.1080/00958970903019509

assumption that many neoplastic cell types exhibit reversible defects in differentiation, which upon appropriate treatment results in tumor reprogramming and a concomitant loss in proliferative capacity and induction of terminal differentiation or apoptosis (programmed cell death). Laboratory studies that focus on elucidating mechanisms of action are demonstrating the effectiveness of differentiation therapy, which is now beginning to show translational promise in the clinical setting. The ability of coumarin derivatives to bind metal ions has increasing interest since their pharmacological response was found to be improved upon complexation [10–13]. Recently, it has been shown that many lanthanide(III) complexes of coumarins might have a potential role in the treatment of different tumor cell lines. Quantitative study on the relationship between structure and reactivity for those compounds requires more derivatives to include into the database besides a large amount of experimental and theoretical data.

In continuation of our ongoing efforts to synthesize metal complexes of different coumarin derivatives [13–21], herein we report the synthesis, chemical, vibrational, and biological properties of Sm(III) and Gd(III) complexes with the biologically active coumarin, Acenocoumarol. Coumarins and their derivatives, natural or synthetic, have diverse toxicities and carcinogenicities with the biological activity depending on constituents of the benzopyrone ring, leading to diverse properties, serving, for example, as fungicide, antibacterial and anti-HIV agents, possessing antithrombotic action, reducing total cholesterol and triglycerides, and causing hypotension *in vivo*, among others. One of the more than 1300 coumarins identified is Acenocoumarol, a 4-hydroxycoumarin that exhibits important biological activities such as anticancer and anticoagulant. Acenocoumarol belongs to the group of vitamin K antagonists, for example Phenprocoumon, Warfarin, and Coumachlor, and is widely used for therapeutic anticoagulation and clinically administered as a racemate.

The vibrational analysis performed here for Acenocoumarol sodium salt helped to explain the vibrational behavior of its complexes, giving evidence for coordination of the ligand to lanthanide ions. Due to their high sensitivity to the structural changes, the FT-Raman and FT-IR spectroscopy were employed for a complete vibrational characterization of the Acenocoumarol sodium structure. Spectra and the marker bands of characteristic functional groups are identified in order to use them as a data bank for further application in trace analysis of rare-earth complexes of Acenocoumarol. The promising biological activity of these compounds led us to perform detailed spectroscopic investigations on their molecular and vibrational structures. Reliable vibrational assignment of the ligand is important to have a good basis for comparison with the vibrational spectra of the complexes. To the best of our knowledge, no detailed vibrational analysis data for Acenocoumarol sodium were yet published. With the present study, we provide deeper insight into the vibrational spectra and suggest the type of binding mode of this biologically active molecule in complex formation.



## 2. Experimental

### 2.1. *Chemistry*

**2.1.1. Reagents.** The compounds used for preparing the solutions were Merck (Germany) products, p.a. grade: $Sm(NO_3)_3 \cdot 6H_2O$, $Gd(NO_3)_3 \cdot 6H_2O$, and

4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one (Acenocoumarol). 4-Hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one sodium salt (Acenocoumarol sodium) was used as ligand for the preparation of metal complex.

**2.1.2. Synthesis.** The synthesis, analytical, and spectral procedures for characterization of the sodium salt of Acenocoumarol sodium salt have been presented earlier [21]. The compound has been characterized and identified based on elemental analysis, conductivities, and NMR spectroscopy.

The complexes were synthesized by mixing water solutions of the respective Ln(III) salts and the ligand, in amounts equal to metal : ligand molar ratio of 1 : 3. The reaction mixture was stirred with an electromagnetic stirrer at 25°C for 1 h. At the moment of mixing of the solutions, precipitates were obtained. The precipitates were filtered, washed several times with water, and dried in a desiccator to constant weight. The complexes were insoluble in water, slightly soluble in methanol and ethanol, and well soluble in DMSO.



**2.1.3. Analytical and spectral methods and instruments.** The carbon, hydrogen, and nitrogen were determined by elemental analysis. The water content was determined by Metron Herizall E55 Karl Fisher Titrator and thermogravimetrically. The metal ion was determined after mineralization and thermogravimetrically. The presence of sodium ion was checked by flame photometry.

The FT-IR spectra of powder samples of Acenocoumarol sodium salt and its complexes were recorded using a FT-IR Equinox 55 Bruker spectrometer with an attenuated total reflectance (ATR) module. An integrated FRA-106 S Raman module was employed for recording the FT-Raman spectra using a Nd : YAG laser operating at 1064 nm for excitation. The laser power was 300 mW and 50 scans were collected for each spectrum. The detection of the Raman signal was carried out with a nitrogen-cooled Ge detector. The spectral resolution was 2 $cm^{-1}$.

The $^1$H-NMR spectra were recorded at room temperature on a Bruker WP 100 (100 MHz) spectrometer in DMSO-$d_6$; chemical shifts are given in ppm.

## 2.2. *Pharmacology*

**2.2.1. Cell culture maintenance.** Mouse melanoma cell line B16 and fibrosarcoma L929 were grown in Dulbeco's modified Eagle's medium (DMEM, Sigma Chemical Co., St Louis, MO, USA), supplied with 10% fetal bovine serum (Sigma), antibiotics (100 U $mL^{-1}$ penicillin, 100 μg $mL^{-1}$ streptomycin; Serva, Feinbiochemica GmbH & Co., Heidelberg, Germany), and 2 mM L-glutamine. The cells grew attached to the plastic at 37°C in a 5% $CO_2$ atmosphere and monolayers were kept in log phase via passage three times per week when 80–85% confluence was reached. Cells were harvested using 0.25% trypsin.

**2.2.2. Macrophage isolation.** Peritoneal exudate cells were obtained by lavage with 10 mL cold Hanks' balanced salt solution (HBSS). After three washings, the cell suspension was adjusted to $4 \times 10^7$ $mL^{-1}$ and seeded (0.1 mL) in 96-well flat-bottom

plates (Corning Inc., Corning, NY, USA). Macrophages were allowed to adhere for 2 h, and nonadherent cells were removed by washing with HBSS. The viability of macrophages was over 95% by trypan blue exclusion test. Various concentrations of the complexes were added to the wells and their cytotoxicity was determined using MTT assay.

**2.2.3. Assay for cytotoxicity.** The MTT reduction assay of Mosmann [22] was used to determine the cytotoxicity of complexes of Acenocoumarol with lanthanum and dysprosium. Briefly, exponentially growing tumor cells were seeded in 96-well plates at a density of $2 \times 10^5$ per well and allowed to adhere for 2 h. Each test compound was dissolved in DMSO and diluted in the cell culture medium. Stock solutions of tested compounds were freshly prepared in DMSO and diluted with the cell culture medium in order to achieve the desired final concentrations. The serial dilutions of tested compounds were prepared immediately before use. At the final dilutions obtained the concentrations of DMSO never exceeded 1%. Then the complexes were added to the wells at concentrations varying from 200 to 6.25 μM and plates were incubated for 72 h at 37C in a humidified 5% $CO_2$ atmosphere. The MTT solution (5 mg mL$^{-1}$) was added to each well (20 μL) and the plates were incubated for an additional 4 h, supernatants were aspirated, and the formazan crystals formed were dissolved by 100 μL acidic (0.04 M HCl) 2-propanol. The optical density was measured at 570 nm.



**2.2.4. Assay for NO synthesis inhibition.** The inhibition of NO synthesis was determined by assay for nitrite, a stable reaction product of NO with molecular oxygen, as previously described [23]. Briefly, peritoneal macrophages ($2.5 \times 10^5$ per well) were activated by incubation in the presence or absence of test complexes for 72 h in the cell culture medium containing 2 μg mL$^{-1}$ lipopolysaccharide (LPS from Salmonella Minnesota, Sigma) and 10 U mL$^{-1}$ recombinant mouse IFN-$\gamma$ (Genzyme, Cambridge, USA). After incubation, 50 μL of supernatants were reacted with an equal volume of Griess reagent (0.5% sulfanilamide, 0.05% N-(1-naphthyl)-ethylenediamine dihydrochloride in 2.5% $H_3PO_4$) for 10 min at room temperature in the dark. The optical densities at 570 nm were measured and nitrite was calculated using $NaNO_2$ to construct the standard curve (200–1.6 μM).

**2.2.5. Statistics.** Values are expressed as mean ± SD. Student's *t*-test was used for statistical analysis. Probability ($p$) values less than 0.05 were considered significant.

## 3. Results and discussion

### 3.1. *Chemistry*

**3.1.1. Coordination of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one to Ln(III).** Reaction of Ln(III) and sodium salt of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one (figure 1) afforded complexes stable both in solid state and in solution. They are insoluble in water and most common organic solvents and slightly soluble in DMSO. Elemental analysis data of the Ln(III)

complexes (table 1) are in agreement with the formula $Ln(L)_3{\cdot}nH_2O$, where $L = C_{19}H_{14}NO_6^-$.

**3.1.2. Vibrational spectral analysis.** The mode of bonding of the ligand to Sm(III) and Gd(III) was elucidated by IR and Raman spectra of the complexes compared with the spectra of the free ligand. The weak band at 3577 $cm^{-1}$ in the IR spectrum of the free ligand shifted to lower wavenumber in the complexes. A broad band characteristic of $\nu$(OH) of crystalline or coordinated water was observed at 3400 $cm^{-1}$ in the IR spectra of the complexes and had higher intensity than the free ligand, attributed to the presence of crystalline water.

The C=O stretching vibration can be easily identified from the IR and Raman spectra, and because of the degree of conjugation, strength, and polarization increase. The carbonyl vibration in ketones normally have strong intensity in the region 1715–1680 $cm^{-1}$. The $\nu$(C=O) bands have high IR intensity in the ligand and in the complex and, therefore, their changes could be tracked. A band at 1713 $cm^{-1}$ in the IR spectrum of the ligand is attributed to stretching of the keto-carbonyl group in the side chain. The band shifted with 20–30 $cm^{-1}$ to lower wavenumber values in the complexes, evidence for the participation of this C=O in coordination. The band at 1648 $cm^{-1}$ corresponds to the carbonyl in the lactone ring and this band is not changed on complex formation.

The O–Na stretch of Acenocoumarol sodium appears at 357 $cm^{-1}$ as a weak intensity band in the Raman spectrum. This band is not observed in the spectra of complexes and hence bidentate binding of the ketone carbonyl group and deprotonated hydroxyl can be suggested. Our calculations for similar complexes predict that $\nu(Ln–O)_{water}$ modes should appear at about 250 $cm^{-1}$. Vibrational modes in Sm(III) and Gd(III) complexes are observed at ~280 $cm^{-1}$. Obviously, the assignment of the Ln–O bands is uncertain and they cannot be used for reliable prediction of the ligand-binding mode.



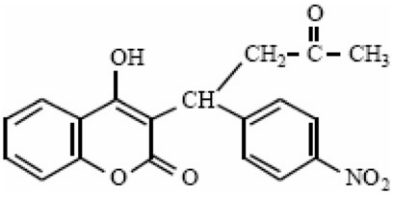



Figure 1. Molecular structure of Acenocoumarol ($C_{19}H_{15}NO_6$).

Table 1. Elemental analysis data for the Sm(III) and Gd(III) complexes of Acenocoumarol.

| | Found/calculated (%) | | | | |
|---|---|---|---|---|---|
| Complex | C | H | N | $H_2O$ | Ln |
| $Sm(L)_3 \cdot 7H_2O$ | 51.33 | 3.82 | 2.99 | 9.25 | 10.97 |
| | 51.35 | 4.20 | 3.15 | 9.46 | 11.26 |
| $Gd(L)_3 \cdot 6H_2O$ | 51.69 | 4.24 | 2.88 | 8.12 | 12.02 |
| | 51.73 | 4.08 | 3.18 | 8.17 | 11.87 |

$L = C_{19}H_{14}NO_6^-$.

The band at 1596 $cm^{-1}$ in the IR spectrum of the free ligand, related to the stretching vibrations of the conjugated olefinic system, remains almost the same in spectra of the complexes. The ring C–H stretch is observed in the spectra of the ligand as a medium intensity band at 3118 $cm^{-1}$ in IR and a weak band in Raman at 3090 $cm^{-1}$ for Ph1 and Ph2, respectively. The vibrations in the region 1500–1400 $cm^{-1}$ correspond to the aromatic system. The C–C stretch of Ph1 is observed at 1505 and 1512 $cm^{-1}$ in IR and Raman, respectively, mixed with the C–H stretch; the C–C stretch of Ph2 is observed as a weak band in Raman at 1482 $cm^{-1}$. All of the above-discussed bands are slightly changed in complexes.

Generally the asymmetric stretch of aromatic nitro gives a very strong band between 1560 and 1500 $cm^{-1}$ [24]. The most intense band measured at 1596 and 1593 $cm^{-1}$ (as a medium in IR and strong in Raman, respectively) can be ascribed as the asymmetric stretch of $NO_2$ group. The symmetric stretch of this vibration usually appears as intense band in IR and Raman spectra between 1390 and 1330 $cm^{-1}$, respectively [25]. In Acenocoumarol sodium the $NO_2$ symmetric stretch is identified at 1338 $cm^{-1}$ as a medium band in IR. All the bands attributed to $NO_2$ show insignificant change in spectra of the lanthanide complexes, suggesting that this group does not participate in the complex formation.

The vibrational wavenumbers, their IR intensities, and Raman activities, as well as the tentative assignments for Acenocoumarol sodium and its Sm(III) and Gd(III) complexes, are presented in tables 2 and 3. The different regions of the FT-IR and FT-Raman spectra are given in Supplementary Material.



**3.1.3. $^1$H-NMR analysis.** Proton spectra of the compounds, recorded at 100 MHz in DMSO-$d_6$, were used to confirm formation of the complexes. The chemical shifts are given in $\delta$-scale. The typical chemical shifts of the $^1$H-NMR spectra in DMSO-$d_6$ solvent are shown in table 4. Differences of the chemical shifts confirmed the expected coordination of the ligand through deprotonated hydroxyl and carbonyl oxygens in agreement with the literature data [13, 21]. Comparison of the $^1$H-NMR spectra of the complexes with those of the ligand and its sodium salt reveals that resonances due to protons of the ligand are considerably broadened and shifted indicating complexation. This is consistent with previously reported values on similar compounds available

Table 2. The FT-IR band positions and assignments of Acenocoumarol sodium and its Sm(III) and Gd(III) complexes.

| $\nu_{IR}$ ($cm^{-1}$) | | | | |
|---|---|---|---|---|
| Acenocoumarol sodium | Sm(III) complex | Gd(III) complex | IR Int. | Assignments |
| 3118 m | 3240 | 3230 | 1.2 | C–H stretch of Ph2 |
| 2932 w | 2930 | 2946 | 5.5 | $CH_3$ sym. stretch + $CH_2$ sym. Stretch |
| 1713 m | 1699 | 1694 | 124.1 | C = O (keto) stretch |
| 1648 m | 1651 | 1651 | 456.5 | C = O (lactone) stretch |
| 1596 m | 1595 | 1596 | 140.3 | C–C stretch of Ph2 + $NO_2$ asym. Stretching |
| 1505 s | 1510 | 1511 | 379.3 | C–C stretch of Ph1 + C–C stretch of lactone ring |
| 1338 m | 1343 | 1344 | 454.1 | $NO_2$ sym. stretching + C–H in plane bending |
| 993 m | 1004 | 1004 | 3.5 | Radial skeletal of Ph2 |
| 755 m (splitting) | 761 | 761 | 44 | C–H out of plane of Ph1 |

Ph2 is phenyl of the side chain and Ph1 is phenyl of the coumarin structure.

in the literature, namely, lanthanide(III) complexes of Warfarin (4-hydroxy-3-(3-oxo-1-phenylbutyl)-2H-1-benzopyran-2-one) [20], Coumachlor (4-hydroxy-3[1-(4-chlorophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one) [20], and Ce(III), La(III), and Nd(III) complexes of Acenocoumarol (4-hydroxy-3[1-(4-nitrophenyl)-3-oxobutyl]- 2H-1-benzopyran-2-one) [13, 21]. $^1$H-NMR shifts of this order are typical for lanthanide coordination compounds and different metal complexes of coumarins [13–21].

Dimethyl sulfoxide (DMSO) was used for the NMR for solubility, but could bind to the metal, fast on the NMR timescale. Recently, we reported a thorough study on the stability of Nd(III) complex of Acenocoumarol in DMSO, and the stepwise formation of this complex was studied in detail by spectrophotometric method [13]. The present work agrees with those reported in the literature.

Table 3. The FT-Raman band positions and assignments of Acenocoumarol sodium and its Sm(III) and Gd(III) complexes.

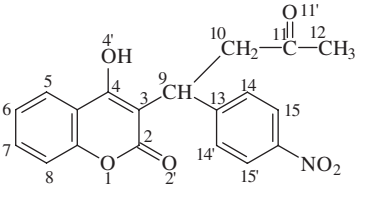
| $\nu_{Raman}$ ($cm^{-1}$) | | | | |
|---|---|---|---|---|
| Acenocoumarol sodium | Sm(III) complex | Gd(III) complex | Raman Act. | Assignments |
| 3083 w | 3077 | 3079 | 250 | C–H stretch of Ph1 |
| 3062 w | | | 51.1 | C–H stretch of Ph2 |
| 2931 w | 2917 | 2918 | 175.4 | $CH_3$ sym. stretch + $CH_2$ sym. stretch |
| 2888 w | | | 62.2 | |
| 1593 s | 1594 | 1594 | 11.7 | C–C stretch of Ph2 + $NO_2$ asym. stretching |
| 1482 w | 1484 | 1485 | 10.8 | C–C stretch of Ph2 |
| 1338 m | 1344 | 1344 | 1029.2 | $NO_2$ sym. stretching + C–H in plane Bending |
| 1117 s | 1110 | 1111 | 14 | C–H in plane bending of Ph1 |
| 357 w | – | – | 8.4 | O–Na stretch |
| – | 285 | 288 | 8.4 | O–Ln stretch |

Ph1 is the phenyl of the coumarin structure and Ph2 is the phenyl of the side chain.

Table 4. The $^1$H-NMR spectral data (100 MHz; DMSO-$d_6$) of Acenocoumarol sodium and its complexes.

| | $\delta$ (ppm)[c] | | | | |
|---|---|---|---|---|---|
| Compound | $H_5$–$H_8$ | $H_9$ | $H_{10}$ | $H_{12}$ | $H_{14,15}$ |
| $C_{19}H_{15}NO_6$[a] | 7.36–7.66 m | 4.18 s | 3.47 | 1.94 | 7.83–8.12 dd |
| $C_{19}H_{14}NaNO_6$[b] | 6.95–7.40 m | 5.05 t | 3.40 d | 3.05 s | 7.60–8.10 dd |
| $Sm(L)_3 \cdot 7H_2O$ | 7.00–7.37 m | 5.08 s | 3.38 b s | 2.07 s | 7.57–8.04 dd |

[a]$C_{19}H_{15}NO_6$–4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one.
[b]$C_{19}H_{14}NaNO_6$–4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one sodium salt.
[c]The atom numbering is in agreement with the following scheme:



Unfortunately, we were not able to obtain suitable single crystals for X-ray diffraction. Determination of the binding mode or structure based on physicochemical and spectroscopic methods, when crystal structure data are not available, is not a trivial task. Therefore, molecular modeling of the molecular structure was used to suggest the type of the metal coordination. In previous investigation, we performed an accurate density functional theory study of the ligand [18]. The results obtained showed that the most probable reactive sites for electrophilic attack (metal binding) in the ligand are the hydroxyl and carbonyl oxygens. Our IR and Raman spectral data confirm that the hydroxyl and carbonyl oxygens were coordinated. Thus, based on the experimental and theoretical results, we suggest the most probable binding in the metal complexes studied, which is in accord with previously published data on related compounds [13–21].

## 3.2. *Pharmacology*

**3.2.1. Cytotoxicity evaluation.** The cytotoxic/cytostatic activities of the newly synthesized complexes of Acenocoumarol were determined by MTT reduction assay against two mouse tumor cell lines, melanoma B16 and fibrosarcoma L929. Their proliferation inhibitory effect was compared to that of the ligand. They were also tested for cytotoxicity against normal mouse peritoneal macrophages.

Concentation–response curves were constructed (figure 2; Supplementary material). From the curves, $IC_{50}$ values are derived ($n = 3$) and presented in table 5.

Acenocoumarol concentrations exceeding 12.5 μM inhibited proliferation of both tumor cell lines; at 50 μM only *ca* 45% of melanoma B16 and fibrosarcoma L929 cells

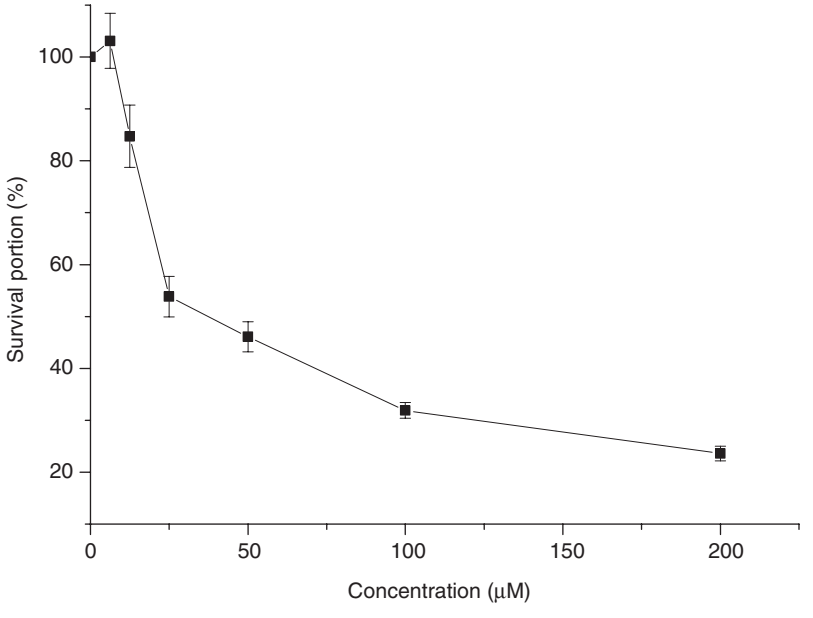



Figure 2. Cytotoxic effect of the gadolinium complex of Acenocoumarol against fibrosarcoma L929 cells. The effect of the compound on the growth and viability of tumor cells was assessed by MTT reduction assay after incubation for 72 h. Results represent mean ± SD of at least three independent experiments.

remained viable and metabolically active. At concentrations over 100 μM, only 35% of tumor cells survived. Acenocoumarol showed less cytotoxicity against normal mouse macrophages with over 60% surviving at the highest concentration of Acenocoumarol tested (200 μM).

Gadolinium(III) complex of Acenocoumarol did not differ significantly from the ligand in its cytotoxicity pattern. At concentrations over 100 μM, ~30% of both tumor cells survived. The complex was less toxic to normal macrophages since the portion of nonviable cells was less than 40% at 200 μM.

According to $IC_{50}$ values, samarium(III) complex of Acenocoumarol was more toxic to melanoma B16 and fibrosarcoma L929 tumor cells than the ligand. At the highest concentration tested (200 μM), less than 20% of both tumor cell lines survived. Samarium(III) complex was much less toxic to normal macrophages.

The results show that both the ligand and its complexes are more toxic to tumor cells than to normal macrophages. Samarium(III) complex of Acenocoumarol showed the highest toxicity to both tumor cell lines.



**3.2.2. NO synthesis inhibition.** The effect of complexes on NO release from IFN-$\gamma$ and LPS-activated macrophages is presented in table 6. The complexes and the ligand were tested in concentrations, which were not toxic for the macrophages. Acenocoumarol at a concentration of 20 μM showed weak, but statistically significant, inhibition in NO release. Gadolinium(III) complex showed similar inhibitory effect at the same concentration. In contrast, samarium(III) complex was able to inhibit NO release at both concentrations tested (20 and 10 μM).

Nitric oxide is a pleiotrophic regulator of physiological functions such as vasorelaxation, bronchodilatation, platelet aggregation, etc, implicated in the regulation and execution of the immune response of the host against pathogens and arising tumors. Macrophage-generated NO mediates the cytotoxic effect of macrophages against tumor cells [26–28]. NO inhibits mitochondrial respiration and DNA replication

Table 5. The $IC_{50}$ values of Acenocoumarol and its gadolinium and samarium complexes derived from the concentration–response curves.

| | $IC_{50}$ value (μM) | | |
|---|---|---|---|
| Cells | Lig | Gd | Sm |
| Melanoma B16 | 45.70 | 45.90 | 35.17 |
| Fibrosarcoma L929 | 41.90 | 37.51 | 32.48 |
| Normal murine macrophages | >200 | >200 | >200 |

Table 6. Nitrite release by IFN-$\gamma$ and LPS-activated peritoneal macrophages in the presence of Acenocoumarol and its gadolinium and samarium complexes.

| Concentration | 20 μM | 10 μM |
|---|---|---|
| Ligand | 37.65 ± 1.27 | 38.47 ± 1.41 |
| Gd | 36.63 ± 1.89 | 38.89 ± 1.03 |
| Sm | 36.02 ± 1.38 | 36.22 ± 2.63 |
| Control | 41.73 ± 1.06 | |

in tumor cells via interaction with intracellular iron–sulfur prosthetic groups of the involved enzymes [29–31]. NO is considered to contribute to the mode of action of some anticancer immunotherapeutic agents with clinical application [32, 33].

Excessive or inappropriate generation of NO is harmful to the host leading to tissue damage and immunosuppression. NO modulates the local and systemic immune response by inhibition of T-cell proliferative responses, decrease in cytotoxicity of NK, and T-cells in tumor-bearing host, suppression of cytokine production, etc [34–38].

Besides their cytotoxicity to tumor cells, Acenocoumarol and its gadolinium and samarium complexes are able to modulate NO generation in activated macrophages.

## 4. Conclusions

The coordination ability of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one was proved in complexation with lanthanide(III) ions. The IR, Raman, and $^{1}$H-NMR spectral analyses confirmed the coordination of 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one through both the hydroxyl and carbonyl oxygens.

Preliminary screening revealed that the lanthanide(III) complexes with 4-hydroxy-3-[1-(4-nitrophenyl)-3-oxobutyl]-2H-1-benzopyran-2-one are potent cytotoxic agents. Taken together, our data give us reason to conclude that the newly synthesized lanthanide(III) complexes should be subjected to more detailed pharmacological and toxicological evaluation.



## Acknowledgements

We gratefully acknowledge the financial support from the Bulgarian Ministry of Education and Science (through the Indo-Bulgarian Project BIn-8/07 under Indo-Bulgarian Intergovernmental Programme of Cooperation in Science & Technology).

## References

[1] I. Kostova. *Curr. Med. Chem. – Anti-Cancer Agents*, **5**, 29 (2005).
[2] I. Kostova. *Mini-Rev. Med. Chem.*, **6**, 365 (2006).
[3] I. Kostova. *Curr. HIV Res.*, **4**, 347 (2006).
[4] I. Kostova. *Exp. Opin. Drug Discov.*, **2**, 1605 (2007).
[5] A.F. Cálgaro-Helena, K.F. Devienne, T. Rodrigues, D.J. Dorta, M.S.G. Raddi, W. Vilegas, S.A. Uyemura, C. Curti. *Chem. Biol. Inter.*, **161**, 155 (2006).
[6] J.H. Lee, H.B. Bang, S.Y. Han, J.-G. Jun. *Tetrahedron Lett.*, **48**, 2889 (2007).
[7] K.F. Devienne, A.F. Cálgaro-Helena, D.J. Dorta, I.M.R. Prado, M.S.G. Raddi, W. Vilegas, S.A. Uyemura, C. Curti. *Phytochemistry*, **68**, 1075 (2007).
[8] J. Yu, L. Wang, R.L. Walzem, E.G. Miller, L.M. Pike, B.S. Patil. *J. Agric. Food Chem.*, **53**, 2009 (2005).
[9] B. Thati, A. Noble, B.S. Creaven, M. Walsh, M. McCann, K. Kavanagh, M. Devereux, D.A. Egan. *Cancer Lett.*, **250**, 128 (2007).
[10] B.S. Creaven, D.A. Egan, K. Kavanagh, M. McCann, A. Noble, B. Thati, M. Walsh. *Inorg. Chim. Acta*, **359**, 3976 (2006).

[11] E. Budzisz, M. Małecka, I.-P. Lorenz, P. Mayer, R.A. Kwiecień, P. Paneth, U. Krajewska, M. Rózalski. *Inorg. Chem.*, **45**, 9688 (2006).
[12] B. Thati, A. Noble, B.S. Creaven, M. Walsh, M. McCann, K. Kavanagh, M. Devereux, D.A. Egan. *Cancer Lett.*, **248**, 321 (2007).
[13] I. Kostova, I. Manolov, M. Radulova. *Acta Pharm.*, **54**, 119 (2004).
[14] I. Kostova, I. Manolov, G. Momekov. *Eur. J. Med. Chem.*, **39**, 765 (2004).
[15] I. Kostova, N. Trendafilova, G. Momekov. *J. Inorg. Biochem.*, **99**, 477 (2005).
[16] I. Kostova, G. Momekov, M. Zaharieva, M. Karaivanova. *Eur. J. Med. Chem.*, **40**, 542 (2005).
[17] I. Kostova, N. Trendafilova, T. Mihailov. *Chem. Phys.*, **314**, 73 (2005).
[18] I. Hubert Joe, I. Kostova, C. Ravikumar, M. Amalanathan, S. Cîntă Pînzaru. *J. Raman Spectrosc.*, (2009). DOI: 10.1002/jrs.2226.
[19] I. Kostova, G. Momekov. *Eur. J. Med. Chem.*, **43**, 178 (2008).
[20] I. Kostova, I. Manolov, S. Konstantinov, M. Karaivanova. *Eur. J. Med. Chem.*, **34**, 63 (1999).
[21] I. Manolov, I. Kostova, S. Konstantinov, M. Karaivanova. *Eur. J. Med. Chem.*, **34**, 853 (1999).
[22] T. Mosmann. *J. Immunol. Methods*, **65**, 55 (1983).
[23] T.H. Stefanova, N.J. Nikolova, R.A. Toshkova, H.O. Neychev. *J. Exp. Ther. Oncol.*, **6**, 107 (2007).
[24] J. Clarkson, W.E. Smith. *J. Mol. Struct.*, **655**, 413 (2003).
[25] M.Ch.R. Delgado, V. Hernandez, J.T.L. Navarrete, S. Tanaka, Y. Yamashita. *J. Phys. Chem. B*, **108**, 2516 (2004).
[26] J.B. Hibbs Jr, R.R. Taintor, Z. Vavrin, E.M. Rachlin. *Biochem. Biophys. Res. Commun.*, **157**, 87 (1988).
[27] J.E. Albina, J.S. Reichner. *Cancer Metastasis Rev.*, **17**, 39 (1998).
[28] C.I. Chang, J.C. Liao, L. Kuo. *Cancer Res.*, **61**, 1100 (2001).
[29] J.C. Drapier, J.B. Hibbs Jr. *J. Clin. Invest.*, **78**, 790 (1986).
[30] M. Wharton, D.L. Granger, D.T. Durack. *J. Immunol.*, **141**, 1311 (1988).
[31] M. Lepoivre, F. Fieschi, J. Coves, L. Thelander, M. Fontecave. *Biochem. Biophys. Res. Commun.*, **179**, 442 (1991).
[32] T. Oshikawa, M. Okamoto, T. Tano, S. Uddin Ahmed, A. Sasai, S. Kan, Y. Moriya, Y. Ryoma, M. Saito, M. Sato. *Int. Immunopharmacol.*, **6**, 764 (2006).
[33] D. Mitropoulos, D. Petsis, A. Kyroudi-Voulgari, M. Kouloukoussa, A. Zervas, C. Dimopoulos. *Nitric Oxide*, **13**, 36 (2005).
[34] J.E. Albina, J. Abate Jr, W. Henry. *J. Immunol.*, **147**, 144 (1991).
[35] H. Bauer, T. Jung, D. Tsikas, D.O. Stichtenoth, J.C. Frölich, C. Neumann. *Immunology*, **90**, 205 (1997).
[36] M.J. Medot Pirenne, M. Heilman, M. Saxena, P.E. McDermott, C.D. Mills. *J. Immunol.*, **163**, 5877 (1999).
[37] P. Lejeune, P. Lagadec, N. Onier, D. Pinard, H. Ohshima, J.F. Jeannin. *J. Immunol.*, **152**, 5077 (1994).
[38] P. Hegardt, B. Widegren, H.-O. Sjögren. *Cell Immunol.*, **200**, 116 (2000).