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New saccharide derivatives of indolo[2,3-b]quinoline as cytotoxic compounds and topoisomerase II inhibitors

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Some of alkyl- and alkylamino- derivatives of 6H-indolo[2,3-b]quinolines are known to be active antiproliferative and cell cycle modulating compounds. Their cytotoxic properties are, at least in part, due to DNA intercalation ability and topoisomerase II inhibition activity. To improve physicochemical and biological properties of 6H-indolo[2,3-b]quinolines the series of new, saccharide (C-2, C-9 or N-6) derivatives were designed and synthesized. The influence of different carbohydrate units (D-glucose, D-lactose, L-rhamnose, L-acosamine, L-daunosamine), position of attachment and linker size on cytotoxic properties and topoisomerase II inhibition activity were tested. Among compounds tested there were 2-deoxy-α-D-glucopyranoside (1-6), 2-deoxy-α-L-rhamnopyranoside (7-12) and 2-deoxy-α-D-lactopyranoside (13-18) derivatives in the group of saccharide moiety containing compounds and a-L-daunosaminide (19-24) and a-L-acosaminide (25-27) in the aminosaccharide derivatives series as well.

Key words: DNA topoisomerases, type II – antagonists and inhibitors; indols; quinones

Introduction

Indolo[2,3-b]quinoline derivatives are a group of compounds being the synthetic analogs of a plant alkaloid neocryptolepine (5-methyl-5H-indolo[2,3-b]quinoline), which (together with cryptolepine) is present in *Cryptolepis sanguinolenta* extracts used in natural medicine in Africa.

Cryptolepine (5-methyl-5H-indolo[3,2-b]quinoline) - major *Cryptolepsis sanguinolenta* alkaloid - displays a plenty of pharmacological effects, such as antimuscarinic, noradrenergic receptor antagonistic, antihypertensive, vasodilative, antithrombotic, antipyretic and antiinflammatory properties.

Neocryptolepine and cryptolepine derivatives reveal antiplasmodial and antitrypanosomal[1] and, first of all, cytotoxic activities.[2]

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Indolo[2,3-b]quinoline derivatives can also be considered as the analogs of the DNA intercalating anticancer drug, ellipticine, and its natural isomer, olivacine. The alkaloids of the pyrido[4,3-b]carbazole group are able to stabilize *in vitro* the topoisomerase II-DNA cleavable complexes. One of the cytotoxic olivacine derivatives - S 16020 - showing no immunogenicity in clinical phase I trial was qualified for phase II studies.[3]

Previous studies revealed that topoisomerase II is in fact a cellular target involved in the mechanism of cytotoxic action of 5,11-dimethyl-5H-indolo[2,3-b]quinoline (DiMIQ), first examined cytotoxic compound of the group.[4]

Further investigation into the impact of the substituents introduced into the indolo[2,3-b]quinoline was conducted. Consequently, the series of different derivatives (including various types of substituents and position of their attachment) of 5H- and 6H-indolo[2,3-b]quinolines were synthesized and their cytotoxic activity and ability to induce topoisomerase II-dependent DNA cleavage *in vitro* were tested.

It was initially stated that among methyl-substituted indolo[2,3-b]quinolines, only derivatives belonging to the 5H series (and none of the 6H series), display cytotoxicity against human cervix carcinoma KB cells - ID_{50} (inhibitory dose 50%) values were in the range of 2 to 9 μM - and against several human cancer cell lines of different origin (ID_{50} values varied from 0.6 to 1.4 μM), as well as stimulate the formation of calf thymus topoisomerase II-mediated DNA cleavage at concentrations between 0.2 and 10 μM.[5,6]

Further SAR (structure-activity relationship) studies conducted on 6H series showed that the introduction of an alkyl-amino-alkyl substituent at the N-6 position of indolo[2,3-b]quinoline accounts for the appearance of the cytotoxic properties against KB cell line. ID_{50} values obtained were in the range of 2.0 to 9.0 μM. These results indicate on a strong relation between 6H-indolo[2,3-b]quinoline derivatives structure and their cytotoxic activity, corresponding well with the ability to bind DNA and to inhibit topoisomerase II activity.[7]

To avoid problems connected with poor solubility and resulting inefficient bioavailability, another members of the cytotoxic indolo[2,3-b]quinoline family, well soluble in water in a non-pH-dependent manner, were synthesized and tested.[8]

Simultaneously, attempts at preparing liposomally-formulated 5H-indolo[2,3-b]quinolines[9] and obtaining cytotoxic 6H sugar and aminosugar bearing indolo[2,3-b]quinoline derivatives were undertaken. The results of research on the 6H series are the subject of present publication.

Material and methods

Compounds

All compounds tested were synthesized at Pharmaceutical Research Institute in Warsaw.[10]

Human topoisomerase II activity inhibition assay

Topoisomerase II Assay Kit (Catalog No. 1001-2) and Human Type II Topoisomerase (p170 Form) (Catalog No. 2000H-2); 2U/ml, used in the assays were purchased from TopoGEN, Inc. (Columbus, Ohio, USA).

The start solutions of compounds tested (10 mM) were prepared by dissolving the substances in DMSO (dimethyl sulfoxide, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) and diluting in reaction buffer to the concentrations of 0.0005, 0.0025, 0.005, 0.025, 0.05, 0.25 and 0.5 mM.

Electrophoresis was run in 1% agarose gel (Roth, Karlsruhe, Germany) with ethidium bromide (0.5mg/ml, Sigma-Aldrich Chemie Gmbh, Steinheim, Germany) at 150V. The same amount of ethidium bromide was added

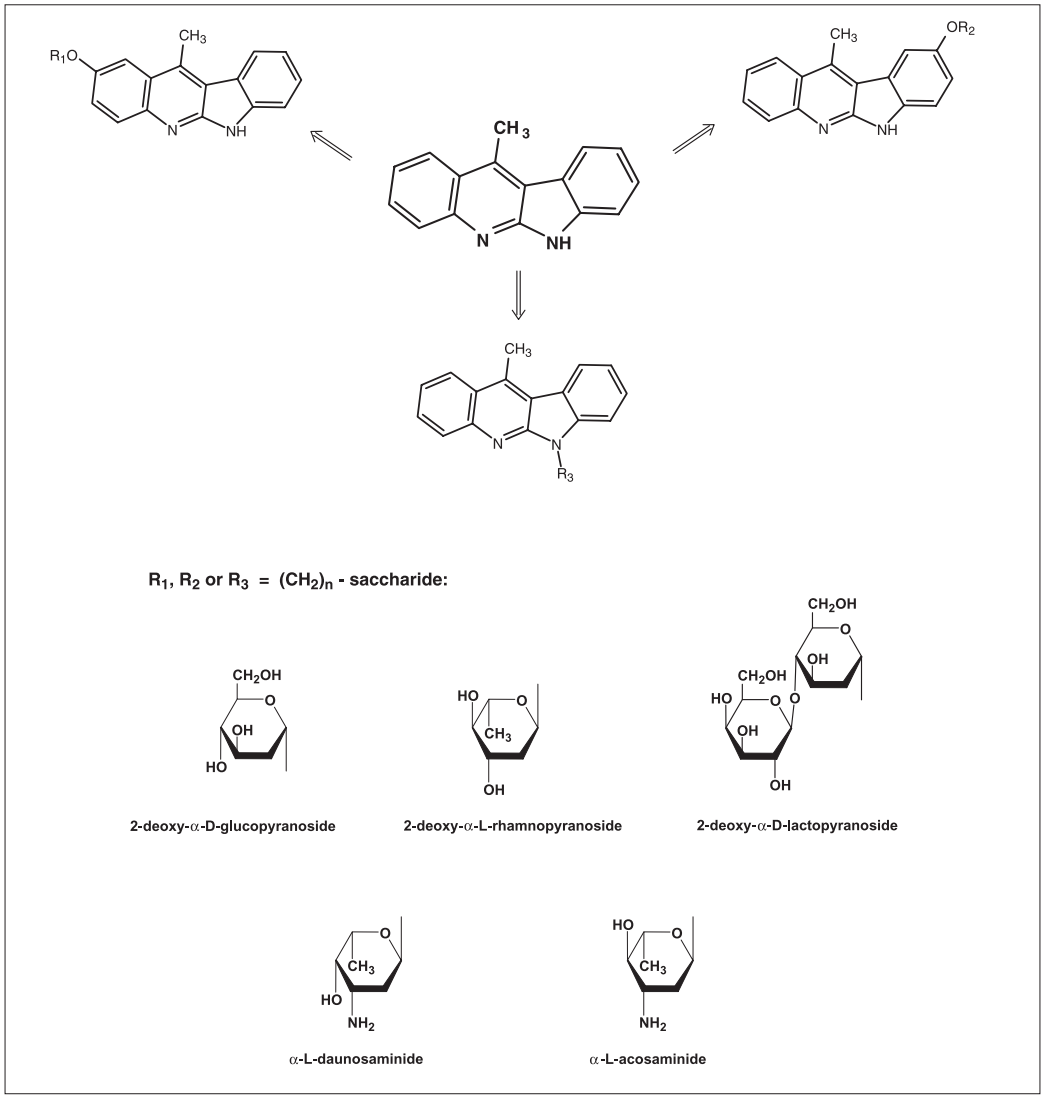


Figure 1. The structures of saccharide and aminosaccharide derivatives studied.

to electrophoresis buffer - 1x TBE (89mM Tris-borate, 1mM EDTA, pH 8.0).

For results acquisition Typhoon 8600 Variable Mode Imager (Molecular Dynamics Inc, Sunnyvale, CA, USA) was applied.

Inhibition of topoisomerase II activity by compounds tested was measured as a degree of inhibition of the kinetoplast DNA decatenation. It was assumed that total enzyme activity inhibition resulted in the same outcomes as achieved for sample with the substrate kinetoplast DNA only.[11,12]

Cell lines

Established *in vitro,* human cervix carcinoma (KB) and Jurkat (T-cell leukemia) cell lines were used. Both lines were obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.) and maintained in the Cell

Table 1. Cytotoxicity and inhibition of topoisomerase II activity revealed by D-glucose indolo[2,3-b]quinoline saccharide derivatives.

Compound tested	Cytotoxity - ID_{50} [μM]	Total topoisomerase II inhibition [mM]
(No. 1) **R_3** = 2-deoxy-α-D-glucopyranoside **n = 2** .	98.30 ± 31.95	n.a.
(No. 2) **R_1** = 2-deoxy-α-D-glucopyranoside **n = 2**	n.a.	n.t.
(No. 3) **R_2** = 2-deoxy-α-D-glucopyranoside **n = 2**	15.40 ± 5.05	n.a.
(No. 4) **R_3** = 2-deoxy-α-D-glucopyranoside **n = 5**	68.20 ± 1.24	n.a.
(No. 5) **R_1** = 2-deoxy-α-D-glucopyranoside **n = 5**	46.30 ± 2.05	n.a.
(No. 6) **R_2**= 2-deoxy-α-D-glucopyranoside **n = 5**	57.60 ± 8.50	n.a.

ID_{50}[1)] – compound concentration leading to 50% inhibition of cell proliferation;
n – number of carbon atoms in linker moiety (as it is shown in Figure 1);
n.a. - not active;
n.t. - not tested.

Culture Collection of the Institute of Immunology and Experimental Therapy, Wroclaw, Poland. The cells were cultured in the RPMI 1640 + Opti-MEM (KB) or RPMI 1640 (Jurkat) medium supplemented with 2mM glutamine (Gibco, Warsaw, Poland), streptomycin (100 mg/ml), penicillin (100U/ml) (both antibiotics from Polfa, Tarchomin, Poland) and 5% (KB) or 10% (Jurkat) fetal calf serum (Gibco, Grand Island, U.S.A.). The cell cultures were maintained at 37°C in humid atmosphere saturated with 5% CO_2.

Anti-proliferative assay in vitro

Test solutions of the compounds (1 mg/ml) were prepared by dissolving the substances in 100 μl of DMSO completed with 900 μl of tissue culture medium. Afterwards, the tested compounds were diluted in culture medium to reach the final concentrations of 100, 10, 1, and 0.1 μg/ml. Results were converted into μM concentrations.

The details of the SRB assay were described by Skehan *et al*.[13] Twenty-four hours before addition of the tested agents, the cells were plated in 96-well plates (Sarstedt, U.S.A.) at a density of $1x10^4$ cells per well. The cytotoxicity assay was performed after 72-hour exposure of the cultured cells to varying concentrations (from 0.1 to 100 μg/ml) of the tested agents. The cells attached to the plastic were fixed by gently layering cold 50% TCA (trichloroacetic acid, Aldrich-Chemie, Germany) on the top of the culture medium in each well. The plates were incubated at 4°C for 1 hour and then washed five times with tap water. The background optical density was measured in the wells filled with culture medium, without the cells. The cellular material fixed with TCA was stained with 0.4% sulforhodamine B (SRB, Sigma, Germany) and dissolved in 1% acetic acid (POCh, Gliwice, Poland) for 30 minutes. Unbound dye was removed by rinsing (4x) with 1% acetic acid. The protein-bound dye was extracted with 10mM unbuffered Tris base (POCh, Gliwice, Poland) for determination of

Table 2. Cytotoxicity and inhibition of topoisomerase II activity revealed by L-rhamnose indolo[2,3-b]quinoline saccharide derivatives.

Compound tested	Cytotoxity - ID_{50} [μM]	Total topoisomerase II inhibition [mM]
(No. 7) **R_3** = 2-deoxy-α-L-rhamnopyranoside **n = 2**	n.a.	n.t.
(No. 8) **R_1** = 2-deoxy-α-L-rhamnopyranoside **n = 2**	n.a.	n.t.
(No. 9) **R_2** = 2-deoxy-α-L-rhamnopyranoside **n = 2**	43.70 ± 8.41	n.a.
(No. 10) **R_3** = 2-deoxy-α-L-rhamnopyranoside **n = 5**	70.80 ± 0.90	n.a.
(No. 11) **R_1** = 2-deoxy-α-L-rhamnopyranoside **n = 5**	n.a.	n.t.
(No. 12) **R_2** = 2-deoxy-α-L-rhamnopyranoside **n = 5**	39.60 ± 13.80	n.a.

ID_{50}[1)] – compound concentration leading to 50% inhibition of cell proliferation;
n- number of carbon atoms in linker moiety (as it is shown in Figure 1);
n.a. - not active;
n.t. - not tested.

optical density (at 540 nm) in a computer-interfaced, 96-well microtiter plate reader Multiskan RC photometer (Labsystems, Helsinki, Finland). Each compound in given concentration was tested in triplicates in each experiment. Every experiment was repeated 3-5 times.

Fluorescent microscopy studies

The cultured cells were washed twice in PBS (phosphate buffer saline, Ca^{2+}, Mg^{2+} - free) and centrifuged. Then the cells were resuspended in the same buffer and incubated for 10, 20 and 60 seconds with 1μM of compound 24. Axioskop 20 (Zeiss, Germany) was used for results acquisition and imaging.

Results and discussion

The results of cytotoxic activity *in vitro* were expressed as ID_{50} – the dose of compound (in mM) that inhibits proliferation rate of the tumour cells by 50% as compared to control untreated cells. The results are presented in Tables 1-4.

Inhibition of the topoisomerase II activity by compounds tested was measured as a degree of the inhibition of kinetoplast DNA decatenation. Some of the compounds tested inhibited the activity of topoisomerase II and the results are presented in Tables 1-4.

As it is shown in Table 1, D-glucose derivatives (1-6) revealed moderate cytotoxic but no topoisomerase inhibitory activity.

Among L-rhamnose containing compounds, only 3 out of 6 (9-11) revealed moderate antiproliferative activity - one with N-6 pentyl linker moiety and two (C-9 substituted) – containing pentyloxy and ethyloxy chains. No one compound from this group revealed any ability to inhibit activity of topoisomerase II.

D-lactose derivatives are the least active cytotoxic compounds (Table 3). Only one compound - N-6 pentyl derivative - shows moderate anti-proliferative activity (16, ID_{50} =

Table 3. Cytotoxicity and inhibition of topoisomerase II activity revealed by D-lactose indolo[2,3-b]quinoline saccharide derivatives.

Compound tested	Cytotoxity - ID_{50} [μM]	Total topoisomerase II inhibition [mM]
(No. 13) R_3 = 2-deoxy-α-D-lactopyranoside **n = 2**	n.a.	n.t.
(No. 14) R_1 = 2-deoxy-α-D-lactopyranoside **n = 2**	n.a.	n.t.
(No. 15) R_2 = 2-deoxy-α-D-lactopyranoside **n = 2**	n.a.	0.5
(No. 16) R_3 = 2-deoxy-α-D-lactopyranoside **n = 5**	45.90 ± 5.73	n.a.
(No. 17) R_1 = 2-deoxy-α-D-lactopyranoside **n = 5**	n.a.	n.t.
(No. 18) R_2 = 2-deoxy-α-D-lactopyranoside **n = 5**	n.a.	n.t.

ID_{50} [1)] – compound concentration leading to 50% inhibition of cell proliferation;
n - number of carbon atoms in linker moiety (as it is shown in Figure 1);
n.a. - not active;
n.t. - not tested.

45.90 ± 5.73 μM) but no topoisomerase inhibition is observed. Surprisingly, one of the compounds, which did not reveal antiproliferative activity, inhibited topoisomerase II activity in the highest concentration tested (compound 15, 0.5 mM). This derivative contains ethyloxy chain attached to indolo[2,3-b]quinoline C-9 carbon atom.

It is evident that for the saccharide containing compounds the crucial for cytotoxic and topoisomerase inhibition activities are C-9 and N-6 substitution positions, and that in the case of N-6 derivatives only five carbon units chain leads to the compounds with biological activity.

Interestingly, substitutions with aminosaccharide moieties give an important increase of cytotoxic properties. All compounds are active and their ID_{50} values are few times lower compared to "non-amino" monosaccharide derivatives. These compounds reveal also strong topoisomerase II inhibitory activity what confirms thesis that there is a third condition (apart from position of substituent and size of linker chain) essential for specific activities of indolo[2,3-b]quinolines derivatives. This condition concerns the type of sugar residue constituted.

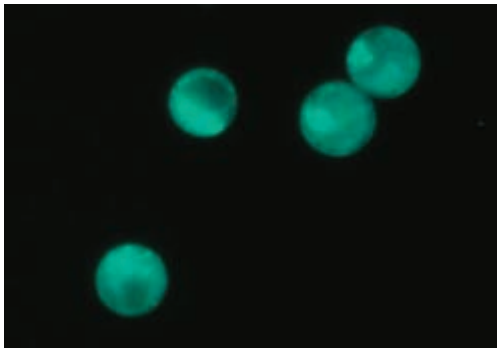
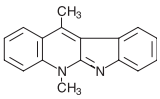


Figure 2. Jurkat cells after 10 seconds of incubation in 1.0μM of compound 24. The cultured cells were washed twice in PBS (phosphate buffer saline, Ca^{2+}, Mg^{2+} - free) and centrifuged. Then the cells were resuspended in the same buffer and incubated for 10, 20 and 60 seconds with 1μM of compound 24. This compound is a fluorophore, so lighting areas in the cells indicate places of its intake. Axioskop 20 (Zeiss, Germany) was used for results acquisition and imaging (Zeiss Filterset 02 - max excitation: $\lambda = 365$; emmission: $\lambda > 420$ nm – was applied).

Table 4. Cytotoxicity and inhibition of topoisomerase II activity revealed by L-daunosamine and L-acosamine indolo[2,3-b]quinoline saccharide derivatives.

Compound tested	Cytotoxity - ID_{50} [μM]	Total topoisomerase II inhibition [mM]
(No. 19)		
R_3 = α-L-daunosaminide	69.42 ± 9.77	0.025
n = 2		
(No. 20)		
R_1 = α-L-daunosaminide	6.41 ± 0.86	0.005
n = 2		
(No. 21)		
R_2 = α-L-daunosaminide	10.26 ± 2.82	0.025
n = 2		
(No. 22)		
R_3 = α-L-daunosaminide	12.80 ± 0.47	0.500
n = 5		
(No. 23)		
R_1 = α-L-daunosaminide	7.05 ± 0.15	0.050
n = 5		
(No. 24)		
R_2 = α-L-daunosaminide	7.05 ± 0.33	0.025
n = 5		
(No. 25)		
R_3 = α-L-acosaminide	7.16 ± 0.002	0.025
n = 2		
(No. 26)		
R_1 = α-L-acosaminide	12.20 ± 1.33	0.025
n = 2		
(No. 27)		
R_2 = α-L-acosaminide	10.12 ± 2.25	0,050
n = 2		
DIMIQ* 	1.14 ± 0.61	0.500
(No. 28)		

ID_{50}[1)] – compound concentration leading to 50% inhibition of cell proliferation;
n - number of carbon atoms in linker moiety (as it is shown in Figure 1);
n.a. - not active;
n.t. - not tested;
*DiMIQ - referential compound - 5,11-dimethyl-indolo[2,3-b]quinoline;

Within range of aminosaccharide derivatives of indolo[2,3-b]quinolines, the most promising are compounds 20 and 21 – C-2 and C-9 substituted ethyloxy, as well as 23 and 24 - C-2 and C-9 substituted pentyloxy L-daunosamine derivatives (Table 4). In the series of L-acosamine derivatives, the best properties were revealed by compound 25 with five carbon linker chain attached to N-6 position of indolo[2,3-b]quinoline.

Cytotoxic activities observed for the active compounds in the series are comparable to values estimated previously for 5H and 6H derivatives.[5-7] The most cytostatic against KB cells and inhibiting activity of topoisomerase II are aminosaccharide substituted indolo[2,3-b]quinolines.

Since some of previously tested indolo[2,3-b]quinoline derivatives show poor solubility (data not shown) what causes the problems connected with bioavailability, next step of the studies was to examine the cell intake of the compound 24. These results are shown in Figure 2. It can be seen that the compound is

well distributed in cytosol and accumulated in some subcellular structures.

Although a great effort have been made to find an effective anticancer chemotherapeutics, the number of clinically active drugs remains quite small and their spectrum of antitumor activity is rather limited. Because of that, there are still many projects and research aiming to discover the new, more effective or more selective anticancer compounds.[14]

Beside the cytotoxic properties assessed in *in vitro* tests, the candidates for the new anticancer drugs should reveal some special features, as good solubility and bioavailability and of course low toxicity. Structure-activity relationship studies are one of the ways to select the compound of the best properties.

On the basis of presented here results, the most promising indolo[2,3-b]quinoline derivatives were chosen for further preclinical *in vitro* and *in vivo* studies.

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