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Synthesis, Structure Elucidation and Cytotoxicity of (+)-Usnic Acid Derivatives on U87MG Glioblastoma Cells

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Abstract

A series of new usnic acid ketamine compounds [1-8] and their oxime analogues [9-12] were synthesized by reacting (+)-usnic acid with various amines and subsequent treatment with hydroxylamine hydrochloride. They were evaluated on the human glioblastoma-astrocytoma cell line (U87MG) by a MTT assay for cell viability in vitro. The ketamine-derivatives (Schiff bases) show significant cytotoxicity on U87MG cells. A novel N-heterocyclic derivative (1,4-diazepine) showed an interesting tautomeric structure and displayed more activity on cancer cell line than (+)-usnic acid itself.

Keywords: Usnic acid derivative; DFT calculations; Glioblastoma cells; Cell viability

Introduction

Usnic acid (2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyl-1,3(2H,9bH)-dibenzo[d,f]furan-5(6H)-one) (Scheme 1) is a lichen metabolite and both the R (+) and S (-) isomers can be found in nature. (+)-Usnic acid and its derivative exhibit a wide range of biological activities [1] such as antibiotic [2], antiviral [3], apoptotic [4, 5], analgesic [6], antipyretic [6] and anti-proliferative [7] activities. The toxicity in vitro and in vivo has been evaluated by Guo et al. [8] and hepatotoxicity but no general toxicity was observed. Antitumor activity of usnic acid was displayed for the first time by Kupchan and Kopperman [9] against Lewis lung carcinoma in mice. In addition, cytotoxicity activity of usnic acid and usnic acid-amine derivatives has been determined against a wide range of murine and human cancer cell lines [4]. Although the cytotoxicity of usnic acid has been extensively reviewed, no data regarding the effects of usnic acid on glioblastoma cells (formally glioblastoma multiform, GBM) exists. GBM represents both the most common and most malignant variant among a number of primary brain tumors. In this study, usnic acid and ten derivatives of (+)-usnic acid were evaluated on a human glioblastoma - astrocytoma cell line (U87MG) by a MTT assay for cell viability. The N-heterocyclic derivative (1,4-diazepine) showed higher cytotoxicity on this cancer cell line than temozolomide (TMZ), which is the drug currently used against this cancer form.

The reaction between usnic acid and primary amines has been studied in a number of papers [10-21] leading to enamines and Schiff bases. One inherent problem with biological testing of usnic acid and some of its derivatives is a very low solubility in water [22]. The aim of this study is to synthesize new (+)-usnic acid derivatives with potential biological activities and better solubility. Ten derivatives of (+)-usnic acid were synthesized with ethylene diamine, series of aromatic amines and hydroxylamine.

Experimental

(+)-usnic acid was obtained from Sigma (USA). Ethylene diamine, 2-aminobenzotri fluoride, 3-aminobenzotri fluoride, 4-aminobenzotri fluoride, 3-chloro-2-methylamine, 5-chloro-2-methylam inine, hydroxylamine hydrochloride, benzylamine. Solvents and other reactants of high quality were purchased from Merck (Germany) and JT Baker (USA).

Melting points were measured on a Mettler Toledo (Switzerland) melting apparatus. IR spectra in KBr discs were recorded on a Nicolet 760 (USA) instrument. Preparative chromatography was performed on a Shimadzu LC-20AP preparatory system (Japan) with Agilent Prep SIL column (250 × 21.2 mm; 10 µm). High resolution mass (HRMS) measurements were recorded on a LCMS-IT-TOF Shimadzu (Japan) with an electrospray ion source (ESI). 1H NMR and 13C NMR spectra were measured at 500 and 125 MHz, respectively, on a Bruker AC-500 MHz (USA) or at 600 and 125 MHz on a Varian Inova instrument in DMSO-d₆ or CDCl₃ using TMS as reference. 2D NMR spectra, HSQC and HMBC were recorded according to standard protocols.

Scheme 1: Synthesis of (+)-usnic acid derivatives 1-10. Reagents and conditions: (i) RNH₂ 1.2 equiv., refluxing EIOH, 4 hrs; (ii) ethylene diamine 1.2 equiv., refluxing EIOH/THF 5:1, 4 hrs; (iii) hydroxylamine 1.2 equiv. in 5% methanolic potassium hydroxide-water (2:1), 50°C, N₂, 2 hrs.; (a) The compound is tautomeric; (b) The compound is tautomeric [24].

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General methods

Procedure (A) for the preparation of compounds (1-2): A suspension of (+)-usnic acid (1 mmol) in a mixture of tetrahydrofuran and absolute ethyl alcohol (15:12 ml) was treated with ethylene diamine (1.2 mmol, pure liquid), and heated to reflux for 4 hrs with stirring. After the end of the reaction, the mixture was concentrated under reduced pressure. The obtained residue was purified by preparative chromatography.

(S,E)-6-acetyl-2-(1-((E)-1-(R)-6-acetyl-7,9-dihydroxy-8,9b-dimethyl-1,3-dioxo-3,9b-dihydrobenzo[b,d]fur-2(1H)-ylidene)ethyl)amino)(ethyl)amino)(ethylidene)-7,9-dihydroxy-8,9b-dimethylbenzo[b,d]fur-an,1,3(2H,9bH)-dione (1): White solid (163 mg, 46%); mp. 186-187°C; Rf = 0.51 (CH3Cl/MeOH/NaOH/HH, 95:5:1); IR ν (cm^-1) 3270, 3086, 2986, 1696, 1635, 1545; ESI-HRMS (m/z) calcld. for C18H19N3O6, M+ = 468.1213, found 468.1218.

(E)-6-acetyl-7,9-dihydroxy-8,9b-dimethyl-2(1-(4-trifluoromethyl)phenylamino)(ethylidene) dibenzo[b,d]fur-an,1,3(2H,9bH)-dione (5): White crystals (317 mg, 65%); mp. 171-171.5°C; Rf = 0.48 (ethyl acetate/hexane/MeOH, 3:7:0.1); IR ν (cm^-1) 3477, 2925, 1699, 1635, 1545; ESI-HRMS (m/z) calcld. for C29H26F4N3O9 (M+H) 488.1321, found 488.1320.

Procedure (B) for the preparation of ketamines 3-7 and (11): A suspension of (+)-usnic acid (1 mmol) in absolute ethyl alcohol (10 ml) was treated with amines (1.2 mmol, neat) and heated to reflux for 4 hrs with stirring. After the end of the reaction, the mixture was concentrated under reduced pressure. The obtained residue was purified by preparative chromatography.

(E)-6-acetyl-7,9-dihydroxy-8,9b-dimethyl-2-(1-(2-trifluoromethyl)phenylamino)(ethylidene)dibenzo[b,d]fur-an,1,3(2H,9bH)-dione (3): White crystals (204 mg, 42%); mp. 150-151°C; Rf = 0.36 (ethyl acetate/hexane/MeOH, 7:3:0.1); IR ν (cm^-1) 2971, 2971, 1693, 1635, 1548; ESI-HRMS (m/z) calcld. for C18H19N3O6, M+ = 468.1321, found 488.1325. 'H NMR (500 MHz, DMSO-d6, δ ppm) (J Hz) 1.76 (s, 3H, CH-10); 2.03 (s, 3H, CH-15); 2.46 (s, 3H, CH-14); 2.69 (s, 3H, CH-12); 6.02 (s, 1H, H-4); 7.71 (m, 2H, H-6, 4'); 7.86 (t, 1H, J = 7.5, H-5'); 7.93 (d, 1H, J = 7.5, H-5'); 11.92 (s, 1H, OH-9); 13.40 (s, 1H, OH-5); 13.59 (s, 1H, OH-3); 13.60 (s, 1H, OH-5); 13.65 (s, 1H, OH-3); 13.80 (s, 1H, OH-1); 14.59 (s, 1H, NH). 13C NMR (125 MHz, CDCl3, δ ppm) (J Hz) 17.5 (C-15); 20.8 (CH-12); 31.3 (CH-14); 31.9 (CH-10); 57.7 (C-9b); 101.4 (C-6); 102.2 (C-2); 103.0 (C-4); 104.8 (C-9a); 108.3 (C-8); 122.9 (CF, J = 271.5); 127.0 (C-2', J = 30.8); 127.25 (C-3', J = 50.8); 128.6 (C-6'); 128.9 (C-4'); 133.1 (C-5'); 134.5 (C-1'); 155.8 (C-5a); 158.1 (C-9); 163.6 (C-7); 173.5 (C-4a); 175.1 (C-11); 191.2 (C-3); 199.0 (C-1); 200.7 (C-13).

(E)-6-acetyl-7,9-dihydroxy-8,9b-dimethyl-2-(1-(3-trifluoromethyl)phenylamino)(ethylidene)dibenzo[b,d]fur-an,1,3(2H,9bH)-dione (4): White crystals (326 mg, 67%); mp. 160-160.5°C; Rf = 0.43 (ethyl acetate/hexane/MeOH, 7:3:0.1); IR ν (cm^-1) 3107, 3086, 2925, 1698, 1628, 1549; ESI-HRMS (m/z) calcld. for C18H19N3O6, M+ = 468.1321, found 488.1323. 'H NMR (500 MHz, DMSO-d6, δ ppm) (J Hz) 1.71 (s, 3H, CH-10); 1.98 (s, 3H, CH-15); 2.53 (s, 3H, CH-14); 2.65 (s, 3H, CH-12); 6.01 (s, 1H, CH-3'); 7.75 (s, 1H, H-2'); 7.80 (d, 2H, J = 5.5, H-4', 6'); 7.86 (t, 1H, J = 5.5, H-5'); 11.92 (s, 1H, OH-9); 13.40 (s, 1H, OH-7); 14.84 (s, 1H, NH). 13C NMR (125 MHz, CDCl3, δ ppm) (J Hz) 7.5 (CH-15); 20.6 (CH-12); 31.3 (CH-14); 31.9 (CH-10); 57.7 (C-9b); 101.4 (C-6); 102.2 (C-2); 103.0 (C-4); 104.8 (C-9a); 108.4 (C-8); 122.9 (C-3', J = 38.3); 123.3 (CF, J = 272.3); 125.0 (C-4', J = 33.2); 129.2 (C-6'); 130.4 (C-5'); 132.4 (C-3', J = 32.9); 136.8 (C-1'); 155.7 (C-5a); 158.1 (C-9); 163.7 (C-7); 173.9 (C-4a); 175.1 (C-11); 191.2 (C-3); 199.0 (C-1); 200.6 (C-13).
were concentrated under reduced pressure. The obtained residue was purified by preparative chromatography.

\[(E)-7,9\text{-dihydroxy-6-(1\text{-hydroxyimino})ethyl}-8,9\text{-}b\text{-dimethylidencybenzo[b,d]furan-1,3(2H,9bH)-dione} (8)\]: White solid (67 mg, 70%); mp: 152-153°C; Rf = 0.35 (ethanol acetic acid/hexane/MeOH, 3:7:0.11); IR (cm\(^{-1}\)) 3398, 3029, 2929, 2854, 1698, 1607, 1530; ESI-HRMS (m/z) calcd. for C\(_{25}\)H\(_{20}\)NO\(_{10}\), found 503.1425.

\[\delta (ppm) 7.62 (d, 2H, J=5.5, H-2'); 5.89 (s, 1H, H-4); 7.34 (d, 2H, J=5.5, H-5'); 11.18 (s, 1H, OH-9); 11.65 (s, 1H, O\(\text{O}^+\)-13); 12.09 (s, 1H, OH-7); 14.91 (s, 1H, NH).

Cell viability by the MTT assay: U87MG glioblastoma cells were plated into 12-well culture plates and were incubated in 10% serum culture at 37°C under a 5% CO\(_2\)/95% humidified air incubator for 24 hrs. Cells were then incubated with TMZ, (+)-usnic acid or compounds 1-10 at indicated concentrations in serum free DMEM for an additional 24 hrs. The compounds were dissolved in DMSO and the level of DMSO in treatments did not exceed 0.1%. Cell viability was determined using the MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide assay, which yields a blue formazan product in living cells, but not in dead cells or their lytic debris. Then, cells were washed with PBS (phosphate buffered saline) and dissolved in DMSO (dimethyl sulfoxide, Sigma Aldrich). Absorbance was determined at 540 nm. The results were expressed as a percentage of MTT assay activity relative to the non-treated control. CC\(_{50}\) of the specific compound was calculated, based on three concentrations using Origin software (www.Originlab.com). The concentration of treating compounds at which 50% cell alive compared to non-treated samples was calculated from formula in corresponding to corresponding curve formed individually, being considered as CC\(_{50}\) of each compound. The experiments were repeated three times independently.

Preparative chromatography

High-speed fractionation was performed using a column with a high separation capacity so the obtained compounds could be achieved with high purity. The obtained residue was purified on a Shimadzu LC-20A using a silica column at ambient temperature. Separation was achieved using the mobile phases in Table 3 at a flow rate of 12 ml/min and was monitored using a PDA detector at 254 nm. In the purification step the yields were finally obtained in the range from 33% to 72%.

Cell culture and drug treatment: The biological studies were conducted in department of Pharmacology, School of Medicine, Kangwon National University, Chuncheon, Korea.

Human cancer cell line: U87MG glioblastoma cells were obtained from the Korean Cell Line Bank (KCLB), cultured in Dulbecco's modified Eagle's medium (DMEM; Gibco BRL, Grand Island, NY, USA) plus 10% fetal bovine serum (FBS; Hyclone Laboratories, Inc.), 100 U/mL of penicillin, 100 mg/mL of streptomycin (Gibco BRL, Grand Island, NY, USA). Cells were maintained at 37°C under a humidified 5% CO\(_2\) atmosphere.

Results and Discussion

Synthesis

The reaction of (+)-usnic acid (2.0 equivalents) and ethylene diamine (1.0 equivalent) in a mixture of absolute ethyl alcohol and tetrahydrofuran (5:1) at reflux for 4 hr produced compound 1 only [28]. In our hands, the reaction of (+)-usnic acid (1.0 equivalent) and ethylene diamine (1.2 equivalents) gave compound 1 and the N-heterocyclic derivative 2 under the same conditions. The latter was predominant.

Compounds 3-5 were synthesized in absolute ethyl alcohol at reflux for 4 hrs. The reaction leading to compound 3 (42%) showed lower yield than compounds 4 (m-CF\(_2\)J; 67%) and 5 (p-CF\(_2\)J; 65%) (Scheme 1).

Under similar conditions 3-chloro-2-methylaniline and 5-chloro-2-methylaniline gave compounds 6 and 7 in good yields, bright crystal needles 78%, 86%, respectively.

Treatment of compounds 4, 7 and (+)-usnic acid with hydroxyamine hydrochloride in 5.0% methanic potassium hydroxide - water (2:1) at 50°C [10] resulted in good yields of the corresponding oximes (8, 9 and 10).

Structure elucidation and NMR chemical shift assignments

As shown by Kutney and Sanchez [11,14,15,19,20] does the...
reaction with primary amines occur at the carbonyl group C(11)=O leading to an enamine. This is confirmed in this study. Schiff base formation involves the amine attacking at C=C=O. C-11 is part of a triketone system and therefore is more reactive than C-13 being part of an aromatic systems. This means that the Schiff base is formed at C-11.

The assignment of the NMR chemical shifts is central to the assignment of structures. Following the assignments of Ref. [19] it can be seen that the chemical shifts of the A-ring (Scheme 1) of mono substituted derivatives are very similar to those of usnic acid itself. The assignments of the other compounds are based on chemical shifts of usnic acid and this reasoning. For the benzene rings of compounds 3-7, C-F coupling constants are of great help in derivatives 3-5 and the resonances of the benzene rings of compounds 6 and 7 can then be assigned based on substituent effects.

The structure and assignment of compound 2 will be discussed separately as the structure is rather complex. Based upon the HSQC spectrum (Table 1) C-4 can be assigned unambiguously and the methylene carbons can be identified. Based on the HMBC spectrum (Table 1) long-range correlations from OH-7 confirm the assignment of C-6, C-7 and C-8. Knowing the assignment of C-6 the cross-peak from CH$_3$-14 assigns H-14 as well as C-13. Knowing the position of C-8, cross-peaks from CH$_3$-15 assigns H-15. The cross-peak from OH-9 confirms that the resonances in the HMBC spectrum overlap, although they are separate in the 1D spectrum at a lower field. H-4 helps to assign C-2 and C-4a. As H-10 and H-15 are now assigned, the remaining methyl signal must be those of H-12 and H-14. Cross-peaks from these assign C-13 and C-11. Cross-peaks from H-10 assign C-9a, C-4a and C-1. The latter is very important and the assignment is confirmed by cross-peaks to the CH$_3$ protons. The chemical shifts in DMSO-d$_6$ are very similar to those in CDC$_3$ (Table 1) so we may assume that the structure is similar in the two solvents.

Deuterium isotope effects on chemical shifts have also been studied in order to support the structural assignment. The deuteration is done by dissolving the compound in CH$_3$OD and removing the methanol by rotary evaporation. This type of isotope effect is useful in the study of intramolecularly hydrogen bonded compounds [29,30]. The deuterium isotope effect at C-9 (0.6 ppm) is unusually large. Also C-11 shows large isotope effects although somewhat broad. The large effect at C-9 indicates a strong intramolecular hydrogen bond or an equilibrium system [31,32]. The former is in line with the large OH chemical shift of 15.6 ppm. Both the OH-7 and the NH resonance show splittings due to isotope effects.

Based on the above information a structure can be suggested as seen in Figure 2. However, upon cooling to -60°C two broad resonances appear in the $^1$H spectrum at 12.7 and 9.95 ppm integrating roughly 1:1. These are ascribed to NH resonances. Furthermore, the CH$_2$ resonances merge to one broad resonance. In the $^13$C spectrum many resonances are now broad: C-1, C-4, C-9, C-10, C-11 and CH$_2$CH$_3$, indicating that an exchange situation is at hand, suggesting that two different tautomer’s exist as seen in Figure 1.

A comparison between calculated and experimental data for structure A shows a very good correlation (Figure 2). This correlation is not improved by adding data for structure B, so the former must dominate at ambient temperature. Kutney and Sanchez [10] found that using hydroxylamine hydrochloride in 5.0% methanolic potassium hydroxide - water (2:1) with the dibenzyl ether of usnic acid resulted in oxime formation at carbon 13 although a more logical result would have been at C-11 as amines react at this position (see previously). However, HMBC spectra confirmed that for 10 the reaction had occurred at C-13 (Scheme 1).

### Biological activity

The MTT assay [29] was applied to evaluate the cytotoxicity of the derivatives on the human glioblastoma-astrocytoma cell line (U87MG) (Table 2). The cytotoxicity of (+)-usnic acid and derivatives has been

| Compounds | Conc. (µM) | Cell viability (% control) 24 hrs | CC$_{50}$ (µM) | DMSO | Conc. (µM) | Cell viability (% control) 24 hrs | CC$_{50}$ (µM) |
|-----------|----------|----------------------------------|----------------|------|----------|----------------------------------|----------------|
| (+)-Usnic acid | 10 | 100 | 114.0 ± 14.3 | 34.3 ± 8.1 | 20.0 ± 7.8 | 46.99 | 101.0 ± 10.0φ |
| 50 | 100 | 102.3 ± 7.7 | 33.6 ± 7.0 | 11.3 ± 0.7 | 33.14 | >100 | |
| 100 | 100 | 102.4 ± 1.4 | 104.63 ± 10.7 | 75.71 ± 12.0 | >400 | >200 | |
| OH-7 | 10 | 100 | 102.4 ± 1.4 | 104.63 ± 10.7 | 75.71 ± 12.0 | >400 | >200 | |
| 100 | 400 | 83.2 ± 1.3 | 29.1 ± 6.6 | 8.0 ± 2.5 | 44.17 | >200 | |
| 10 | 100 | 102.7 ± 4.7 | 71.3 ± 6.7 | 48.3 ± 0.8 | 94.13 | >100 | |
| TMZ | 100 | 400 | 83.3 ± 14.5 | 61.0 ± 14.1 | >100 | >100 | |

*Data represent means of three independent experiments ± SD. Compounds were tested at the highest possible concentration keeping the DMSO concentration at 0.1% v/v.

Table 2: Cytotoxicity of (+)-usnic acid and of ten derivatives 1-10 on the U87MG cell line.
Ethyl acetate - hexane (4:6)

Chloroform - Methanol – Ammonia (95:5:0.1)

Ethyl acetate - hexane (3:7)

Mobile phase

Ethyl acetate - hexane (4:6)

6, 7, 9

50

8

9

10

Table 3: Mobile phases for preparative chromatography.

| Compound | Mobile phase |
|----------|--------------|
| 1        | Chloroform - Methanol – Ammonia (95:5:0:1) |
| 2        | Chloroform - Methanol – Ammonia (95:5:0:1) |
| 3        | Ethyl acetate - hexane (5:5) |
| 4        | Ethyl acetate - hexane (4:6) |
| 5        | Ethyl acetate - hexane (3:7) |
| 8        | Ethyl acetate - hexane (4:6) |
| 9        | Ethyl acetate - hexane (4:6) |
| 10       | Ethyl acetate - hexane (4:6) |

**Figure 1:** Tautomeric structures of compound 2.

**Figure 2:** Plot of calculated (B3LYP-631G*) 13C nuclear shielding's vs. experimental 13C chemical shift.

The sensitivity of (+)-usnic acid is varying from one cell line to the next. The CC50 observed in U87MG cells after 24 hrs. (47 µM) is corresponding to previously published IC50 values in K-562 and DU145 cells (53 and 57 mM, respectively), but after 72 hrs. [4]. The time effects of (+)-usnic acid need to be addressed. In human keratinocytes (HaCaT) IC50 was estimated to 70 µM for (+)-usnic acid after 24 hrs. [20]. Both the (+) and (-) isomer of usnic acid are found naturally and the (-)-isomer shows generally a lower cytotoxicity compared to (+)-isomers [4,35]. Although a recent study relating to Mycobacterium tuberculosis showed similar activity for the two isomers [35]. A recent paper by Bruno et al. showed less cytotoxicity in rat myoblasts (L6 cells) of Schiff bases of usnic acid relative to the mother compound itself [19].

Our preliminary bioactivity data suggests that (+)-usnic acid and its derivatives could be potential cancer chemical therapy agent although a profound study is needed to examine the specificity of cytotoxicity on cancer cell lines as well as mechanism of activities in vitro and in vivo.

Conclusions

Condensation of (+)-usnic acid with amines and hydroxylamine gave a series of ketamine derivatives and its oxime derivatives. Of particular interest is the formation of a new type of compound 2. In addition, the cytotoxic activity of (+)-usnic acid and ketamine derivatives was evaluated using a MTT assay. The new compounds 2 and 8 were more active compounds in terms of cytotoxicity and they had an improved solubility. Compound 2 showed better activities (CC50=33.14 µM<46.99 µM) than (+)-usnic acid in this study. However, further investigations are needed to evaluate the biological activity of these compounds in relation to U87MG cancer cells and to improve the activity of usnic acid derivatives in vivo.

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References

1. Araújo AA, De Melo MG, Rabelo TK, Nunes PS, Santos SL, et al. (2015) Review of the biological properties and toxicity of usnic acid. Nat Prod Res 29: 2167-2180.

2. Cocchiello M, Skerti N, Nimis PL, Sava GA (2002) Review on usnic acid, an interesting natural compound. Naturwissenschaften 89: 137-146.

3. Cardarelli M, Serino G, Campanella L, Ercole P, De Cicco Nardone F, et al. (1997) Antimitotic effects of usnic acid on different biological systems. Cell Mol Life Sci 53: 667-672.

4. Guo L, Shi Q, Fang JL, Mei N, Ali AA, et al. (2008) Review of usnic acid and Usnea barbata toxicity. J Environ Sci Health C Environ Carcinog Ecotoxicol Rev 26: 317-338.

5. Kupchan SM, Kopperman HL (1975) l-usnic acid: tumor inhibitor isolated from Pseudomonas species. Experientia 31: 625.

6. Kutney JP, Leman JD, Salisbury PJ, Shanchez IH, Yee T (1977) Studies in the usnic acid series. VII. The biodegradation of (+)-usnic acid by a Pseudomonas species. Isolation, structure determination, and synthesis of (+)-6-desacetylusnic acid. J Can Chem 55: 2336-2352.
11. Kutney JP, Shanchez IH (1976) Studies in the usnic acid series. I. The condensation of (+)-usnic acid with aliphatic and aromatic amines. Can J Chem 54: 2795-2803.

12. Kutney JP, Shanchez IH (1977) Studies in the usnic acid series. VI. The preparation of some other derivatives of (+)-usnic acid. Can J Chem 55: 1073-1078.

13. Kutney JP, Shanchez IH, Yee T (1976) Studies in the usnic acid series. VII. The biodegradation of (+)-usnic acid by a Pseudomonas species. Isolation, structure determination, and synthesis of (+)-6-desacetylusnic acid. Can J Chem 54: 3713-3720.

14. Luzina OA, Polovinka MP, Salakhutdinov NF, Tolstikov GA (2007) Chemical modification of usnic acid 2. Reactions of (+)-usnic acid with amino acids. Russ Chem Bull 56: 1249-1251.

15. Luzina OA, Polovinka MP, Salakhutdinov NF, Tolstikov GA (2009) Chemical modification of usnic acid: III. Reaction of (+)-Usnic Acid with Substituted Phenylhydrazines. Russ J Org Chem 45: 1783-1789.

16. Mayer M, O'Neill MA, Murray KE, Santos-Magalhães NS, Carneiro-Leão AM, et al. (2005) Usnic acid: a non-genotoxic compound with anti-cancer properties. Anticancer Drugs 16: 805-809.

17. Tazetdinova AA, Luzina OA, Polovinka MP, Salakhutdinov NF, Tolstikov GA (2009) Amino-derivatives of usnic acid. Chem Nat Comp 45: 800-804.

18. Sokolov DN, Luzina OA, Salakhutdinov NF (2012) Usnic acid: preparation, structure, properties and chemical transformations. Russ Chem Rev 81: 747-788.

19. Bruno M, Trucchi B, Monti D, Romeo S, Kaiser M, et al. (2013) Synthesis of a Potent anti-malarial Agent through Natural Products Conjugation. ChemMedChem. 8: 221-225.

20. Bruno M, Trucchi B, Burlando B, Ranzato E, Martinotti S, et al. (2013) (+)-Usnic acid enaminone with remarkable citoactivating properties. Bioorg Med Chem 21: 1834-1843.

21. Luzina OA, Sokolov DN, Pokrovskii MAAG, Pokrovskii OB, Bekker VN, et al. (2015) Synthesis and Biological Activity of Usnic Acid Enamine Derivatives Chemistry of Natural Compounds. Chem Nat Comp 51: 646-651.

22. Kristmundsdóttir T, Aradóttir HA, Ingólfsdóttir K, Ogmundsdóttir HM (2002) Solutibilization of the lichen metabolite (+)-usnic acid for testing in tissue culture. J Pharm Pharmacol 54: 1447-1452.

23. Frisch MJ, Trucks GW, Schlegel HB, Scuseria GE, Robb MA, et al. (2009) Gaussian, Inc., Wallingford CT.

24. Becke AD (1988) Density-functional exchange-energy approximation with correct asymptotic behavior. Phys Rev A Gen Phys 38: 3098-3100.

25. Lee C, Yang W, Parr RG (1988) Development of the Colle-Salvetti correlation-energy formula into a functional of the electron density. Phys Rev B Condens Matter 37: 785-789.

26. Wolinski K, Hilton F F, Pulay P (1990) Efficient implementation of the gaugeindependent atomic orbital method for NMR chemical shift calculations. J Am Chem Soc 112: 8251-8260.

27. Ditchfield R (1974) Self-consistent perturbation theory of diamagnetism. I. A gageinvariant LCAO (linear combination of atomic orbitals) method for NMR chemical shifts. Mol Phys 27: 789-807.

28. Kruger MC, Tomasi S, Uriac P, Toupet L, Wenge P (2008) Preparation and characterization of copper(II) and nickel(II) complexes of a new chiral salen ligand derived from (+)-usnic acid. RSC Dalton Trans 46: 6524-6526.

29. Hansen PE (1986) Deuterium Isotope Effects on 13C Nuclear Shielding of Intra molecular Hydrogenbonded Systems. Magn Reson Chem 24: 903-910.

30. Bolvig S, Hansen PE (2000) Isotope Effects on chemical shifts as an analytical tool in structural studies of intramolecularly hydrogenbonded compounds. Curr Org Chem 4: 19-54.

31. Mosmann T (1983) Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 65: 55-63.

32. O'Neill MA, Mayer M, Murray KE, Rolim-Santos HM, Santos-Magalhães NS, et al. (2010) Does usnic acid affect microtubules in human cancer cells? Braz J Biol 70: 659-664.

33. Takai M, Uehara Y, Beisler JA (1979) Usnic acid derivatives as potential antineoplastic agents. Med Chem 22: 1380-1384.

34. Koparal AT, Ayaz Tüylü B, Türk H (2006) In vitro cytotoxic activities of (+)-usnic acid and (-)-usnic acid on V79, A549, and human lymphocyte cells and their non-genotoxicity on human lymphocytes. Nat Prod Res 20: 1300-1307.

35. Bekker O, Sokolov DN, Luzina OS, Komarova NI, Galtsov YV, et al. (2015) Synthesis and activity of (+)-usnic acid and (-)-usnic acid derivatives containing 1,3-thiazole cycle against Mycobacterium tuberculosis. Med Chem Res 24: 2926-2938.