Journal of the Serbian Chemical Society
JSCS-info@shd.org.rs • www.shd.org.rs/JSCS
J. Serb. Chem. Soc. 86 (5) 459–468 (2021)
JSCS–5434
Original scientific paper

Synthesis, characterization and biological activity of Pt(II) complexes with steroidal thiosemicarbazones

BOŽIDAR R. ČOBELJIĆ[1]*#, MARIJANA B. ŽIVKOVIĆ[2#], IVANA Z. MATIĆ[3], IRENA T. NOVAKOVIĆ[2#], DUŠAN M. SLADIĆ[1#], KATARINA K. ANĐELKOVIĆ[1#] and NATALIJA M. KRSTIĆ[2#]

[1]*University of Belgrade, Faculty of Chemistry, Studentski trg 12–16, P. O. Box 158, Belgrade, Serbia,* [2]*University of Belgrade-Institute of Chemistry, Technology and Metallurgy, Department of Chemistry, Njegoševa 12, 11000 Belgrade, Serbia and* [3]*Institute of Oncology and Radiology of Serbia, Pasterova 14, Belgrade, Serbia*

(Received 11 December, revised and accepted 14 December 2020)

Abstract: In this work, Pt(II) complexes of previously synthesized steroidal thiosemicarbazones were synthesized and characterized. The ligands and their metal complexes were studied by analytical and spectroscopic data (elemental analysis, IR, 1D-NMR and 2D-NMR, HSQC, HMBC, NOESY, COSY), the analysis of which enabled complete ^{1}H and ^{13}C assignments of each compound including *E* and *Z* isomers. All the synthesized ligands and complexes were screened for their cytotoxic and antimicrobial activity. The results demonstrate that the new steroidal thiosemicarbazone complexes were significantly less cytotoxic than the corresponding steroidal thiosemicarbazones. In addition, complexes showed lower antimicrobial activity than the standard drugs, similar to the activity of the starting thiosemicarbazones.

Keywords: 3-oxo-*α*,*β*-unsaturated steroids; hydrazones; square–planar complexes; cytotoxicity; antimicrobial activity.

INTRODUCTION

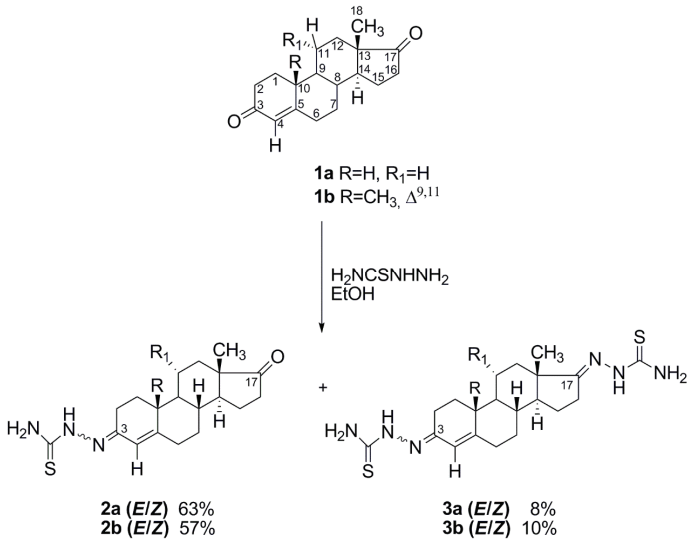
Steroids are a group of biologically active molecules widespread in nature that play a very important role in biological systems. In addition, steroid-based chemotherapeutics are widely used in medicine. Therefore, certain functional and structural modifications of the steroid core by the addition of new functional groups or heterocyclic systems could be very useful, giving compounds with new and more pronounced biological activity.[1] Likewise, substituted thiosemicarbazone derivatives have proven to be very useful because of their interesting biological behaviour.[2] These compounds may act as antidepressants,[3] muscle relax-

* Corresponding author. E-mail: bozidar@chem.bg.ac.rs
# Serbian Chemical Society member.
https://doi.org/10.2298/JSC201211083C

ants, psychoeptic, hypnotics,[4–7] and also show antimicrobial,[2,8,9] antiamoebic,[10] anti-inflammatory,[11,12] and cytotoxic activities.[13] Thiosemicarbazones are compounds that could be used as possible ligands for metal complexes and for the syntheses of heterocyclic compounds.[14–20]

Taking all this into consideration, new steroidal mono- (**2a**,**b**) and bis(thiosemicarbazones) (**3a**,**b**), obtained from 19-norandrost-4-ene-3,17-dione (**1a**) and androsta-4,9(11)-diene-3,17-dione (**1b**), have recently been synthesized[21] (Scheme 1). All these compounds were fully characterized and their biological activity was examined. It was found that 3-thiosemicarbazones **2a** and **2b** exhibit very high cytotoxic actions against all examined cancer cell lines, much higher than the corresponding starting steroids **1a** and **1b** or thiosemicarbazide itself.

In the late 1960s, Rosenberg[22–25] discovered the anticancer activity of cisplatin which began to be used in the treatment of cancer. Since the discovery of cisplatin, to this day, a large number of metal complexes have been synthesized in order to find potential chemotherapeutics with better antitumor potential, higher selectivity in killing cancer cells and fewer side effects.[26]



Scheme 1. Synthesis of steroidal thiosemicarbazones.

Motivated by the aforementioned issues and as a continuation of our work on new hetero-steroid derivatives as biologically active molecules,[1,21,27–30] it was decided to prepare new steroidal complexes with platinum, in the reaction of previously synthesized 3-thiosemicarbazones **2a** and **2b** with cisplatin, and to examine their biological activity and compare the results with the activity of those reported earlier.[21] To the best of our knowledge, very few Pt steroidal complexes have been prepared to date.[31,32]

EXPERIMENTAL

Chemistry

The melting points were determined on a Digital melting point WRS-1B apparatus and are uncorrected. The IR spectra were recorded on a Perkin–Elmer FT-IR 1725X spectrophotometer. The NMR spectra were recorded on a Bruker Avance 500 MHz spectrometer (^{1}H at 500 MHz and ^{13}C at 125 MHz) in DMSO-d_6 at room temperature using $SiMe_4$ as the internal standard, δ/ppm, J/Hz. The HRMS spectra were recorded on an Agilent 6210 LC ESI-MS TOF spectrometer. The elemental analyses (C, H, N and S) were performed by standard micro-methods on a Vario EL III analyzer. The molar conductivities were measured at room temperature (25 °C) on a digital Cond 330i conductivity meter. Thin-layer chromatography (TLC) was performed using aluminum plates coated with Merck silica gel 60 F_{254} and flash column chromatography (FCC) was performed on silica gel Merck 0.040–0.063 mm. The TLC spots were detected with 50 % aq. H_2SO_4 followed by heating. 19-Norandrost-4-ene-3,17-dione and androsta-4,9(11)-diene-3,17-dione were purchased from Galenika AD ($_{Belgrade}$) and recrystallized from a suitable solvent. c*is*-Diaminedichloridoplatinum(II) ($[Pt(NH_3)_2Cl_2]$) was obtained from Sigma–Aldrich.

General procedure for the synthesis of thiosemicarbazones

Thiosemicarbazones **2a** and **2b** were prepared as described earlier.[21] To a solution of steroid (**1a**,**b**, 1 mmol) in dried ethanol (50 mL), thiosemicarbazide (1 mmol) was added. The solution was then allowed to reflux for 5 h under stirring. The pH of the mixture was adjusted to ≈4.5 with CH_3COOH (about 3 mL). After completion of the reaction (monitored by TLC), the solvent was removed under reduced pressure. The residue was chromatographed by FCC using the indicated solvent system. In both cases the products were obtained as inseparable mixtures of *E* and *Z* diastereoisomers.

*19-Norandrost-4-ene-3,17-dione 3-thiosemicarbazone (**2a**) (E/Z=7:3)*

Starting with 270 mg 19-norandrost-4-en-3,17-dione (**la**), elution with toluene/EtOAc (8/2) afforded compound **2a** (217 mg, 63 %).

*Androsta-4,9(11)-diene-3,17-dione 3-thiosemicarbazone (**2b**) (E/Z=8:2)*

Starting with 285 mg androsta-4,9(11)-diene-3,17-dione (**1b**), elution with toluene/ /EtOAc (85/15) afforded compound **2b** (189 mg, 53 %).

*Synthesis of complexes **4** and **5***

*Complex **4** (Pt(II) with ligand **2a**).* Into a solution of 19-norandrost-4-ene-3,17-dione 3-thiosemicarbazone (**2a**, 0.1 mmol, 34.5 mg) in dichloromethane (10 mL) was added cisplatin ($[Pt(NH_3)_2Cl_2]$, 0.1 mmol, 30 mg). The mixture was stirred under reflux for 3 h and filtered. The solution was then allowed to cool slowly to room temperature and then placed in a refrigerator. After six days, a solid yellow precipitate was obtained. The precipitate was filtered off, washed with a small amount of methanol and dried over silica gel to give 16.9 mg (19.4 %) of complex **4.**

*Complex **5** (Pt(II) with ligand **2b**).* Into a solution of androsta-4,9(11)-diene-3,17-dione 3-thiosemicarbazone (**2b**) (0.1 mmol, 35.7 mg) in methanol (10 mL), cisplatin ($[Pt(NH_3)_2Cl_2]$, 0.1 mmol, 30 mg) was added. The reaction mixture was stirred with heating for 3 h at 65 °C and filtered. The solution was then allowed to cool slowly to room temperature and then placed in the refrigerator. After slow evaporation of the solvent in the refrigerator (≈ 7 °C) during six days yellow solid precipitate was obtained. The precipitate was filtered off, washed with a small amount of methanol and dried over silica gel to give 14.8 mg (23.9%) of complex **5**.

The analytic and spectral data for ligands **2a** and **b** and complexes **4** and **5** are given in the Supplementary material to this paper.

Biology

Cytotoxicity assay. The cytotoxic activity of the compounds was evaluated against three human malignant cell lines: cervical adenocarcinoma (HeLa), chronic myelogenous leukemia (K562), and acute T-cell leukemia Jurkat cell line. The cytotoxicity assay procedure has been described elsewhere.[1,21,30] The positive control was the chemotherapy drug cisplatin. Survival of cells was determined by the MTT assay after 72 h of continuous action, according to the method of Mosmann,[33] which was modified by Ohno and Abe,[34] and described in detail in previous studies.[28–30] All tested cell lines were obtained from the American Type Culture Collection (Manassas, VA, USA).

Antimicrobial activity. The antibacterial activity was evaluated using two different strains of bacteria, one Gram-positive bacteria: *Clostridium sporogenes* (ATCC 19404), and one Gram-negative bacteria: *Pseudomonas aeruginosa* (ATCC 9027). Amikacin (30 μg/100 μL H_2O) was used as the positive control, while water and DMSO served as negative controls. Antibacterial activity was determined by the well diffusion method,[35] as described in detail in a previous study.[1] The fungus tested was *Aspergillus brasiliensis* (ATCC 16404). Nystatin (30 μg/100 μL DMSO) was used as the positive control, while DMSO served as the negative control. The antifungal activity was determined according to the method described in detail in a previous study.[1]

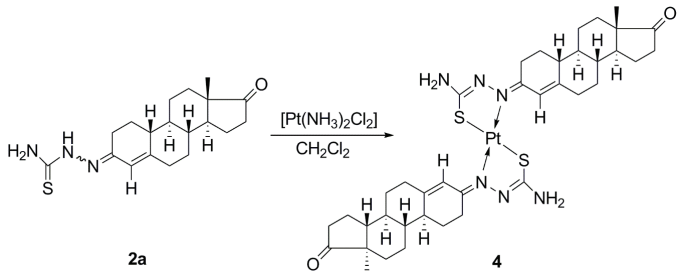
The brine shrimp test. The brine shrimp test of toxicity was performed against freshly hatched nauplii of *Artemia salina*.[36] The method was slightly modified as described earlier.[29] The compounds were dissolved in DMSO and diluted by artificial seawater until the concentration range of 0.01–0.50 mg mL^{-1} was obtained. The final concentration of DMSO was 1 % and did not cause changes of viability of nauplii. The number of nauplii was approximately 20. Surviving nauplii were counted after 24 h, and LC_{50} (concentration lethal to 50 % of the nauplii) were determined after statistical analysis. All the tests were performed in triplicate.

RESULTS AND DISCUSSION

Steroidal thiosemicarbazones **2a** and **b** were prepared as described earlier,[21] starting from 19-norandrost-4-ene-3,17-dione (**1a**) or androsta-4,9(11)-diene--3,17-dione (**1b**) and thiosemicarbazide in EtOH in the presence of CH_3COOH. As the *α*,*β*-unsaturated 3-carbonyl group is more active than the 17-carbonyl group, the reaction was conducted by controlling an equimolar ratio of **1a**,**b** and thiosemicarbazide (1:1) to give **2a**,**b** in the yields of 63 and 57 %, respectively. Nevertheless, bis(thiosemicarbazones) **3a**,**b** were obtained as well in a small amount (8 and 10 %), even under such conditions (Scheme 1).

All synthesized compounds were fully characterized by their analytical and spectroscopic data (HRMS, IR, 1D-NMR and 2D-NMR, HSQC, HMBC, NOESY, COSY). The ^{1}H- and ^{13}C-NMR analysis revealed the presence of two diastereoisomers, which could not be separated.[21] Therefore, compounds **2a** and **b** were used as ligands for complexation reactions with $[Pt(NH_3)_2Cl_2]$ in the form of mixtures of both isomers (*E* and *Z*).

Reaction of 19-norandrost-4-ene-3,17-dione 3-thiosemicarbazone **2a** with $[Pt(NH_3)_2Cl_2]$ (mole ratio 1:1) in dichloromethane (CH_2Cl_2) gave amorphous solid compound **4** soluble in DMSO. Elemental analysis showed that complex **4** contains two molecules of the ligand (Scheme 2).



Scheme 2. Synthesis of complex **4**.

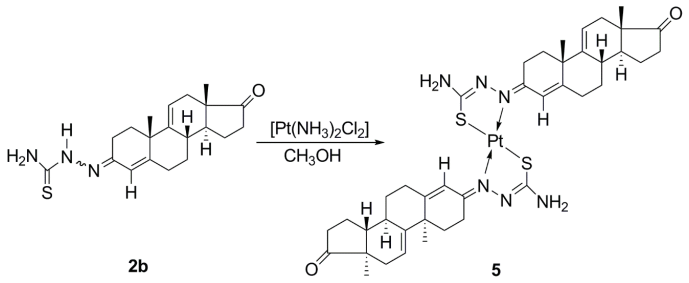
In the IR spectrum of **4**, the absorption band at 1736 cm^{-1} for the C(17)=O carbonyl was unchanged as well as sharp bands in the region 3246–3422 cm^{-1}, originating from the ν(N–H) stretching. On the other hand, the absorption bands attributed to the ν(C=N) stretching vibration appeared at higher frequencies (1528 and 1607 cm^{-1}) compared to the ones for the ligand (1497 and 1586 cm^{-1}), indicating an interaction between the azomethine nitrogen and platinum. The ^{1}H--NMR spectrum of complex **4** contained only one set of signals even though the reaction was performed with a mixture of the diastereomers (*E* and *Z*). The signal for Hα-2 proton was shifted to a lower field and was now at about 3.40 ppm, partially covered by a signal from DMSO in which the spectra were recorded. The singlet for the olefin H-4 proton occurs at δ = 6.52 ppm and the signal for H_2N protons at 6.70 ppm as an extended singlet. Besides, there is a noticeable lack of signal for the H-N proton, which in the ligand was at δ = 10.07 ppm for (*E*)- and at 10.32 ppm for (*Z*)-isomer.

In the ^{13}C-NMR spectrum, again only one set of signals was observed, indicating that the newly formed complex was symmetric. The characteristic signals were at δ / ppm = 24.8 (C-2), 25.7 (C-1), 41.4 (*d*, C-10), 122.1 (C-4), 153.3 (C-5), 161.3 (C-3), 172.3 (C=S) and 219.3 (C-17). Furthermore, the values for C-4 and C-10 suggest that the ligand in complex **4** is the (*E*)-isomer.

Complex **5** was obtained in the direct reaction of androsta-4,9(11)-diene--3,17-dione 3-thiosemicarbazone (**2b**) and cisplatin (mole ratio 1:1) in methanol (CH_3OH). The reaction mixture was stirred for 3 h at 65 °C. After filtration and slow evaporation of the solvent in a refrigerator (≈7 °C) during six days, yellow solid precipitate, complex **5**, soluble in DMSO was obtained (Scheme 3).

The band at 1739 cm^{-1} corresponding to the carbonyl group at C-17 is still visible in the IR spectrum of the synthesized complex. Moreover, the sharp bands in the 3289–3455 cm^{-1} region remain unchanged and are attributed to ν(NH_2)

vibrations. In addition, the absorption bands attributed to the ν(C=N) vibrations appeared at higher frequencies (1518 and 1608 cm^{-1}) compared to those in the corresponding ligand (1502 and 1585 cm^{-1}), indicating an interaction between the azomethine nitrogen and platinum.



Scheme 3. Synthesis of complex **5**.

The ^{1}H-NMR spectrum of complex **5** also contains only one set of signals. The spectrum showed doublet for the H-11 olefinic proton at 5.31 ppm (J = 5 Hz), the singlet for H-4 proton at δ 6.38 ppm and an extended singlet at 6.78 ppm for the NH_2 protons. The signal for Hα-2 proton was shifted to a lower field and was now at 3.36 ppm, partially covered by a signal from DMSO in which the spectra were recorded. There is also a noticeable lack of a signal for the H-N proton, which in the ligand was at δ 10.09 ppm for the (*E*)- and 10.36 ppm for the (*Z*)-isomer.

In accordance with the above, the ^{13}C-NMR spectrum also showed only one set of signals indicating that, regardless of the fact that the reaction was performed with a mixture of isomers, in the complex **5** formation only one, probably (*E*)-isomer, participates. The characteristic signals were at δ/ppm: 24.3 (C-2), 40.4 (C-10), 121.3 (C-4), 160.6 (C-3), 156.6 (C-5), 172.9 (C=S) and 220.2 (C-17).

The molar conductivity values of **4** and **5** in DMSO were 3.4 and 1.9 μS cm^{-1}, respectively, indicating that both complexes are non-electrolytes and are stable in DMSO. Bearing all these facts in mind, as well as the results of elemental analysis, the square–planar complexes **4** and **5** consist of two deprotonated semicarbazone ligands coordinated to the metal ion *via* two thiolate sulfur atoms in the *trans* position and two azomethine nitrogen atoms.

In vitro *cytotoxic activity*

The cytotoxic activities of steroidal thiosemicarbazones **2a** and **2b**, and their Pt(II) metal complexes **4** and **5** were examined against cervical adenocarcinoma (HeLa), chronic myelogenous leukemia (K562) and acute T-cell leukemia Jurkat cell line with cisplatin used as the positive control. As shown in Table I, the steroidal platinum(II) complexes **4** and **5** were almost inactive against HeLa and K562 cells, while both complexes exhibited low cytotoxicity against the Jurkat

cell line. These results demonstrate that new steroidal thiosemicarbazone complexes were significantly less active than the corresponding steroidal thiosemicarbazones **2a** and **2b**.

TABLE I. The *in vitro* cytotoxic activity of compounds **2a**, **2b**, **4** and **5** (concentration which induced 50 % decrease (IC_{50}±*SD*, μM) in malignant cell survival); CDDP: c*is*-diamine-dichloridoplatinum(II)

Compound	HeLa	K562	Jurkat
4	>200	187.99±16.98	139.91±9.48
5	>200	>200	164.60±22.50
2a	18.1±3.3	11.3±2.2	n.a.
2b	17.3±6.8	6.7±0.3	n.a.
CDDP	4.60±0.07	6.00±0.59	3.44±0.19

In vitro *antimicrobial activity*

The *in vitro* antimicrobial activity of steroidal thiosemicarbazones and their metal complexes were assayed by the agar well diffusion method using cultures of *C. sporogenes*, *P. aeruginosa* and *A. brasiliensis*. The results (Table II) showed that only complex **5** had a very weak antibacterial activity, similar to the activity of the starting thiosemicarbazones, while neither of the complexes exhibited any antifungal activity.

TABLE II. Antimicrobial activity (Inhibition zone, mm) of the investigated compounds tested by the agar well diffusion method

Compound	Culture		
	C. sporogenes	*P. aeruginosa*	*A. brasiliensis*
4	–	–	–
5	10	10	–
2a	10	10	10
2b	10	10	10
Amikacin	22	20	–
Nystatin	–	–	30

The brine shrimp test

The LC_{50} values obtained for the ligands and newly synthesized complexes are given in Table III. All the examined compounds were found to be less toxic

TABLE III. Results of the Brine shrimp test for the investigated compounds; CDDP: c*is*-diaminedichloridoplatinum(II)

Compound	LC_{50} / mM
4	0.079
5	0.035
2a	0.597
2b	0.663
CDDP	0.006

when compared to cisplatin. However, the complexes were found to be ten times more active in the brine shrimp assay compared to the ligands.

CONCLUSION

The reactions of 19-norandrost-4-ene-3,17-dione 3-thiosemicarbazone and androsta-4,9(11)-diene-3,17-dione 3-thiosemicarbazone with cisplatin gave neutral square–planar Pt(II) complexes that consist of two deprotonated hydrazone ligands coordinated to metal ion *via* two thiolate sulfur and two azomethine nitrogen atoms.

The new steroidal thiosemicarbazone complexes were almost inactive against HeLa and K562 cells, while both complexes exhibited some cytotoxicity against the Jurkat cell line. Complex **5** showed a very weak antibacterial activity, similar to the activity of the starting thiosemicarbazones, while neither of the complexes had any antifungal activity.

Platinum complexes mostly exert their activity by covalent modification of DNA. The relatively low activity of the complexes synthesized in this work might be attributed to the absence of a good leaving group and/or high stability of the chelate complexes. Since it is excepted that platinum steroidal complexes can readily pass through cell membranes, bind to cytosolic steroid receptors and migrate to the cell nucleus, further research will be directed to the synthesis of platinum steroid complexes with enhanced reactivity with DNA.

Acknowledgment. This work was financially supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia (Grant Nos. 451-03-68/ /2020-14/200168 and 451-03-68/2020-14/200026).

И З В О Д

СИНТЕЗА, КАРАКТЕРИЗАЦИЈА И БИОЛОШКА АКТИВНОСТ КОМПЛЕКСА Pt(II) СА СТЕРОИДНИМ ТИОСЕМИКАРБАЗОНИМА

БОЖИДАР Р. ЧОБЕЉИЋ[1], МАРИЈАНА Б. ЖИВКОВИЋ[2], ИВАНА З. МАТИЋ[3], ИРЕНА Т. НОВАКОВИЋ[2], ДУШАН М. СЛАДИЋ[1], КАТАРИНА К. АНЂЕЛКОВИЋ[1] и НАТАЛИЈА М. КРСТИЋ[2]

[1]Универзитет у Београду, Хемијски факултет, Студентски трг 12–16, 11000 Београд, [2]Универзитет у Београду-Институт за хемију, технологију и металургију, Центар за хемију, Његошева 12, 11000 Београд и [3]Институт за онкологију и радиологију Србије, Пастерова 14, 11000 Београд

Почевши од претходно синтетисаних стероидних тиосемикарбазона, у овом раду су синтетисани и окарактерисани комплекси платине(II). Лиганди и њихови метални комплекси проучавани су аналитичким и спектроскопским методама (елементална анализа, ИЦ, 1D NMR и 2D NMR, HSQC, HMBC, NOESY, COSY). Анализом добијених података омогућена је потпуна ^{1}H и ^{13}C асигнација свих једињења укључујући *E* и *Z* изомере. За синтетисане лиганде, као и њихове комплексе испитивана је цитотоксична и антимикробна активност. Резултати указују на то да нови стероидни тиосемикарбазонски комплекси испољавају значајно нижу цитотоксичност од одговарајућих стероидних тиосемикарбазона. Поред тога, комплекси поседују антимикробну активност сличну активности полазних тиосемикарбазона, а нижу од стандардних лекова.

(Примљено 11. децембра, ревидирано и прихваћено 14. децембра 2020)

REFERENCES

1. M. B. Živković, I. T. Novaković, I. Z. Matić, D. M. Sladić, N. M. Krstić, *Steroids* **148** (2019) 36 (https://doi.org/10.1016/j.steroids.2019.04.010)
2. S. A. Khan, P. Kumar, R. Joshi, P. F. Iqbal, K. Saleem, *Eur. J. Med. Chem.* **43** (2008) 2029 (https://doi.org/10.1016/j.ejmech.2007.12.004)
3. N. Ergenç, N. S. Günaya, R. Demirdamar, *Eur. J. Med. Chem.* **33** (1998) 143 (https://doi.org/10.1016/S0223-5234(98)80039-1)
4. T. A. Voronina, N. M. Tozhanova, *Farmakol. Toksikol.* **44** (1981) 155 (https://www.ncbi.nlm.nih.gov › pubmed)
5. K. Swathi, M. Sarangapani, *World J. Pharm. Pharm. Sci.* **3** (2014) 2720 (https://www.wjpps.com/wjpps_controller/archive_show/2014/VOLUME%203,%20FEBRUARY,%20ISSUE%202)
6. V. Mishra, S. N. Pandeya, *Acta Pharm.* **51** (2001) 183 (http://acta.pharmaceutica.farmaceut.org/18301.html)
7. N. Ergenç, G. Çapana, N. S. Günaya, S. Özkırımlı, M. Güngör, S. Özbey, E. Kendic, *Arch. Pharm. (Weinheim, Ger.)* **332** (1999) 343 (https://doi.org/10.1002/(SICI)1521-4184(199910)332:10<343::AID-ARDP343>3.0.CO;2-0)
8. S. A. Khan, A. M. Asiri, *Chin. J. Chem.* **30** (2012) 1901 (https://doi.org/10.1002/cjoc.201200126)
9. E. Bermejo, R. Carballo, A. Castiñeiras, R. Domínguez, A. E. Liberta, C. Maichle-Mössmer, D. X. West, *Z. Naturforsch.* **54b** (1999) 777 (https://doi.org/10.1515/znb-1999-0613)
10. S. Singh, F. Athar, M. R. Maurya, A. Azam, *Eur. J. Med. Chem.* **41** (2006) 592 (https://doi.org/10.1016/j.ejmech.2006.01.014)
11. M. Asif, A. Husain, *J. Appl. Chem. (London, U. K.)* **2013** (2013), Article ID 247203 (http://dx.doi.org/10.1155/2013/247203)
12. K. Verma, S. N. Pandeya, U. K. Singh, S. Gupta, P. Prashant, Anurag, G. Bhardwaj, *Int. J. Pharm. Sci. Nanotech.* **1** (2008) 357 (https://doi.org/10.37285/ijpsn.2008.1.4.7)
13. J. A. Lessa, I. C. Mendes, P. R. O. da Silva, M. A. Soares, R. G. dos Santos, N. L. Speziali, N. C. Romeiro, E. J. Barreiro, H. Beraldo, *Eur. J. Med. Chem.* **45** (2010) 5671 (https://doi.org/10.1016/j.ejmech.2010.09.021)
14. J. M. Pérez, A. I. Matesanz, A. Martín-Ambite, P. Navarro, C. Alonso, P. Souza, *J. Inorg. Biochem.* **75** (1999) 255 (https://doi.org/10.1016/S0162-0134(99)00096-3)
15. L. H. Hall, S. Y. Cheen, J. B. Barnes, D. X. West, *Met.-Based Drugs* **6** (1999) 143 (http://dx.doi.org/10.1155/MBD.1999.143)
16. K. H. Reddy, P. S. Reddy, P. R. Babu, *J. Inorg. Biochem.* **77** (1999) 169 (https://doi.org/10.1016/S0162-0134(99)00188-9)
17. P. F. Kelly, A. M. Z. Slawin, A. Soriano-Rama, *J. Chem. Soc. Dalton Trans. (1972––1999)* (1996) 53 (https://doi.org/10.1039/DT9960000053)
18. D. X. West, S. B. Pardhye, P. B. Sonawane, *Struct. Bonding (Berlin, Ger.)* **76** (1991) 1 (https://doi.org/10.1007/3-540-53499-7_1)
19. A. E. Liberta, D. X. West, *BioMetals* **5** (1992) 121 (https://doi.org/10.1007/BF01062223)
20. D. X. West, A. E. Liberta, S. B. Padhye, R. C. Chikate, P. B. Sonawane, A. S. Kumbhar, R. G. Yerande, *Coord. Chem. Rev.* **123** (1993) 49 (https://doi.org/10.1016/0010-8545(93)85052-6)
21. M. B. Živković, I. Z. Matić, M. V. Rodić, I. T. Novaković, D. M. Sladić, N. M. Krstić, *RSC Adv.* **6** (2016) 34312 (https://doi.org/10.1039/c6ra01516f)

22. B. Rosenberg, L. Van Camp, T. Krigas, *Nature* **205** (1965) 698 (https://doi.org/10.1038/205698a0)
23. B. Rosenberg, L. Van Camp, J. E. Trosko, V. H. Mansour, *Nature* **222** (1969) 385 (https://doi.org/10.1038/222385a0)
24. P. J. Loehrer, L. H. Einhorn, *Ann. Intern. Med.* **100** (1984) 704 (https://doi.org/10.7326/0003-4819-100-5-704)
25. Z. Guo, P. J. Sadler, *Angew. Chem. Int. Ed.* **38** (1999) 1512 (https://doi.org/10.1002/(SICI)1521-3773(19990601)38:11<1512::AID-ANIE1512>3.0.CO;2-Y)
26. A. R. Savić, *PhD Thesis*, University of Belgrade, Faculty of Chemistry, 2014 (in Serbian) (https://cherry.chem.bg.ac.rs/handle/123456789/2703)
27. N. M. Krstić, M. S. Bjelaković, M. M. Dabović, V. D. Pavlović, *Molecules* **15** (2010) 3462 (https://doi:10.3390/molecules15053462)
28. N. M. Krstić, M. S. Bjelaković, V. D. Pavlović, K. Robeyns, Z. D. Juranić, I. Matić, I. Novaković, D. M. Sladić, *Steroids* **77** (2012) 558 (https://doi.org/10.1016/j.steroids.2012.02.001)
29. N. M. Krstić, V. D. Pavlović, I. T. Novaković, I. Z. Matić, D. M. Sladić, *Mol. Diversity* **17** (2013) 547 (https://doi.org/10.1007/s11030-013-9455-9)
30. N. M. Krstić, I. Z. Matić, Z. D. Juranić, I. T. Novaković, D. M. Sladić, *J. Steroid. Biochem. Mol. Biol.* **143** (2014) 365 (http://dx.doi.org/10.1016/j.jsbmb.2014.06.005)
31. A. Murugkar, B. Unnikrishnan, S. Padhye, R. Bhonde, S. Teat, E.Triantafillou, E. Sinn, *Met.-Based Drugs* **6** (1999) 177 (http://dx.doi.org/10.1155/MBD.1999.177)
32. Y. Huang, E. Kong, C. Gan, Z. Liu, Q. Lin, J. Cui, *Bioinorg. Chem. Appl.* (2015), Article ID 742592 (http://dx.doi.org/10.1155/2015/742592)
33. T. Mosmann, *J. Immunol. Methods* **65** (1983) 55 (https://doi:org/10.1016/0022-1759(83)90303-4)
34. M. Ohno, T. Abe, *J. Immunol. Methods* **145** (1991) 199 (https://doi:org/10.1016/0022-1759(91)90327-C)
35. C. Perez, M. Pauli, P. Bazerque, *Acta Biol. Med. Exp*. **15** (1990) 113
36. B. N. Meyer, N. R. Ferrigni, J. E. Putnam, L. B. Jacobsen, D. E. Nichols, J. L. McLaughlin, *Planta Med.* **45** (1982) 31 (http://dx.doi.org/10.1055/s-2007-971236).