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Synthesis of conjugates of (−)-cytisine derivatives with ferrocene-1-carbaldehyde and their cytotoxicity against HEK293, Jurkat, A549, MCF-7 and SH-SY5Y cells

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ABSTRACT

First example of conjugation of quinolizidine alkaloids with ferrocene was presented. The cytotoxic properties of the obtained conjugates against cell lines HEK293, Jurkat, A549, MCF-7 and SH-SY5Y were studied. It was found, that conjugate **17** (having ferrocenyl methylene fragment and phenyl carboxamide moiety) produced a relatively higher cytotoxicity towards cancer Jurkat and SH-SY5Y cells with weak activity against non-cancerous HEK293 cells, suggesting the selectivity of this substance to inhibit certain tumor cells. The effect of leading compound **17** on cell cycle progression of HEK293, Jurkat, A549, MCF-7 and SH-SY5Y lines in a time-dependent fashion was studied. It was shown that hit-compound **17** caused a prominent arrest of MCF-7, Jurkat and A-549 cells in S phase along with a decrease of cells in G1 and a nearly total decline of cells in G2/M; the induction of apoptosis in HEK293 and SH-SY5Y cells was cell-cycle independent.

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1. Introduction

Improving the effectiveness of chemotherapy of cancer is a priority for global health [1a,1b], because a number of patients diagnosed with this disease is growing steadily according to WHO data [1c], and almost of all clinically used anticancer drugs have significant disadvantages – they are toxic and cause severe side effects [1d,1e]. Therefore, the search and development of novel effective and specifically targeted agents for chemotherapy of cancer are a pick challenge for modern medicinal and pharmaceutical chemistry.

Despite the various existing trends in the area of anticancer drug development, two approaches, namely the synthesis of metal complexes and chemical transformations of natural compounds, are at the forefront of the recent worldwide research [2a-2g]. This state of affairs is not surprising. Various complexes of metals have been widely tested as anticancer agents since the discovery of cisplatin [3a,3b]. And very soon biological active compounds of iron (namely ferrocene derivatives) have written a new amazing chapter in this story. Why? The answer is - due to the specific physical and chemical properties of this molecule [3c] which lead to the manifestation of the highest antitumor properties of its salts, derivatives and conjugates [3d-3g].

Indeed, ferrocene derivatives are stable in biological media, exhibit oxidative-reductive activity; have high lipophilicity (contributing to their easy penetration through cell membranes) [3f]. Often starting ferrocenes are commercially available and their chemical potential is wide: a variety of chemical transformations can be realized with their participation. Significant number of 'ferrocene containing' compounds with pronounced antitumor properties have been synthesized at the present time: there are the simple amides [3h], nitro-imidazole and 1,2,4-triazole derivatives [3i,3j] as well as the ferrocenes with various hetero- and aromatic fragments [3k-3n]. Some examples of conjugation of ferrocene moiety with impiridones, steroids and even with paclitaxel were presented recently [3o-3q].

It is known that the ferrocene itself does not exhibit any cytotoxicity [4a-4d] and for the first time this type of activity was found (*in vitro* and *in vivo*) only for ferricinium salts [5a,5b]. It is considered that the cytotoxic activity of these salts is not related to their

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direct interaction with DNA, but is due to their ability to generate reactive oxygen species (ROS). ROS (superoxide ions) attack of tumor DNA lead to oxidative destruction of the sugar phosphate backbone and triggering the apoptosis mechanism [6].

In some cases, alkylation of DNA (the main targets are nucleic bases) was postulated for ferrocene derivatives as the basis of their antitumor action [7]. It is considered, that such mechanism is usually attributed to highly toxic 'classical' alkylating agents (such as cyclophosphane, embikhin or nitrosomethyl urea), but quite non-toxic compounds with noticeable anti-tumor activity are found among ferrocene derivatives [3f].

Several processes directed to initiating of tumor cells apoptosis, in which ferrocene derivatives with low toxicity can take a key participation, are postulated. First is an ability to influence the mechanism of tumor DNA reparation via the interaction with telomerase RNA, or with a DNA matrix fragment and a DNA/RNA duplex fixed on hTERT. As a result of suppression of reparation process, the tumor cell apoptosis is activated. Nonspecific interaction of ferrocene derivatives with the plasmatic membrane of a tumor cell also is considered to be one of the possible mechanisms of their cytotoxic action. Accumulation of ferrocene derivatives in the thickness of the cell membrane (due to their physico-chemical properties) leads to the effect of membrane 'fluidity', which activates phagocytic cells (neutrophils and macrophages). In parallel, there is a deterioration of the supply energy of the cell and its metabolic activity decreases. Interaction of ferrocene derivatives with mitochondria seems quite possible [3f]. It is suggested that this type of potential antitumor agents can accumulate in mitochondrial membranes, depolarizing their internal layer and violating the integrity of the external one. After that, some proteins, including the apoptosis-inducing factor, come out into the cytoplasm [3f].

On the other hand, natural compounds (and especially alkaloids) also are widely used for chemotherapy of cancer for a long time [8a,8b]. As is known, many antitumor drugs were created on the basis of these plant metabolites [8c-8f]. All antitumor alkaloids are divided into two groups according to their mechanism of action: alkaloids acting on microtubules (alkaloids of *Vinca rosea* L., *Colchicum Speciosum Stev., Taxus brevifolia)* and topoisomerase I and II inhibitors (alkaloids of *Podophyllum peltatum* L., *Camptotheca acuminate).* Some compounds with noticeable activity against intestinal cancer cells and hepatocellular carcinoma, multiple myeloma [8g-8j] were found among quinolizidine alkaloids - secondary metabolites of *Fabaceae* plants [9]. The cytotoxic properties of quinolizidine alkaloid (−)-cytisine, which is the main subject of our ongoing research, have also been studied. It turned out that (−)-cytisine **1** (Fig. 1) is active against A-549 and MCF-7 tumor cell lines (in high (mMolar) concentrations) [10a,10b]. Some natural derivative of (−)-cytisine - tonkinensine B, also is active against human cervical carcinoma HeLa and human breast tumor MDA-MB-231 cell lines [10c].

Moreover the conjugation of (−)-cyisine with pterocarpan or with some pyrimidines is quite successful; it was shown, that the one of conjugates with pterocerpan is active against breast cancer

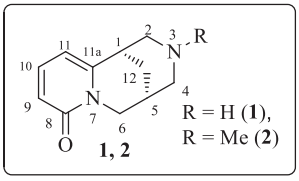


Fig. 1. (−)-Cytisine **1** and methylcytisine **2**.

cells (MCF-7) with IC_{50} = 31.4 ± 0.63 μM [10a] and one of conjugates of 9-amino derivative of **1** with 1,3-dimehtyl-5-formyl uracil inhibits the growth of lymphoblastic leukemia cells (Jurkat) with IC_{50} = 20.6 ± 2.1 μM [10d].

Thus, according to the literature data discussed above and our own experience, we assume, that synthesis of 'hybrid molecules' on the basis of quinolizidine alkaloid (−)-cytisine and ferrocene derivatives can be a one of promising variant of a creation of new effective antitumor agents [3o,3p,3q,10a,10d]. And the conjugation of (−)-cytisine **1** and 3-amines **3**–**9**, **11** (Fig. 2) with commercial available ferrocene-1-carbaldehyde **12** was chosen for realization of this approach.

2. Results and discussion

The reductive alkylation of the primary or secondary amino group by aldehydes was used for the conjugation of ferrocene containing fragment with the 2-pyridone core of derivatives **3**–**9, 11** (Fig. 2) and with (−)-cytisine **1** [10e]. Starting amines **3**–**9** were obtained according to the procedures described earlier [10d,10e]; **11** was obtained from the nitro derivative **10** [10f] by analogy.

The final conjugation of the synthesized amines **3**–**9** and **11** with a formyl ferrocene **12** was carried out by boiling of their equimolar amounts in benzene yielded the azomethine intermediate, which further (without isolation) was reduced with $NaBH_4$ to give compounds **13**–**21** (Fig. 2). The yields of conjugates **13**–**21** were 23–77%.

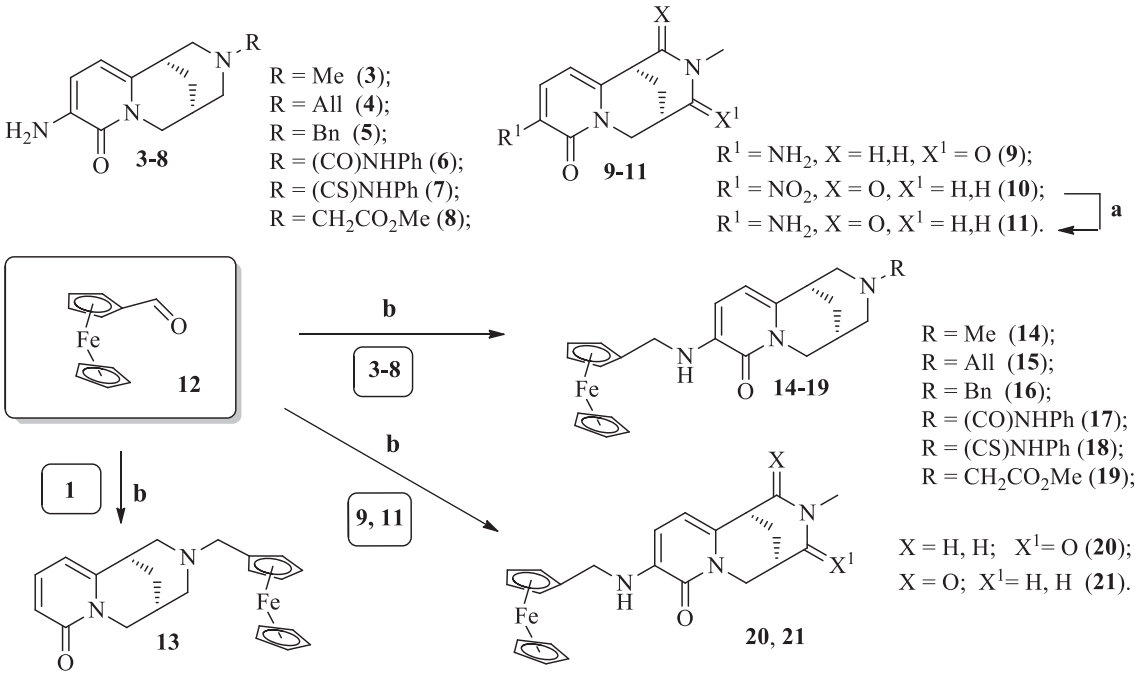
All the prepared compounds **13**–**21** and starting **1** and **2** were screened for their cytotoxic activity against conditionally-normal human embryonic kidney HEK293 cells as well as leukaemic Jurkat, lung A549, breast MCF-7 and neuroblastoma SH-SY5Y cancer cell lines according to MTT method [11].

The summarized in Table 1[a] results, revealed that three from nine of synthesized (−)-cytisine derivatives - compound **13** (with ferrocene fragment at 3 position), **14** and **20** with ferrocene fragment at C-9 were nontoxic against listed above cell lines as well as starting methylcytisine **2**. In contrast with 4-oxo **20** weak cytotoxicity was appeared in 2-oxo derivative **21**; it was active only against non-cancerous HEK293 cells with IC_{50} = 26.37 ± 2.01 μM.

Ester **19** has not affected the viability of HEK293, A549, MCF-7 and SH-SY5Y cell lines, but inhibited the metabolic activity of Jurkat cells (IC_{50} = 48.39 ± 9.19 μM). Protection of the secondary amino group in compounds **15** and **16** by allyl and phenyl fragments lead to increasing of their cytotoxicity: both of samples demonstrated the moderate antiproliferative properties against all cell lines tested (Table 1).

Of the entire series only compound **17** (having a phenyl carboxamide moiety at *N*-3) produced a noticeable cytotoxicity towards cancer Jurkat and SH-SY5Y cells in comparison with HEK293 cells, suggesting the selectivity of this substance to inhibit certain tumor cells with IC_{50} = 55.90 ± 2.36 μM (HEK293), 15.29 ± 4.09 μM (Jurkat) and 16.06 ± 5.46 μM (SH-SY5Y) accordingly. Similar type of selectivity was observed in benzyl compound **16** (it's IC_{50} = 22.76 ± 1.38 μM and 13.85 ± 5.47 μM for HEK293 and Jurkat). Compound **18** (thio analogue of **17**) was nearly equipotent to produce the mild antitumor activity (Table 1), but no selectivity was observed in this case.

Based on the cytotoxic activity data of synthesized compounds **13**–**21**, the preliminary conclusions about SAR can be drawn. Most importantly, C-9 conjugation of the 2-pyridone core of (−)-cytisine or its 3-*N*-substituted derivatives with ferrocene by the aminomethylene spacers contributes to the appearance of their cytotoxic properties. Moreover, the binding of allyl, benzyl, phenyl urea and phenyl thiourea (groups bearing linear or aromatic π-systems) to the secondary nitrogen atom (*N*-3) of compounds **15**–**18** enhance



Reagents and conditions: **a**) $FeCl_3$, Zn, DMF/H_2O (2:1), 100 °C; **b**) amine, C_6H_6, 80 °C, then $NaBH_4$, MeOH, 0-20 °C.

Fig. 2. Synthesis of conjugates **13**–**21. Reagents and conditions**: **a**) $FeCl_3$, Zn, DMF/H_2O (2:1), 100 °C; **b**) amine, C_6H_6, 80 °C, then $NaBH_4$, MeOH, 0–20 °C.

Table 1
In vitro growth inhibitory effect of compounds **1, 2, 13**–**21** in human cell lines HEK293, Jurkat, A549, MCF-7, SH-SY5Y.

No	IC_{50} (μM)[a]				
	HEK293	Jurkat	A549	MCF-7	SH-SY5Y
1	>100	>100	4.4 ± 1.703 mM[b]	101.7 ± 2.0[c]	>100
2	>100	>100	>100	>100	>100
13	>100	>100	>100	>100	>100
14	>100	>100	>100	>100	>100
15	37.52 ± 11.07	59.3 ± 8.40 (p = 0.008)[d]	33.11 ± 5.14	81.02 ± 8.13 (p = 0.00004)[d]	22.31 ± 3.05
16	22.76 ± 1.38	13.85 ± 5.47	42.10 ± 12.21 (p = 0.001)[d]	32.09 ± 1.93	28.94 ± 3.88
17	55.90 ± 2.36	15.29 ± 4.09 (p = 0.000005)[d]	>100	>100	16.06 ± 5.46 (p = 0.000006)[d]
18	49.29 ± 10.01	72.18 ± 3.38 (p = 0.0003)[d]	>100	63.34 ± 3.11 (p = 0.009)[d]	64.60 ± 5.33 (p = 0.005)[d]
19	>100	48.39 ± 9.19	>100	>100	>100
20	>100	>100	>100	>100	>100
21	26.37 ± 2.01	>100	>100	>100	>100
FU[e]	7.43 ± 0.83	0.70 ± 0.10	0.32 ± =0.02	1.20 ± 0.09	1.97 ± 0.4

[a] IC_{50} (μM) values obtained from MTT assays. Cells were incubated with compounds for 48 h. The results represent the mean ± SD of two independent trials, performed in triplicate.
[b] [6b].
[c] [6a].
[d] IC_{50} values for corresponding cell line versus IC_{50} values for HEK293 cells. IC_{50} values differences for certain cell lines proved by one-way ANOVA with Dunnett's Post Hoc test.
[e] FU - fluor uracile.

their antiproliferative properties. And in the case with benzyl and phenyl carboxamide fragments, cytotoxicity against non-cancerous HEK293 cell line decreases, leads to selectivity of antitumor activity of **16** and **17**.

To further characterize the cell-growth inhibitory activity of this series of compounds, the most active substance **17** was probed for its effect on cell cycle progression of HEK293, Jurkat, A549, MCF-7 and SH-SY5Y lines in a 'time-dependent fashion' using flow cytometry and propidium iodide staining (Fig. 3 and Table 1).

In non-cancerous HEK293 as well as in neuroblastoma SH-SY5Y cells (Fig. 3**A**, **E**) compound **17** elicited mainly an increase of cells in sub-G1 phase without prominent disturbance of cell cycle, suggesting that the induction of apoptosis in HEK293 and SH-SY5Y cells is cell-cycle independent. More substantial changes were observed in tumor Jurkat, A549 and MCF-7 cell lines.

The treatment of MCF-7 cells with compound **17** induced the cell-cycle arrest at the S phase, decreased the percentage of cells in G1 and G2/M stages resulted in incremental rise of apoptotic cells (subG1 cell population) within 72 h (Fig. 3**D**). Jurkat cells incubated with compound **17** demonstrated the cell-cycle changes profile similar to that of MCF-7 cells (Fig. 3**B**) with the peak effects upon 48 h.

The exposure of compound **17** to A-549 cells triggered an S phase arrest, drop of G2/M and the decrease the proportion of cells

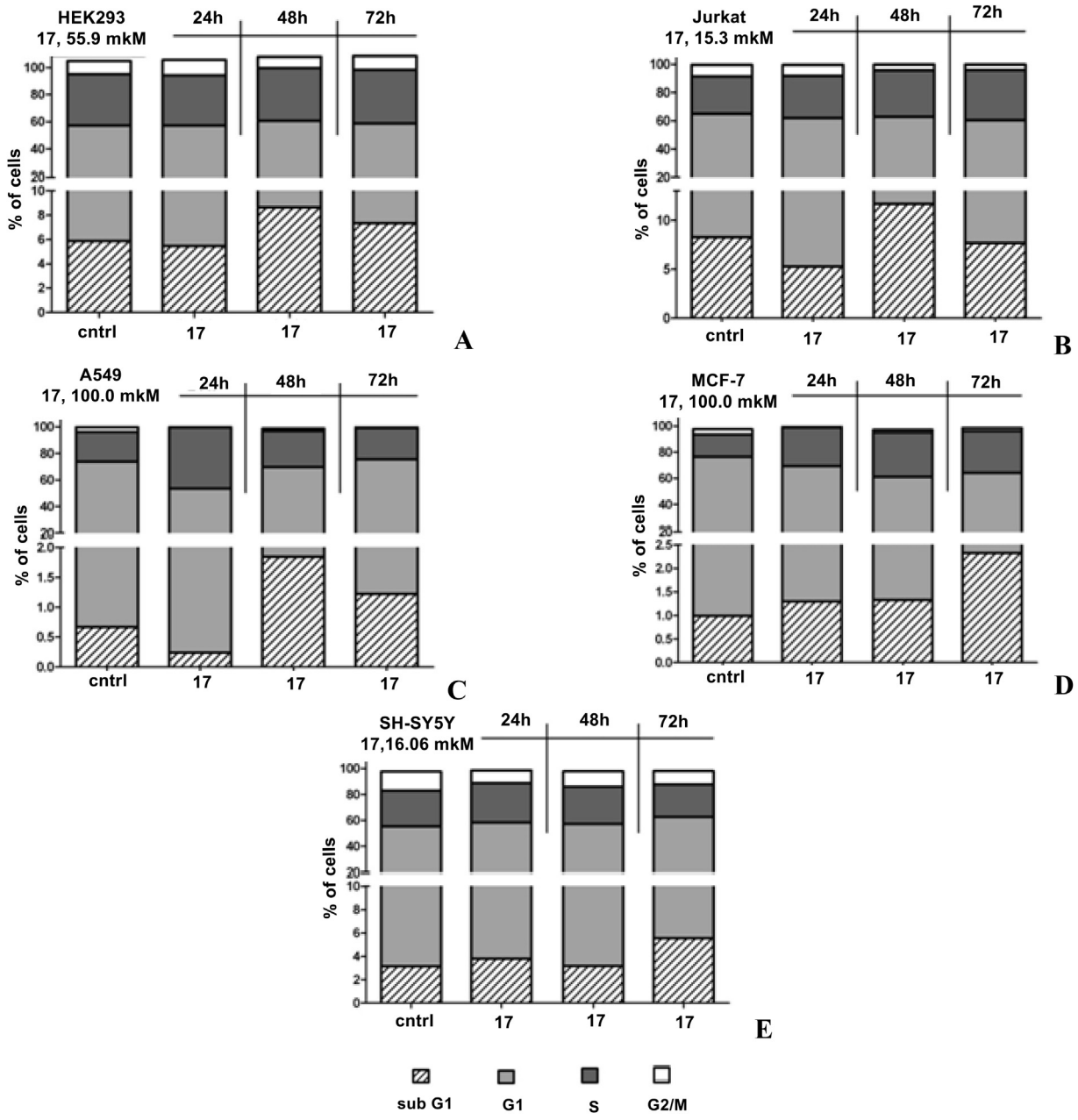


Fig. 3. Effect of compound **17** on cell cycle distribution of different cells.

in the G1 phase at 24 h, however, the rate of apoptotic cells reached a maximum at 48 h (Fig. 3**C**). Collectively the results suggested that compound **17** could promote apoptosis in MCF-7, Jurkat and A549 cells mainly by S phase arrest whereas induction of apoptosis in HEK293 and SH-SY5Y cell is cell-cycle independent supporting a different mechanism of cytotoxic action in cancer and conditionally normal cell lines. S phase arrest in certain cell lines could be a result of DNA damage evoked by compound **17**. Most of existing anti-tumor chemotherapeutics act as DNA damage agents which: 1) directly or indirectly induce DNA strand breaks; 2) covalently modify DNA bases; 3) change the chromatin structure and topology by inhibiting chromatin-modifying enzymes, such as DNA topoisomerases, histone deacetylases, or demethylases. Principal outcomes initiated by DNA damage agents result in proliferation arrest or in slowing down the cell cycle progression that, in case of failure of DNA repair, could activate different cell death pathways such as apoptosis, mitotic catastrophe, or necrosis [12]. Drug-induced DNA damage inhibits the cell cycle progression through the S phase via either positive regulation by G1-specific cyclin-dependent kinases (Cdks) and negatively by the pRb (retinoblastoma tumor suppressor gene). In brief, DNA damage is detected by ATM/ATR kinases which activate checkpoint kinases Chk1/2 with subsequent phosphorylation of Cdc25A phosphatase and its degradation. This leads to prevention of the activation of S-phase specific Cdks, Cdk2/cyclin E, and Cdk2/cyclin A. Additionally, phosphorylation of p53 leads to its stabilization and increased expression of p21, an inhibitor of Cdk2/cyclin E and Cdk4/6/cyclin A activity. Decreased phosphorylation of pRb protein by Cdks blocks the activation of S-phase specific DNA expression. Based on the specific arrest of MCF-7, Jurkat and A549 cells by compound **17** in S-phase observed in the present study, in future studies we will analyze the effects of this novel 'ferrocene-alkaloid' conjugate on specific cell-cycle

regulators (Cdks, cyclins) and replication-start and -progression signals of the S-phase in cancer cells.

Thus, the most obvious approaches to synthesis of the extended library of derivatives of (−)-cytisine with ferrocene fragments follow from the analysis of the preliminary obtained SAR results. First of all, some manipulations with the length and nature of the spacer (amide, thio- and carboxamide, azomethine or alkyl, alkenyl, alkynyl types) for conjugation of ferrocene fragment with alkaloid molecule will be carrying out. In addition, the topology of substituents will change step by step (3, 9 and 11 positions of the (−)-cytisine molecule), as this can significantly affect the cytotoxic properties of novel derivatives. And, of course, the possibility of functionalization of the ferrocene part of the molecules will be exploited exhaustively.

3. Experimental part

3.1. General

Quinolizidine alkaloids (−)-cytisine **1** and methylcytisine **2** were isolated from *Thermopsis Lanceolata* sp. Sibirica grown in the Ufa Botanical Garden-Institute of UFRC of RAS. The physico-chemical properties of **1** and **2** were in accordance with literary data [9]. Ferrocene carboxaldehyde **12** is commercial available (CAS 120930-10-6). Amines **3**–**9** and nitro compound **10** were described earlier [10d,10f]. TLC monitoring was performed using ALUGRAM® precoated aluminium sheets with 0.20 mm silica gel 60 with fluorescent indicator UV_{254} (MACHEREY-NAGEL, Germany). CC was carried out on the 0.05–0.1 mm standard silica 60 (kit I for low-pressure flash chromatography, MACHEREY-NAGEL, Germany). Optical rotation was measured on a PerkinElmer 341 LC digital polarimeter with a sodium lamp (D-line wavelength = 589 nm). IR spectra were recorded using an IR Prestige-21 spectrophotometer (Shimadzu).

Electrospray ionization (ESI) mass spectra were obtained on a HPLC mass-spectrometer LCMS-2010EV (Shimadzu) (direct syringe sample inlet, mobile phase was acetonitrile/water (95:5)) at the ionizing electrode potential of 4.5 kV. The mobile phase flow rate was 0.1 ml min^{-1}. The heater's and the desolvation line's temperature was 200 and 230 °C, respectively. The nebulizer gas (nitrogen) flow rate was 1.5 L min^{-1}.

NMR samples were prepared by dissolving 2 mg of a compound in 0.7 ml of $CDCl_3$ at 273 K. NMR spectra were recorded in 5-mm NMR tubes on a Bruker AVANCE-III 500 spectrometer (z-gradient PABBO) operating at 500.30 MHz (^{1}H) and 125.75 MHz (^{13}C).

The ^{1}H NMR spectra were acquired with a spectral width of 5.6 kHz and 32 k data points and 8 scans, providing a digital resolution of ca. 0.5 Hz (^{1}H 90° pulse width = 11.5 μs). For ^{13}C NMR spectra, a spectral width of 29.7 kHz was used with 64 k data points and 20 k scans (^{13}C 90° pulse width = 9.7 μs).

Gradient selected HSQC spectra were recorded using the standard Bruker sequence (hsqcetgp). These data were collected with 4096 × 256 data points with 2 scans for each increment. The delay d4 was set to 1.72 ms. Gradient selected HMBC spectra (hmbcgpndqf) were collected with 4096 × 256 data points with 4 scans for each increment. The delay d6 was set to 71.4 ms. Spectral widths of 5.6 and 29.7 kHz were used in the *F*2 (^{1}H) and *F*1 (^{13}C) domains, respectively. HSQC and HMBC data were processed using a sine window in the *F*2 and *F*1 dimensions. Phase sensitive gs-COSY data were collected with 4 K × 2 K data points with 2 scans for each increment. For the 2D NOESY NMR experiments, the solutions were degassed to remove any dissolved oxygen. The following parameters and procedures are commonly employed: spectral width 5.6 kHz, 2 K × 2 K data matrix and 256 time increments of 2 transients each, mixing time 0.5s. Fourier transformations were carried out with zero-filling using the shifted sine-bell apodization function in both dimensions.

3.2. Synthesis of compounds

3.2.1. (1S,5S)-9-amino-3-methyl-3,4,5,6-tetrahydro-2H-1,5-methanopyrido[1,2-a] [5]diazocine-2,8(1H)-dione (11)

Solution of 3-nitrolactam **10** (0.1 g, 0.38 mmol) in 1 ml of DMF was added to suspension of $FeCl_3$ hexahydrate (0.19 g, 1.14 mmol) and zinc powder (0.24 g, 3.8 mmol) in mixture of 6 ml of DMF and H_2O (2:1). Reaction mixture was refluxed for 30 min and filtered, precipitate was washed by hot water (1 × 5 ml). Then water lays were neutralized with crystalline Na_2CO_3, and extracted with $CHCl_3$ (5 × 10 ml). Combined organic layers were dried over anhydrous Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by column chromatography on SiO_2 (eluent $CHCl_3$:MeOH = 99:1) to give 0.08 g of product **11** with the yield 95%.

11: $[\alpha]_D^{20} = -214.0$ ($CHCl_3$, *c* 0.60). $R_f = 0.25$ (acetone).

For $C_{12}H_{15}N_3O_2$ calculated: C, 61.79%; H, 6.48%; N, 18.01%. Found: C, 61.76%; H, 6.47%; N, 18.03%. IR spectrum (film, *ν*, cm^{-1}): = 3445, 3327, 2936, 2874, 2471, 2352, 1651, 1592, 1538, 1504, 1470, 1455, 1366, 1350, 1308, 1280, 1244, 1213, 1138, 1196, 1066, 1120, 1107, 971, 916, 831, 791, 683, 664, 515, 460.

^{13}C NMR ($CDCl_3$, *δ* ppm): 23.58 (C12); 25.91 (C5); 34.25 (Me); 42.27 (C1); 50.19 (C6); 55.67 (C4); 106.83 (C11); 112.84 (C10); 130.86 (C11a); 135.74 (C9); 158.26 (C8); 168.39 (C2).

^{15}N NMR ($CDCl_3$, *δ* ppm): 48.80 (NH_2); 104.05 (N3); 159.67 (N7).

^{1}H NMR ($CDCl_3$, *δ* ppm): 2.09 (dtd, 1H, ^2J = 13.1, $^3J_{12syn\text{-}1}$ = 3.2, $^3J_{12syn\text{-}5}$ = 3.2, $^4J_{12syn\text{-}4endo}$ = 1.5, H_{syn}-12); 2.21 (dtd, 1H, ^2J = 13.1, $^3J_{12anti\text{-}1}$ = 3.2, $^3J_{12anti\text{-}5}$ = 3.2, $^4J_{12anti\text{-}6endo}$ = 1.3, H_{anti}-12); 2.80 (m, 1H, H-5); 2.85 (s, 3H, Me); 3.28 (dt, 1H, ^2J = 12.6, $^3J_{4endo\text{-}5}$ = 1.5, $^4J_{4endo\text{-}12syn}$ = 1.5, H_{endo}-4); 3.56 (m, 1H, H-1); 3.63 (ddd, 1H, ^2J = 12.6, $^3J_{4exo\text{-}5}$ = 5.7, $^4J_{4exo\text{-}6exo}$ = 0.9, H_{exo}-4); 3.98 (ddd, 1H, ^2J = 15.7, $^3J_{6exo\text{-}5}$ = 6.7, $^4J_{6exo\text{-}4exo}$ = 0.9, H_{exo}-6); 4.21 (br.s, 2H, NH_2); 4.26 (dt, 1H, ^2J = 15.7, $^3J_{6endo\text{-}5}$ = 1.3, $^4J_{6endo\text{-}12anti}$ = 1.3, H_{endo}-6); 6.18 (d, 1H, $^3J_{11\text{-}10}$ = 7.5, H-11); 6.53 (d, 1H, $^3J_{10\text{-}11}$ = 7.5, H-10).

3.2.2. (1R,5S)-3-(Ferrocenylmethyl)-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (13)

A mixture of amine **1** (0.2 g, 1.1 mmol) and ferrocencarbaldehyde (1.1 mmol) in C_6H_6 was refluxed. When the reaction was completed (TLC monitoring), the benzene was evaporated. The residue was dissolved in MeOH (1 mL), cooled, treated with $NaBH_4$ (10 mmol), stirred on a magnetic stirrer for 1 h and concentrated. The residue was dissolved in H_2O and extracted with $CHCl_3$ (5 × 10 mL). The extracts were combined, dried over Na_2SO_4, and evaporated. The residue was chromatographed over SiO_2 (eluent $CHCl_3$–MeOH) to afford **13** (0.27 g, 65%)

Amorphous compound, $[\alpha]_D^{20} = -204.0$ ($CHCl_3$, *c* 1.6). R_f 0.5 ($CHCl_3$–MeOH, 9:1).

IR spectrum (film, *ν*, cm^{-1}): = 3092, 2937, 2799, 1653, 1569, 1548, 1472, 1424, 1351, 1281, 1223, 1138, 1105, 1059, 999, 797, 751, 664, 484. ESI MS *m/z* (I_{rel}, %): 388 M^+ (12), 411 $[M+Na]^+$ (36), 427 $[M+K]^+$ (31), 452 $[M + Na + CH_3CN]^+$ (100), 799 $[2\,M + Na]^+$ (52).

^{13}C NMR ($CDCl_3$, *δ* ppm): 25.6 (C12); 27.8 (C5); 35.4 (C1); 49.9 (C6); 57.5 (C1'); 58.9 (C4); 59.4 (C2); 67.6 (C4'); 67.7 (C5'); 68.4 (C7'); 69.6 (C3'); 69.6 (C6'); 82.2 (C2'); 104.4 (C11); 116.4 (C9); 138.5 (C10); 151.6 (C11a); 163.5 (C8).

^{15}N NMR ($CDCl_3$, *δ* ppm): 40.46 (N3); 178.75 (N7).

^{1}H NMR ($CDCl_3$, *δ* ppm): 1.64 (br.d, 1H, ^2J = 12.6, H_{anti}-12); 1.78 (br.d, 1H, ^2J = 12.6, H_{syn}-12); 2.24 (br.d, 1H, ^2J = 11.0, H_{exo}-4); 2.28 (br.d, 1H, ^2J = 10.7, H_{exo}-2); 2.34 (m, 1H, H-5); 2.78 (br.d, 1H, ^2J = 10.7, H_{endo}-2); 2.81 (br.d, 1H, ^2J = 11.0, H_{endo}-4); 2.87 (m, 1H, H-1); 3.29 (m, 2H, H-1′); 3.83 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 6.8, H_{exo}-6); 3.93 (m, 1H, H-6′); 3.97 (br.d, 1H, ^2J = 14.9, H_{endo}-6); 3.99 (m, 1H, H-

3′); 4.02 (m, 5H, H-7′); 4.03 (m, 2H, H-4′(5′)); 5.91 (d, 1H, $^3J_{11\text{-}10}$ = 6.9, H-11); 6.41 (d, 1H, $^3J_{9\text{-}10}$ = 8.8, H-9); 7.23 (dd, 1H, $^3J_{10\text{-}9}$ = 8.8, $^3J_{10\text{-}11}$ = 6.9, H-10).

3.2.3. (1R,5S)-9-[(Ferrocenylmethyl)amino]-3-methyl-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a][1,5]diazocin-8-one (14) was synthesized from 3 (0.2 g, 0.9 mmol) to afford 14 (0.29 g, 77%). Amorphous compound, $[\alpha]_D^{20}$ = −33.0 (CHCl$_3$, c 1.4). R_f 0.3 (acetone)

IR spectrum (film, ν, cm^{-1}): = 3394, 3093, 2936, 2779, 1639, 1595, 1565, 1477, 1459, 1420, 1383, 1362, 1342, 1256, 1209, 1151, 1057, 1002, 803, 753, 485. ESI MS *m/z* (I_{rel}, %): 417 M$^+$ (11), 418 [M+H]$^+$ (13), 440 [M+Na]$^+$ (100), 456 [M+K]$^+$ (24), 481 [M + Na + CH$_3$CN]$^+$ (37).

^{13}C NMR (CDCl$_3$, δ ppm): 26.1 (C12); 27.9 (C5); 34.6 (C1); 42.3 (C2'); 46.4 (C1″); 50.1 (C6); 62.3 (C4); 63.25 (C2); 7.63 (C5′(6′)); 67.8 (C4'); 67.9 (C7'); 68. 6 (C8'); 86.3 (C3'); 104.7 (C11); 106.9 (C10); 135.8 (C11a); 136.2 (C9); 158.0 (C8).

^{15}N NMR (CDCl$_3$, δ ppm): 29.02 (N3); 67.08 (N1'); 167.28 (N7).

^{1}H NMR (CDCl$_3$, δ ppm): 1.69 (br.d, 1H, ^2J = 12.6, H$_{anti}$-12); 1.82 (br.d, 1H, ^2J = 12.6, H$_{syn}$-12); 2.10 (s, 3H, H-1″); 2.18 (br.d, 1H, ^2J = 10.8, H$_{exo}$-4); 2.18 (br.d, 1H, ^2J = 10.5, H$_{exo}$-2); 2.38 (m, 1H, H-5); 2.78 (br.d, 1H, ^2J = 10.5, H$_{endo}$-2); 2.86 (m, 1H, H-1); 2.87 (br.d, 1H, ^2J = 10.8, H$_{endo}$-4); 3.86 (m, 1H, H-2′); 3.95 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 6.8, H$_{exo}$-6); 4.13 (m, 2H, H-5′(6′)); 4.14 (br.d, 1H, ^2J = 14.9, H$_{endo}$-6); 4.23 (m, 5H, H-8′); 4.25 (m, 1H, H-7′); 4.25 (m, 1H, H-4′); 5.19 (m, 1H, H-1′); 5.95 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.25 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10).

3.2.4. (1R,5S)-3-allyl-9-[(ferrocenylmethyl)amino]-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a] [1,5]diazocin-8-one (15) was synthesized from 4 (0.2 g, 0.82 mmol) by analogy with compound 13 to afford 15 (0.25 g, 70%). Amorphous compound, $[\alpha]_D^{20}$ = −67.0 (CHCl$_3$, c 1.7). R_f 0.6 (EtOAc)

IR spectrum (film, ν, cm^{-1}): = 3394, 3080, 2979, 2936, 2788, 1640, 1593, 1564, 1559, 1480, 1460, 1398, 1384, 1365, 1256, 1211, 1200, 1181, 1105, 1000, 923, 803, 753, 485. ESI MS *m/z* (I_{rel}, %): 444 [M+H]$^+$ (4), 466 [M+Na]$^+$ (100), 482 [M+K]$^+$ (23), 507 [M + Na + CH$_3$CN]$^+$ (42).

^{13}C NMR (CDCl$_3$, δ ppm): 26.7 (C12); 28.0 (C5); 34.7 (C1); 42.43 (C2'); 50.1 (C6); 59.6 (C4); 61.1 (C2); 61.2 (C1″); 67.6 (C5′(6′)); 67.8 (C4'); 67.9 (C7'); 68.6 (C8'); 86.4 (C3'); 104.6 (C11); 107.0 (C10); 117.0 (C3″); 135.3 (C2″); 136.1 (C11a); 136.2 (C9); 158.0 (C8).

^{15}N NMR (CDCl$_3$, δ ppm): 36.63 (N3); 66.78 (N1'); 167.89 (N7).

^{1}H NMR (CDCl$_3$, δ ppm): 1.72 (br.d, 1H, ^2J = 12.6, H$_{anti}$-12); 1.84 (br.d, 1H, ^2J = 12.6, H$_{syn}$-12); 2.20 (br.d, 1H, ^2J = 10.8, H$_{exo}$-4); 2.22 (br.d, 1H, ^2J = 10.5, H$_{exo}$-2); 2.38 (m, 1H, H-5); 2.83 (br.d, 1H, ^2J = 10.5, H$_{endo}$-2); 2.85 (m, 2H, H-1″); 2.86 (m, 1H, H-1); 2.94 (br.d, 1H, ^2J = 10.8, H$_{endo}$-4); 3.87 (m, 2H, H-2′); 3.94 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 6.8, H$_{exo}$-6); 4.13 (br.d, 1H, ^2J = 14.9, H$_{endo}$-6); 4.13 (m, 2H, H-5′(6′)); 4.23 (m, 5H, H-8′); 4.25 (m, 1H, H-7′); 4.26 (m, 1H, H-4′); 5.01 (dq, 1H, $^3J_{3''cis\text{-}2''}$ = 10.6, ^2J = 1.6, $^4J_{3''cis\text{-}1''}$ = 1.6, H$_{cis}$-3″); 5.02 (dq, 1H, $^3J_{3''trans\text{-}2''}$ = 16.6, ^2J = 1.6, $^4J_{3''trans\text{-}1''}$ = 1.6, H$_{trans}$-3″); 5.19 (m, 1H, H-1′); 5.62 (ddt, 1H, $^3J_{2''\text{-}3''cis}$ = 16.6, $^3J_{2''\text{-}3''trans}$ = 10.6, $^3J_{2''\text{-}1''}$ = 6.2, H-2″); 5.93 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.26 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10).

3.2.5. (1R,5S)-3-benzyl-9-[(ferrocenylmethyl)amino]-1,2,3,4,5,6-hexahydro-8H-1,5-methanopyrido[1,2-a] [1,5]diazocin-8-one (16) was synthesized from 5 (0.2 g, 0.68 mmol) by analogy with compound 13 to afford 16 (0.21 g, 64%). Amorphous compound, $[\alpha]_D^{20}$ = −117.0 (CHCl$_3$, c 1.2). R_f 0.3 (CHCl$_3$–MeOH, 98:2)

IR spectrum (film, ν, cm^{-1}): = 3394, 2936, 2794, 1640, 1593, 1564, 1481, 1457, 1368, 1360, 1255, 1105, 1058, 1041, 804, 753, 698, 484. ESI MS *m/z* (I_{rel}, %): 492 [M − H]$^+$ (55), 493 M$^+$ (26), 494 [M+H]$^+$ (11), 516 [M+Na]$^+$ (100), 532 [M+K]$^+$ (31), 557 [M + Na + CH$_3$CN]$^+$ (22).

^{13}C NMR (CDCl$_3$, δ ppm): 26.72 (C12); 28.1 (C5); 34.7 (C1); 42.6 (C2'); 50.1 (C6); 59.9 (C4); 60.8 (C2); 62.2 (C1″); 67.7 (C5′(6′)); 67.8 (C4'); 7.93 (C7'); 68.6 (C8'); 86.4 (C3'); 104.8 (C11); 107.1 (C10); 126.7 (C5″); 128.1 (C4''(6″)); 128.3 (C3''(7″)); 135.9 (C11a); 136.2 (C9); 138.2 (C2″); 158.0 (C8).

^{15}N NMR (CDCl$_3$, δ ppm): 66.89 (N1'); 167.80 (N7).

^{1}H NMR (CDCl$_3$, δ ppm): 1.75 (br.d, 1H, ^2J = 12.6, H$_{anti}$-12); 1.87 (br.d, 1H, ^2J = 12.6, H$_{syn}$-12); 2.26 (br.d, 1H, ^2J = 10.5, H$_{exo}$-2); 2.30 (br.d, 1H, ^2J = 10.8, H$_{exo}$-4); 2.38 (m, 1H, H-5); 2.81 (br.d, 1H, ^2J = 10.5, H$_{endo}$-2); 2.86 (m, 1H, H-1); 2.91 (br.d, 1H, ^2J = 10.8, H$_{endo}$-4); 3.41 (s, 2H, H-1″); 3.92 (m, 2H, H-2′); 3.94 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 6.3, H$_{exo}$-6); 4.14 (m, 2H, H-5′(6′)); 4.19 (br.d, 1H, ^2J = 14.9, H$_{endo}$-6); 4.24 (m, 5H, H-8′); 4.27 (m, 1H, H-7′); 4.29 (m, 1H, H-4′); 5.23 (br.s, 1H, H-1′); 5.89 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.28 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10); 7.01 (d, 2H, ^3J = 7.5, H-3''(7″)); 7.15 (t, 1H, ^3J = 6.7, H-5″); 7.19 (dd, 2H, ^3J = 7.5, ^3J = 6.7, H-4''(6″)).

3.2.6. (1R,5S)-9-[(Ferrocenylmethyl)amino]-8-oxo-N-phenyl-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a] [1,5]diazocine-3(4H)-carboxamide (17) was synthesized from 6 (0.2 g, 0.62 mmol) by analogy with compound 13 to afford 17 (0.15 g, 45%). Amorphous compound, $[\alpha]_D^{20}$ = −130.0 (CHCl$_3$, c 1.4). R_f 0.3 (EtOAc)

IR spectrum (film, ν, cm^{-1}): = 3398, 3007, 2936, 1639, 1593, 1560, 1533, 1501, 1482, 1477, 1444, 1345, 1235, 1106, 1001, 753, 668, 484. ESI MS *m/z* (I_{rel}, %): 545 [M+Na]$^+$ (56), 561 [M+K]$^+$ (100).

^{13}C NMR (CDCl$_3$, δ ppm): 26.7 (C12); 27.51 (C5); 33.9 (C1); 42.3 (C2'); 49.36 (C6); 50.6 (C4); 52.7 (C2); 67.7 (C5′(6′)); 67.9 (C4'); 67.9 (C7'); 68.6 (C8'); 86.1 (C3'); 105.8 (C11); 106.6 (C10); 120.2 (C4''(8″)); 123.0 (C6″); 128.7 (C5''(7″)); 133.0 (C11a); 136.8 (C9); 138.9 (C3″); 155.7 (C1″); 158.0 (C8).

^{15}N NMR (CDCl$_3$, δ ppm): 68.86 (N1'); 101.74 (N2″); 163.63 (N7).

^{1}H NMR (CDCl$_3$, δ ppm): 1.91 (br.d, 1H, ^2J = 12.6, H$_{anti}$-12); 1.98 (br.d, 1H, ^2J = 12.6, H$_{syn}$-12); 2.42 (m, 1H, H-5); 3.01 (m, 1H, H-1); 3.09 (br.d, 1H, ^2J = 12.8, H$_{exo}$-4); 3.13 (br.d, 1H, ^2J = 12.2, H$_{endo}$-2); 3.84 (m, 2H, H-2′); 3.89 (dd, 1H, ^2J = 15.0, $^3J_{6exo\text{-}5}$ = 6.3, H$_{exo}$-6); 3.99 (br.d, 1H, ^2J = 12.2, H$_{exo}$-2); 4.12 (m, 2H, H-5′(6′)); 4.20 (m, 1H, H-7′); 4.20 (m, 5H, H-8′); 4.22 (m, 1H, H-4′); 4.23 (br.d, 1H, ^2J = 12.8, H$_{endo}$-4); 4.29 (br.d, 1H, ^2J = 15.0, H$_{endo}$-6); 5.20 (br.s, 1H, H-1′); 6.04 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.23 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10); 6.54 (br.s, 1H, H-2″); 6.96 (t, 1H, ^3J = 6.7, H-6″); 7.16 (d, 2H, ^3J = 7.5, H-4''(8″)); 7.18 (dd, 2H, ^3J = 7.5, ^3J = 6.7, H-5''(7″)).

3.2.7. (1R,5S)-9-[(Ferrocenylmethyl)amino]-8-oxo-N-phenyl-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a] [1,5]diazocine-3(4H)-carbothioamide (18) was synthesized from 7 (0.2 g, 0.56 mmol) by analogy with compound 13 to afford 18 (0.07 g, 23%). Amorphous compound, $[\alpha]_D^{20}$ = −155.0 (CHCl$_3$–MeOH, c 1.5). R_f 0.4 (CHCl$_3$–MeOH, 98:2)

IR spectrum (film, ν, cm^{-1}): = 3401, 3273, 2936, 2742, 1636, 1587, 1564, 1521, 1416, 1304, 1218, 1104, 1023, 926, 810, 750, 484. ESI MS *m/z* (I_{rel}, %): 561 [M+Na]$^+$ (100),[M+K]$^+$ (96).

^{13}C NMR (CDCl$_3$, δ ppm): 27.1 (C12); 28.5 (C5); 34.6 (C1); 42.3 (C2'); 48.9 (C6); 55.0 (C4); 56.9 (C2); 67.8 (C5′(6′)); 68.0 (C4'); 68.0 (C7'); 68.7 (C8'); 86.0 (C3'); 106.5 (C11); 106.7 (C10); 123.9 (C4''(8″)); 125.2 (C6″); 128.9 (C5''(7″)); 132.1 (C11a); 137.1 (C9); 139.9 (C3″); 158.1 (C8); 185.0 (C1″).

^{15}N NMR (CDCl$_3$, δ ppm): 69.16 (N1'); 124.27 (N2″); 162.81 (N7).

^{1}H NMR (CDCl$_3$, δ ppm): 2.03 (m, 2H, H-12); 2.55 (m, 1H, H-5); 3.10 (m, 1H, H-1); 3.27 (br.d, 1H, ^2J = 12.8, H$_{exo}$-4); 3.30 (br.d, 1H, ^2J = 12.2, H$_{endo}$-2); 3.87 (dd, 1H, ^2J = 15.0, $^3J_{6exo\text{-}5}$ = 6.3, H$_{exo}$-6); 3.90 (m, 2H, H-2′); 4.16 (m, 2H, H-5′(6′)); 4.24 (m, 5H, H-8′); 4.26 (m, 1H, H-7′); 4.28 (m, 1H, H-4′); 4.41 (br.d, 1H, ^2J = 15.0, H$_{endo}$-6);

4.62 (br.d, 1H, ^2J = 12.2, H_{exo}-2); 4.97 (br.d, 1H, ^2J = 12.8, H_{endo}-4); 5.29 (br.s, 1H, H-1′); 6.09 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.27 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10); 6.98 (d, 2H, ^3J = 8.1, H-4″(8″)); 7.11 (t, 1H, ^3J = 7.4, H-6″); 7.26 (dd, 2H, ^3J = 8.1, ^3J = 7.4, H-5″(7″)).

3.2.8. Methyl[(1R,5S)-9-[ferrocenylmethyl)amino]-8-oxo-1,5,6,8-tetrahydro-2H-1,5-methanopyrido[1,2-a] [1,5]diazocin-3(4H)-yl] acetate (19) was synthesized from 8 (0.2 g, 0.72 mmol) by analogy with compound 13 to afford 19 (0.25 g, 73%). Amorphous compound, $[\alpha]_D^{20}$ = −48.0 (CHCl₃, c 1.3). R_f 0.5 (EtOAc)

IR spectrum (film, ν, cm^{-1}): = 3396, 2938, 2829, 1734, 1639, 1594, 1560, 1477, 1457, 1437, 1420, 1364, 1255, 1194, 1158, 1103, 1003, 805, 752, 479. ESI MS *m/z* (I_{rel}, %):475 M^+ (12), 498 $[M+Na]^+$ (100), 514 $[M+K]^+$ (81).

^{13}C NMR ($CDCl_3$, δ ppm): 26.0 (C12); 28.0 (C5); 34.7 (C1); 42.4 (C2'); 50.0 (C6); 51.2 (C3″); 58.5 (C2″); 58.6 (C4); 59.7 (C2); 67.7 (C5′(6′)); 67.9 (C4'); 67.9 (C7'); 68.6 (C8'); 86.4 (C3'); 104.7 (C11); 106.9 (C10); 135.7 (C11a); 136.3 (C9); 158.0 (C8); 170.8 (C1″).

^{15}N NMR ($CDCl_3$, δ ppm): 28.59 (N3); 67.23 (N1'); 167.49 (N7).

^1H NMR ($CDCl_3$, δ ppm): 1.77 (br.d, 1H, ^2J = 12.6, H_{anti}-12); 1.86 (br.d, 1H, ^2J = 12.6, H_{syn}-12); 2.40 (m, 1H, H-5); 2.78 (br.d, 1H, ^2J = 10.6, H_{endo}-2); 2.83 (br.d, 1H, ^2J = 10.9, H_{exo}-4); 2.86 (br.d, 1H, ^2J = 10.6, H_{exo}-2); 2.87 (m, 1H, H-1); 2.89 (br.d, 1H, ^2J = 10.9, H_{endo}-4); 3.14 (d, 1H, ^2J = 16.7, H_A-2″); 3.21 (d, 1H, ^2J = 16.7, H_B-2″); 3.63 (s, 1H, H-3″); 3.87 (br.s, 2H, H-2′); 3.97 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 7.0, H_{exo}-6); 4.13 (m, 2H, H-5′(6′)); 4.15 (br.d, 1H, ^2J = 14.9, H_{endo}-6); 4.23 (m, 5H, H-8′); 4.25 (m, 2H, H-4′, H-7′); 5.20 (br.s, 1H, H-1′); 5.95 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.26 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10).

3.2.9. (1R,5R)-9-[(Ferrocenylmethyl)amino]-3-methyl-3,5,6-trihydro-2H-1,5-methanopyrido[1,2-a] [1,5]diazocine-4,8(1H)-dione (20) was synthesized from 9 (0.2 g, 0.85 mmol) by analogy with compound 13 to afford 20 (0.28 g, 75%). Amorphous compound, $[\alpha]_D^{20}$ = +40.0 (CHCl₃, c 1.5). R_f 0.5 (acetone)

IR spectrum (film, ν, cm^{-1}): = 3395, 2997, 2941, 2873, 1639, 1595, 1560, 1484, 1461, 1358, 1214, 1105, 1034, 1001, 809, 752, 665, 484. ESI MS *m/z* (I_{rel}, %): 431 M^+ (6), 454 $[M+Na]^+$ (100), 470 $[M+K]^+$ (80), 495 $[M + Na + CH_3CN]^+$ (26), 885 $[2\ M + Na]^+$ (25).

^{13}C NMR ($CDCl_3$, δ ppm): 24.4 (C12); 32.6 (C1); 34.2 (C1″); 36.3 (C5); 42.3 (C2'); 48.6 (C6); 58.8 (C2); 67.8 (C5′(6′)); 67.8 (C4'); 67.9 (C7'); 68.6 (C8'); 85.9 (C3'); 106.1 (C1); 106.6 (C11); 132.4 (C11a); 137.2 (C9); 157.6 (C8); 169.7 (C4).

^{15}N NMR ($CDCl_3$, δ ppm): 69.28 (N1'); 107.29 (N3); 159.83 (N7).

^1H NMR ($CDCl_3$, δ ppm): 2.06 (br.d, 1H, ^2J = 12.6, H_{syn}-12); 2.18 (br.d, 1H, ^2J = 12.6, H_{anti}-12); 2.86 (s, 3H, H-1″); 3.11 (m, 1H, H-5); 3.22 (br.d, 1H, ^2J = 11.8, H_{endo}-2); 3.24 (m, 1H, H-1); 3.68 (dd, 1H, ^2J = 11.8, $^2J_{2exo}$-1 = 3.2, H_{exo}-2); 3.74 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 5.3, H_{exo}-6); 3.88 (m, 2H, H-2′); 4.13 (m, 2H, H-5′(6′)); 4.21 (m, 5H, H-8′); 4.22 (m, 1H, H-7′); 4.23 (m, 1H, H-4′); 4.64 (br.d, 1H, ^2J = 14.9, H_{endo}-6); 5.28 (br.s, 1H, H-1′); 6.06 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11); 6.23 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10).

3.2.10. (1S,5S)-9-[(Ferrocenylmethyl)amino]-3-methyl-3,4,5,6-tetrahydro-2H-1,5-methanopyrido[1,2-a] [1,5]diazocine-2,8(1H)-dione (21) was synthesized from 11 (0.2 g, 0.85 mmol) by analogy with compound 13 to afford 21 (0.22 g, 60%). Amorphous compound, $[\alpha]_D^{20}$ = −77.0 (CHCl₃, c 1.1). R_f 0.5 (CHCl₃–MeOH, 95:5)

IR spectrum (film, ν, cm^{-1}): = 3408, 3097, 2938, 1647, 1595, 1560, 1482, 1474, 1457, 1360, 1268, 1242, 1209, 1104, 1003, 750, 484. ESI MS *m/z* (I_{rel}, %): 431 M^+ (24), 454 $[M+Na]^+$ (100), 470 $[M+K]^+$ (72), 495 $[M + Na + CH_3CN]^+$ (57).

^{13}C NMR ($CDCl_3$, δ ppm): 23.9 (C12); 26.2 (C5); 34.3 (C1″); 42.3 (C1); 42.3 (C2'); 50.2 (C6); 55.7 (C4); 67.8 (C5′(6′)); 67.9 (C4'); 68.0 (C7'); 68.6 (C8'); 86.0 (C3'); 106.6 (C10); 107.3 (C11); 128.2 (C11a); 137.2 (C9); 158.3 (C8); 168.7 (C2).

^{15}N NMR ($CDCl_3$, δ ppm): 69.81 (N1'); 103.67 (N3); 157.03 (N7).

^1H NMR ($CDCl_3$, δ ppm): 2.10 (br.d, 1H, ^2J = 12.6, H_{syn}-12); 2.22 (br.d, 1H, ^2J = 12.6, H_{anti}-12); 2.79 (m, 1H, H-5); 2.86 (s, 3H, H-1″); 3.28 (br.d, 1H, ^2J = 10.8, H_{endo}-4); 3.57 (m, 1H, H-1); 3.62 (dd, 1H, ^2J = 10.8, $^2J_{4exo}$-5 = 5.5, H_{exo}-4); 3.87 (m, 2H, H-2′); 4.00 (dd, 1H, ^2J = 14.9, $^3J_{6exo\text{-}5}$ = 6.8, H_{exo}-6); 4.14 (m, 2H, H-5′(6′)); 4.22 (m, 5H, H-8′); 4.23 (m, 1H, H-7′); 4.24 (m, 1H, H-4′); 4.31 (br.d, 1H, ^2J = 14.9, H_{endo}-6); 5.24 (br.s, 1H, H-1′); 6.22 (d, 1H, $^3J_{10\text{-}11}$ = 7.3, H-10); 6.27 (d, 1H, $^3J_{11\text{-}10}$ = 7.3, H-11).

3.3. Biological assays

3.3.1. Cell culture conditions and treatments

HEK293 (Human Embryonic Kidney 293 cells), Jurkat (Human leukaemic T cell lymphoblast), A549 (Human pulmonary adenocarcinoma), MCF-7 (Human breast adenocarcinoma) and SH-SY5Y (Human neuroblastoma) cell lines were purchased from the Russian Cell Culture Collection (Institute of Cytology Russian Academy of Science, Saint Petersburg, Russia). HEK293, SH-SY5Y, MCF-7, A549 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) (Invitrogen, USA) supplemented with 2 mM L-glutamine (Sigma-Aldrich, UK), 10% fetal bovine serum (FBS; Invitrogen, USA), 50 μg/mL gentamicin sulfate (Invitrogen, USA). Jurkat cells were grown in RPMI 1640 medium (Invitrogen, USA) supplemented with 10% FBS, 50 μg/mL gentamicin sulfate and 2 mM L-glutamine. All cells were cultured and maintained at 37 °C with 5% CO_2 and 95% wet condition. All compounds were dissolved in 100% DMSO (Sigma-Aldrich, UK) to 100 mM stock solutions and diluted in completed DMEM or RPMI immediately before addition to the assay plates. DMSO was maintained at a final concentration of 0.1% unless otherwise specified.

3.3.2. Cell viability

Cells were cultured at appropriate density in 96-well plates (3.0×10^4 cells/well for HEK293 and SH-SY5Y; 1.2×10^4 cells/well for MCF-7 and A549; 1×10^5 cells/well for Jurkat) in a same volume of the proper media. Cells were treated with compounds at final concentrations of 0.1–100 μM for 48 h and cell viability was assessed using the MTT cell viability assay. Experiments were repeated independently two times in triplicate and data were expressed as MTT reduction relative to control (cells treated with 0.1% DMSO). Absorbance was measured within 60 min at 540 nm using the «2300 EnSpire® Multimode Plate Reader» (PerkinElmer, United States). The concentration of the compound that inhibited 50% cell' viability (IC_{50} value) was calculated using nonlinear regression analysis (GraphPad Prism v.5.02; GraphPad Software Inc., USA). The viability of control cells was set as 100%, and the viability in the experimental groups was calculated by comparing the optical density reading with the control. Under similar conditions, cells were treated with 5-Fluorouracil as a positive control for the MTT assay.

3.3.3. Cell cycle analysis

HEK293 (1.75×10^3 cells/well), SH-SY5Y (2.5×10^5 cells/well), MCF-7, A549 (5×10^4 cells/well) cells were seeded in 24-wells plates and cultured for 24 h followed by compound **17** (at IC_{50} values determined for certain cell line) treatment for 24, 48 and 72 h; Jurkat cells (5×10^5 cells/well) were treated with compound **17** for 24, 48 and 72 h immediately after seeding. Cells of control group were treated with 0.1% DMSO. Cells were harvested and fixed in 70% ice-cold ethanol overnight. Subsequently, the cells were centrifuged, the supernatant was discarded and the pellet was treated with RNase A (50 μg/ml) for 5 min at room temperature.

The treated cells were stained with propidium iodide (50 μg/ml) for 15 min at room temperature in the dark. The cells were then analyzed for cell cycle distribution by flow cytometry («Novocyte® 2060», «ACEA Bioscience Inc.», USA) and the changes in the cell cycle profiles were analyzed using « NovoExpress 1.2.5» («ACEA Bioscience Inc.», USA). Experiments were repeated independently two times in triplicate.

4. Conclusions

Thus, novel conjugates of formyl ferrocene with 9-amino 3-*N*-substituted derivatives of (−)-cytisine and its 2-oxo and 4-oxo analogs were synthesized. Evaluation of cytotoxic activity of all synthesized compounds by the ability to inhibit metabolic activity of against cell lines HEK293 (embryonic human kidney), Jurkat (human lymphoblastic leukemia), A549 cells (human alveolar adenocarcinoma), MCF-7 (breast cancer) and SH-SY5Y (human neuroblastoma) was carried out. It was shown, that compound 17 produced a noticeable cytotoxicity towards cancer Jurkat and SH-SY5Y cells with weak activity against non-cancerous HEK293 cells, suggesting the selectivity of this substance to inhibit growth of certain tumor cells. Collectively the results suggested that compound 17 could inhibit cell proliferation and evoke apoptosis in MCF-7, Jurkat and A549 cells by S phase arrest whereas induction of apoptosis in HEK293 and SH-SY5Y cell is cell-cycle independent.

Declaration of competing interest

The authors declare that they have no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.tet.2019.130902.

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