Synthesis, antimicrobial and *in vitro* antiproliferative activity of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) against tumor cells

Petja Marinova, Marin Marinov, Yana Feodorova, Maria Kazakova, Danail Georgiev, Elena Trendafilova, Plamen Penchev, Victoria Sarafian, Neyko Stoyanov

Abstract: *This work presents a method for synthesis, cytotoxicity and antibacterial activity* of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin). *The structure of the obtained product was described by UV-Vis, IR, FT-IR ATR and Raman spectroscopy. In the present investigation we have studied cytotoxic activity of* 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) *on the retinoblastoma cell line WERI-Rb-1 and antibacterial activity against both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli bacteria and the yeasts Candida albicans.*

Key words: *4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin), cytotoxic effect, antimicrobial activity.*

INTRODUCTION

Hydantoin derivatives have important biochemical and pharmacological properties. Several hydantoin derivatives are used as antiepileptic drugs [1,2], others were found to show antiproliferative activity [3,4] and aldosoreductase inhibition [5]. Thioanalogues of hydantoins are also of interest, and their structure and biological activities were also studied [6,7]. Recently, we undertook a systematic study on the synthesis [8], structure [9,10] and complexation properties [11–13] of various dithiohydantoins (imidazolidine-2,4-dithiones) as well as on their complexes with copper, nickel and platinum. Fluorene derivatives attract attention due to their luminescent and electroluminescent properties, caused by the inter- and intra-molecular charge distribution. The most powerful organic light emitting diodes (OLED) are based on fluorene-containing compounds [14,15].

Hydantoin derivatives possess a wide variety of biochemical and pharmacological activities. Several of them are well-known anticonvulsive drugs [16] whereas others have been suggested to act as antiarrhythmics and antimicrobial agents, skeletal muscle relaxants and nonsteroidal antiandrogens [17]. More recently, antitumor effect of hydantoin derivatives has been described by several authors [18,19]. There are not many studies researching their anticancer and antimicrobial effects.

The aim of this investigation is to present a method for synthesis of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin), its structural elucidation and to examine its cytotoxic and antimicrobial activity.

EXPERIMENTAL

Instrumentation and methods

All used chemicals were purchased from Merck and Sigma-Aldrich. Melting point temperature of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) was determined by a SMP-10 digital melting point apparatus. The purity of the compound was checked by thin layer chromatography on Kieselgel 60 F254, 0.2 mm Merck plates, eluent system (vol. ratio): benzene : acetone = 5 : 1. Electronic spectrum was measured on a Lambda 9 Perkin-Elmer UV/Vis/NIR Spectrophotometer from 200 nm to 1000 nm. The IR spectrum of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) was registered in KBr pellet on a Bruker FT-IR VERTEX 70 Spectrometer from 4000 cm^{-1} to 400 cm^{-1} at resolution 2 cm^{-1} with 25 scans. Attenuated Total Reflection FTIR (ATR) spectrum was registered on the same instrument by ATR accessory MIRacle™ with a one-reflection ZnSe element (Pike); the stirred crystals of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) were pressed by an anvil to the reflection element; the spectrum was from 4500 cm^{-1} to 600 cm^{-1} at resolution 2 cm^{-1} with 16 scans. The Raman spectrum of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) (the stirred crystals placed in aluminium disc) was measured on a RAM II (Bruker Optics) with a focused laser beam of 200 mW power of Nd:YAG laser (1064 nm) from 4000 cm^{-1} to 51 cm^{-1} at resolution 2 cm^{-1} with 25 scans.

WST-1 cell proliferation assay

The 4'-bromo-(9'-fluorene)-spiro-5-hydantoin solved in 100% DMSO was diluted in RPMI-1640 culture medium containing penicillin and streptomycin and brought to a concentration of 500 μM. The concentration of DMSO was thus decreased to 0.2 % which did not itself influence cell viability. The cytotoxic effect of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) was determined on the retinoblastoma cell line WERI-Rb-1 using Cell Proliferation Reagent WST-1 (Roche Applied Science). Cells were seeded in triplicates at a density of 6.5 x 10^4 cells/well in a 96-well plate. After a cultivation period of 24 h at 37°C and 5% CO_2, 100 μl of the compound solution were added to the wells making up a final volume of 200 μl. Cells grown in culture medium alone or with appropriate concentrations of DMSO were used as controls. The cytotoxic effect of the compound was measured at three different time points – after 24 h, 48 h and 72 h. At each time point WST-1 reagent was added to the cells. After an incubation period of 4 h at 37°C and 5% CO_2 the absorbance was measured using a microplate ELISA SUNRISE reader at a wavelength of 450 nm with a reference filter at 620 nm.

The percentage of viable cells was calculated as a ratio of the OD value of the sample to the OD value of the control. The data are presented as mean ± standard deviation of the mean.

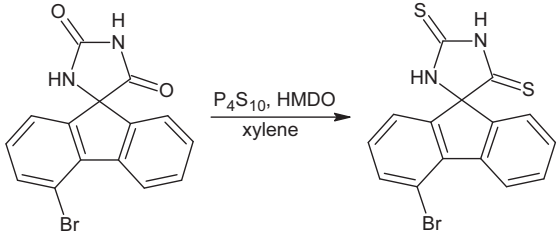
Antimicrobial assay

The antimicrobial effect of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) against clinically isolated Gram-positive, Gram-negative bacteria – *Staphylococcus aureus*, *Escherichia coli* and the yeasts *Candida albicans* was studied. The technique used for this investigation was a disk-diffusion method on agar medium. The substance was dissolved in 15% DMSO solution and added to growing media in concentrations 360 ppm. Agar spill into Petri dishes film thickness 4 mm inoculated in an amount of 1x10^6 CFU (Colony-forming unit) for *E. coli*, 1x10^6 CFU for *S.aureus* and 1x10^6 CFU for *C. albicans*. The plates were cultivated at 37°C for 18-20h and 48h for the bacteria and the yeast, respectively. Two growing media were used for the cultivation of the test microorganisms - Muller-Hinton agar for the bacteria and Sabouraud agar for the yeast.

Synthesis of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin)

4'-Bromo-(9'-fluorene)-spiro-5-hydantoin (9.87 g, 0.03 mol) was mixed with P_4S_{10} (4.89 g, 0.011 mol), hexsametyldisiloxane (21 cm^3, 0.1 mol) and 100 cm^3 of xylene. The mixture obtained was refluxed for 5 hours. After cooling down to room temperature, the product obtained was filtered off and was recrystallized from methanol-water solution.

Yield: 10.2 g (94%), M. p. = 245-6 °C, R_f = 0.63 (benzene : ethanol = 5 : 1).



Scheme 1. Synthesis of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin)
UV-Vis (DMSO): λ_{max} = 279 nm, 230 nm, 205 nm.

IR (v_{max}, cm^{-1}): 3374, 3066, 2919, 2853, 1754, 1726, 1605, 1575, 1515, 1465, 1448, 1407, 1275, 1260, 1232, 1216, 1194, 1167, 1132, 1119, 1102, 1064, 1029, 1003, 982, 946, 912, 874, 827, 773, 754, 744, 730, 682, 638, 580, 563, 536, 501, 473, 454, 424, 411.

FT-ATR (v_{max}, cm^{-1}): 3364, 3063, 2902, 2830, 2740, 1754, 1716, 1605, 1515, 1465, 1448, 1406, 1373, 1261, 1232, 1217, 1185, 1164, 1133, 1119, 1101, 1064, 1028, 1003, 983, 946, 912, 874, 826, 773, 744, 729, 682, 644, 637.

Raman (v_{max}, cm^{-1}): 3048, 2058, 1606, 1485, 1347, 1296, 1218, 1066, 1028, 711, 450.

RESULTS AND DISCUSSION

The synthesis of the target compound, named 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin), was carried out in accordance to Scheme1. 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) was investigated by electronic UV-Vis, IR and Raman spectroscopy. Maxima in the electronic spectrum of the 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) were observed at 279 nm, 230 nm and 205 nm. The IR bands at 3374 cm^{-1} and 3066 cm^{-1} of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) that were observed may refer to the stretching vibrations of the two N-H groups of the hydantoin ring. The one of two vibrational (N^1-H) and (N^3-H) stretching modes did not appear in the Raman spectrum. In the IR spectrum of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) the bands at 1605 cm^{-1} and 1575 cm^{-1} can be attributed to stretching vibration of the two C=S groups of the hydantoin ring. In Raman spectrum of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) the one of the two C=S groups was appeared at 1606 cm^{-1}. The other vibrational (C=S) stretching modes did not appear in the Raman spectrum.

The data from the cytotoxicity assay showed that 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) reduced the number of proliferative cells by around 30% after 24 h and by 50% after 48 h (Figure 1). Results showed that after treatment with this compound, cell viability decreased significantly. The observed inhibition was time-dependent.

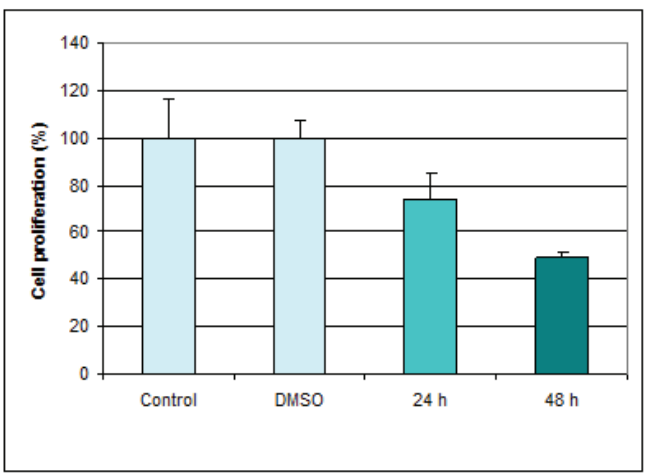


Figure1. Effect of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) on proliferation by WST-1 at 24h and 48h of treatment

Hydantoin derivates are used in treating many human diseases, but still lacks detailed studies to look into their anticancer properties. In the present investigation, we have shown that 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) posses cytotoxic,

time-dependent effects on retinoblastoma cell line. Other researchers had also revealed results for similar compounds tested as potential anticancer drugs [20].

CONCLUSIONS

The synthesis of 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) was described and the various spectral data, UV-Vis, IR, FT-IR ATR and Raman spectroscopy, confirmed its structure. The preliminary results of our study showed that 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) could serve as potential anticancer agent. Further investigations are needed to elucidate the exact mechanisms of this action and to exclude any cytotoxic effect on normal cells. The results for 4'-bromo-(9'-fluorene)-spiro-5-(2,4-dithiohydantoin) showed no antimicrobial activity against the bacteria *Escherichia coli, Staphylococcus aureus* and no activity towards *Candida albicans.*

Acknowledgement

Financial support by the National Science Fund of Bulgaria, (Contract DFNI BO1/0014) is gratefully acknowledged.

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About the authors:

Petja Marinova[1a], University of Plovdiv, Faculty of Chemistry, Department of General and Inorganic Chemistry with Methodology of Chemistry Education, tel:032/261-445, e-mail:marinova@uni-plovdiv.bg

Danail Georgiev[1b], University of Plovdiv, Faculty of Biology, Department of Biochemistry and Microbiology, tel:032/261-486, e-mail: danail_d_g@abv.bg

Plamen Penchev[1c], University of Plovdiv, Faculty of Chemistry, Department of Analytical Chemistry and Computer Chemistry, tel:032/261-446, e-mail:plamen@uni-plovdiv.net

Elena Trendafilova[1c], University of Plovdiv, Faculty of Chemistry, Department of Analytical Chemistry and Computer Chemistry, tel:032/261-442, e-mail:ahet@uni-plovdiv.net

Marin Marinov[2], Agricultural University - Plovdiv, Faculty of Plant Protection and Agroecology, Department of General Chemistry, tel: 032/654-305, e-mail:m_n_marinov@abv.bg

Yana Feodorova[3], Medical University-Plovdiv, Department of Medical Biology, tel: 032/ 602 528, e-mail:yanafeodorova@gmail.com

Maria Kazakova[3], Medical University-Plovdiv, Department of Medical Biology, tel: 032/602 993, e-mail:kazakova25@abv.bg

Victoria Sarafian[3], Medical University-Plovdiv, Department of Medical Biology, tel: 032/602 993, e-mail: sarafian@abv.bg

Neyko Stoyanov[4], University of Ruse - Razgrad Branch, Department of Chemistry and Chemical Technology, e-mail:nstoyanov@uni-ruse.bg

This paper has been reviewed