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Invited review

Rebeccamycin analogues as anti-cancer agents

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Abstract

Rebeccamycin, a microbial metabolite possessing a maleimide indolo[2,3-*a*]carbazole framework with a carbohydrate moiety attached to one of the indole nitrogens, is a well-known topoisomerase I inhibitor. This review reports the various total syntheses of rebeccamycin and structure–activity relationship studies on rebeccamycin analogues. Rebeccamycin analogues were prepared either by semi-synthesis from the natural metabolite or by total synthesis. Different families of rebeccamycin analogues were obtained by modifications at the imide heterocycle, dechlorination and substitutions on the indole moieties, modifications of the sugar residue, construction of dimers, coupling the sugar unit to the second indole nitrogen, changing indolo[2,3-*a*]carbazole skeleton to indolo[2,3-*c*]carbazole, replacing one or both indole moieties by 7-azaindole units. The biological activities of the rebeccamycin analogues are described. According to their chemical structure, the analogues can inhibit topoisomerase I and/or kinases. From the structure–activity relationships, some important rules were established. Several compounds exhibit stronger antiproliferative activities than the natural metabolite with IC_{50} values in the nanomolar range. Some analogues, especially those possessing azaindole moieties, are much more selective than rebeccamycin toward the tumour cell lines tested.

Keywords: Rebeccamycin; Indolocarbazole; Antitumour agents; Topoisomerase I inhibitors; Kinases inhibitors

1. Introduction

Rebeccamycin, a microbial metabolite isolated from cultures of *Saccharothrix aerocolonigenes*, was described in 1987 as a compound able to induce a prolongation of survival of leukemic mice at dose levels ranging from 8 to 256 mg kg^{-1} with P388 leukemia and L1210 leukemia. Rebeccamycin was found to introduce breaks in eukaryotic DNA, however, its weak solubility in aqueous media posed problems in further evaluation [1]. Its structure was determined by spectroscopic means, X-ray crystallography, and total synthesis [2,3]. Rebeccamycin (Fig. 1) possesses an indolocarbazole framework onto which a 4-*O*-methylglucose is attached via a β-*N*-glycosidic bond. Fermentation feeding experiments showed that addition of sodium bromide in the place of sodium chloride in the fermentation broth resulted in isolation of a brominated analogue DBrIC. DBrIC has a similar potency and antitumour activity as rebeccamycin [4]. Biosynthesis studies conducted with radio-labelled components revealed that rebeccamycin is derived from two tryptophan, one glucose, and one methionine molecules [5].

To date four synthetic approaches have been developed (Fig. 2). Kaneko et al. [3] applied the indole Grignard method by coupling 7-chloroindole to *N*-protected dibromomaleimide. The adduct was photocyclised before glycosylation via the Koenigs–Knorr method. The second route [3] utilised a Diels–Alder reaction between maleimide and a 2,2′-bisindole obtained from 7,7′-dichloro-indigo. Danishefsky [6] introduced the sugar moiety using a 1,2-anhydrosugar before photocyclisation. The originality of the synthesis described by Faul et al. [7] was the edification of the indolocarbazole framework after coupling of the carbohydrate part to 7-chloroindole-3-acetamide.

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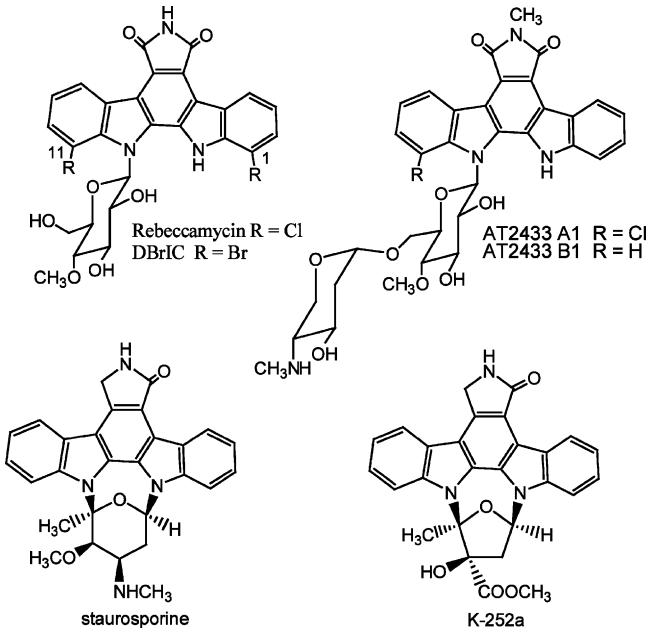


Fig. 1. Rebeccamycin and structurally related microbial metabolites.

Interestingly, rebeccamycin is structurally related to many other microbial metabolites such as staurosporine, AT2433 A1 and B1, K-252a (Fig. 1) [8], but unlike the parent antibiotics, rebeccamycin can be produced in large quantities by fermentation. In a 30 l fermentor, the yield was 663 mg l^{-1}, allowing the preparation of rebeccamycin analogues by semi-synthesis. The main structural differences between rebeccamycin and staurosporine and K-252a are (i) the sugar moiety linked to only one indole nitrogen in rebeccamycin, (ii) an imide function instead of amide function in the upper heterocycle. These differences seem essential for enzyme selectivity. Staurosporine is a non selective kinases inhibitor without efficiency against topoisomerase I whereas rebeccamycin is a topoisomerase I inhibitor without efficiency toward protein kinase C (PKC) and protein kinase A [9]. With the aim of investigating the elements of the rebeccamycin structure that are critical for the biological activity, structure–activity relationship studies were performed by several groups. Rebeccamycin analogues were obtained either by semi-synthesis or total synthesis.

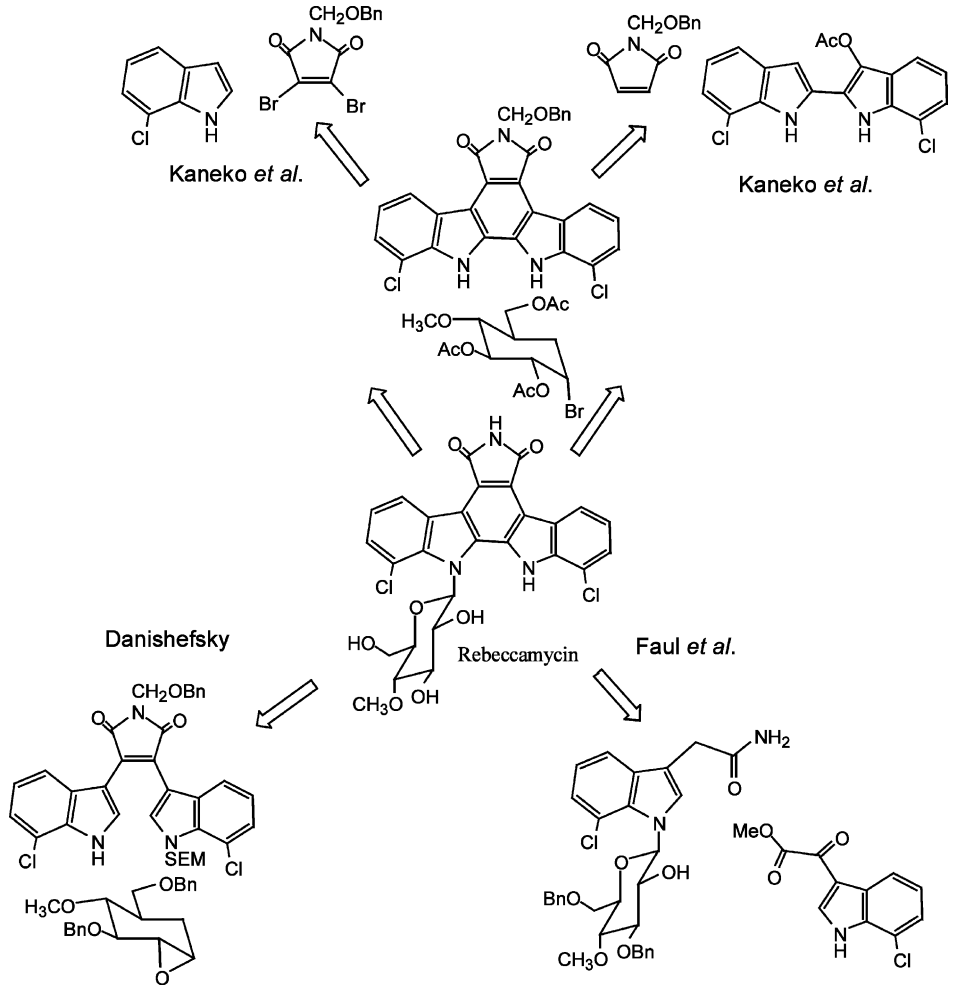


Fig. 2. Total syntheses of rebeccamycin.

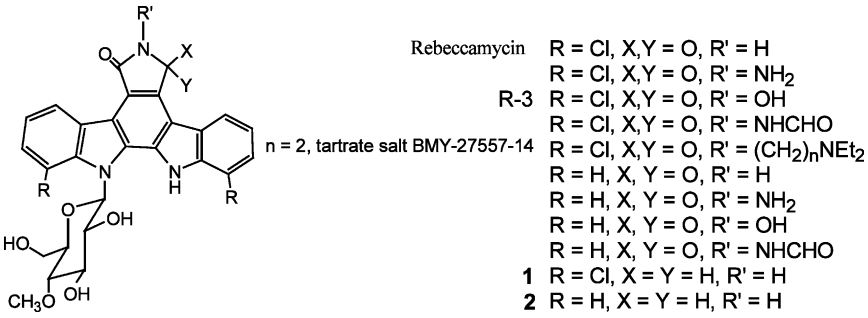


Fig. 3. Modifications at the imide heterocycle and parent aglycones.

2. Modifications at the imide heterocycle

Various substituents were introduced on the imide nitrogen of rebeccamycin, 1,11-dechlorinated rebeccamycin and corresponding aglycones without the sugar moiety (Fig. 3) [9–12]. The induction of topoisomerase I-mediated DNA cleavage by these rebeccamycin analogues was studied. The inhibitory potencies of the compounds were assessed in vitro by comparing the cleavage of DNA by the enzyme in the absence or presence of the drug. Moreover, DNA-binding properties were investigated by various spectroscopic techniques and the DNA sequence selectivity was probed by DNase I footprinting. The results showed that the sugar residue is necessary for the drug ability to interfere with topoisomerase I and to form intercalation complexes. The chlorine atoms reduce the inhibitory activity toward topoisomerase I, and substituents on the imide nitrogen can be varied without reduction of activity [13]. The interaction with DNA of compound **R-3**, bearing a hydroxyl group at the imide nitrogen, was especially examined. The studies indicated that the indolocarbazole chromophore intercalates into DNA and the sugar part contributes to reinforce the affinity for DNA by interacting with the grooves. Modifications of the exocyclic substituents on the bases of DNA in both the major and minor grooves showed that the drug uses both exocyclic substituents exposed in the minor and major grooves to recognise selectively TpG- and GpT-containing sequences [14]. Analysis of the base preferences around topoisomerase I cleavage sites indicated that **R-3** stabilised topoisomerase I preferentially at sites having a T and G on the 5′ and 3′ sides of the cleaved bond.

When the imide function was replaced by an amide function (compounds **1** and **2** Fig. 3), a non negligible inhibition of PKC was observed.

The tartrate salt derivative BMY-27557-14 ($n = 2$) has entered phase II clinical evaluation [4].

3. Substitutions on the indole moieties

Fluoro substituents were introduced in the various positions by feeding experiments with addition of 6-fluoro, 5-fluoro or 4-fluorotryptophan to the fermentation broths (Fig. 4) [4]. Initial studies revealed that 4′-demethylated-3,9-difluoro compound was ca. 10-fold more potent toward topoisomerase I than camptothecin, the prototype inducer of topoisomerase I-mediated DNA cleavage. The 4′-demethylated-4,8-difluoro analogue was 10-fold less potent than camptothecin. The 2,10-difluoro-4′-methylated and 4′-demethylated analogues were equal to camptothecin for inducing topo I-mediated DNA cleavage. It has been shown that the presence of a methyl group at the 4′-*O* position has little effect on potency for inducing topo I-mediated DNA cleavage but increases cytotoxicity against P388 murine leukemia cells almost 10-fold. The most cytotoxic

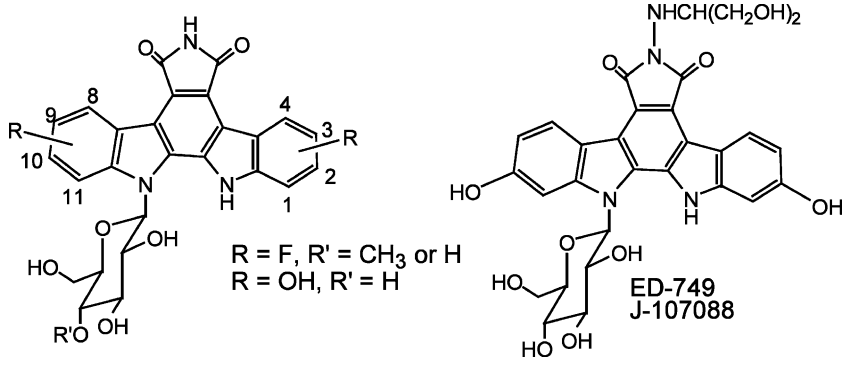
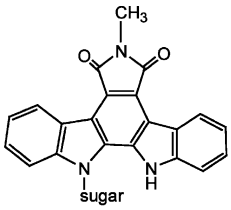
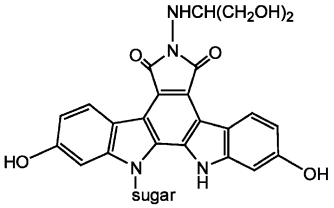


Fig. 4. Substitution on the idole moieties with a fluoro or a hydroxy group.



A

α- and β-glucopyranose
α- and β-galactopyranose
α- and β-fucopyranose



B

α- and β-glucopyranose
α- and β-arabinofuranose
α- and β-xylofuranose
α- and β-allopyranose
α- and β-mannopyranose
α- and β-2-deoxyglucopyranose
α- and β-ribofuranose
β-allofuranose
β-glucofuranose
β-galactopyranose
β-6-deoxyglucopyranose
β-xylopyranose
β-maltose

Fig. 5. Modifications of the sugar moiety.

compound in this series was the 3,9-difluoro-4′-demethylated analogue.

Analogues bearing hydroxy substituents on the indolocarbazole framework were obtained either by semi-synthesis or by total synthesis (Fig. 4) [15,16]. According to the position of the hydroxyl groups, both topo I inhibitory potencies and antiproliferative activities varied in a large extent. The hydroxyl substituents at 3,9-positions induced the strongest cytotoxicity and topo I inhibitory properties as observed for fluoro substituents. ED-749 has been introduced in the clinic by Merck–Banyu as a topo I targeting drug.

4. Modification of the sugar moiety

To indolocarbazoles possessing a methyl group at the imide nitrogen, various sugar moieties have been attached, a glucopyranose, a galactopyranose or a fucopyranose. The carbohydrate units were linked to the indole nitrogen via either a β- or an α-*N*-glycosidic bond [17] (Fig. 5 and Table 1 compounds **A**). Compounds possessing an α-*N*-glycosidic bond do not behave as intercalating agents and have much less effect on topo I but there is no significant differences in terms of cytotoxicities. The β-*N*-glycosidic linkage represents a key element for topo I inhibition. Modification of the sugar moiety in compounds possessing a β-*N*-glycosidic bond does not change significantly the cytotoxicity toward the tumour cell line tested (murine P388 leukemia). Compounds with an α-*N*-glycosidic bond are stronger PKC inhibitors than compounds with a β-*N*-glycosidic bond. Ohkubo et al. [18] synthesised analogues of ED-749 substituted at the indole nitrogen with a variety of carbohydrates linked via either a β- or

Table 1
Inhibitory activities toward Topo I and PKC, antiproliferative activities against murine P388 leukemia cells

Compound **A**	Topo-I MIC (μM)	CTX P388 IC_{50} (μM)	PKC IC_{50} (μM)
α-Glucopyranose	> 20	6.0	59
β-Glucopyranose	2.0	6.0	> 100
α-Galactopyranose	6.0	6.0	62
β-Galactopyranose	0.6	6.0	99
α-Fucopyranose	> 20	6.2	82
β-Fucopyranose	2.0	6.2	> 100
Compound **B**	Topo-I cleavage EC_{50} (μM)	CTX P388 IC_{50} (μM)	PKC IC_{50} (μM)
α-Glucopyranose	0.300	19	23
β-Glucopyranose	0.051	0.0015	200
α-Mannopyranose	0.080	0.015	50
β-Mannopyranose	0.230	0.036	20
α-Allopyranose	0.026	0.018	72
β-Allopyranose	0.026	0.036	20
α-2-Deoxyglucopyranose	0.130	0.016	150
β-2-Deoxyglucopyranose	0.240	0.005	130
β-6-Deoxyglucopyranose	0.140	0.003	60
β-Galactopyranose	0.140	0.007	> 200
β-Xylopyranose	0.100	0.004	> 200
α-Xylofuranose	0.017	0.012	> 200
β-Xylofuranose	0.017	0.00096	> 200
α-Ribofuranose	0.030	0.019	21
β-Ribofuranose	0.008	0.0018	20
β-Allofuranose	0.042	0.017	> 200
β-Glucofuranose	0.020	0.014	> 200
β-Maltose	> 3	0.0035	> 200

an α-*N*-glycosidic bond (Fig. 5 and Table 1, compounds **B**). The β-ribofuranosyl substituent led to the most efficient topo I inhibitor, however, the cytotoxicity against murine leukemia cells P388 was similar to that observed with a β-glucopyranosyl group which is a weaker topo I inhibitor. The β-xylofuranosyl substituent induced the strongest cytotoxicity toward P388 cells. The cytotoxicities cannot be correlated to the anti-topo I activities, probably because of the presence of other mechanisms of cytotoxicity. Depending on the structure of the sugar unit, the α- or β-glycosylated compounds exhibit the stronger inhibitory potency toward topo I, PKC and P388 cells.

The sugar residue of rebeccamycin is located in one of the two helical grooves of DNA, probably the minor groove as is the case with the anthracyclins. However, the exact positioning of the glycosyl residue in the drug–DNA complex was poorly understood. To better understand the interaction of glycosylated indolocarbazoles with DNA, the interaction of a rebeccamycin derivative bearing a 2′-amino group on the carbohydrate moiety was examined. The 2′-amino derivative was synthesised as outlined in Fig. 6 from *N*-methylmaleimide indolocarbazole [19] and a *N*-phtalimido-sugar. Coupling was performed according to a method described for the synthesis of nucleosides [20] using *N*-methylmaleimide indolocarbazole silylated at one of the indole nitrogens in the presence of trimethylsilyltriflate. Reaction of the coupling product with hydrazine hydrate led to the diamine. Binding to DNA and topo I inhibitory capacities showed that the introduction of an amino group on the glycosyl residue contributes to a tighter interaction with DNA and does not prevent the drug from inhibiting topo I. The 2′-amino derivative binds significantly more tightly to DNA than the corresponding analogue bearing only an amino substituent at the imide nitrogen (Fig. 6) [21]. In the presence of formaldehyde, the 2′-amino function allows the formation of covalent drug–DNA complexes. It has been shown that the 2′-amino derivative reacts covalently with the 2-amino group of guanines exposed in the minor groove of DNA. The HCHO-mediated alkylation of the drug with A.T base pairs involves the 6-amino group of adenines exposed in the major groove whereas the covalent attachment to G.C base pairs involves the 2-amino group of guanines exposed in the opposite minor groove. Therefore, the drug is able to bind to both the minor and the major grooves of the double helix [22].

Fluorinated analogues bearing a 6′-amino substituent were also prepared (Fig. 7) [23]. Their EC_{50} values (effective concentration inducing 50% topo I-mediated DNA cleavage) were in the nanomolar range.

With the aim of improving the interaction with DNA possibly via a covalent reaction, compounds bearing a halogenoacetyl substituent at various positions on the sugar residue were prepared, as well as analogues bearing an acetyl group instead of a bromoacetyl group to gain an insight into the role of the halogen atom (Fig. 8 and Table 2) [24]. No covalent reactions with DNA were detected, this could be due to either hydrolysis of

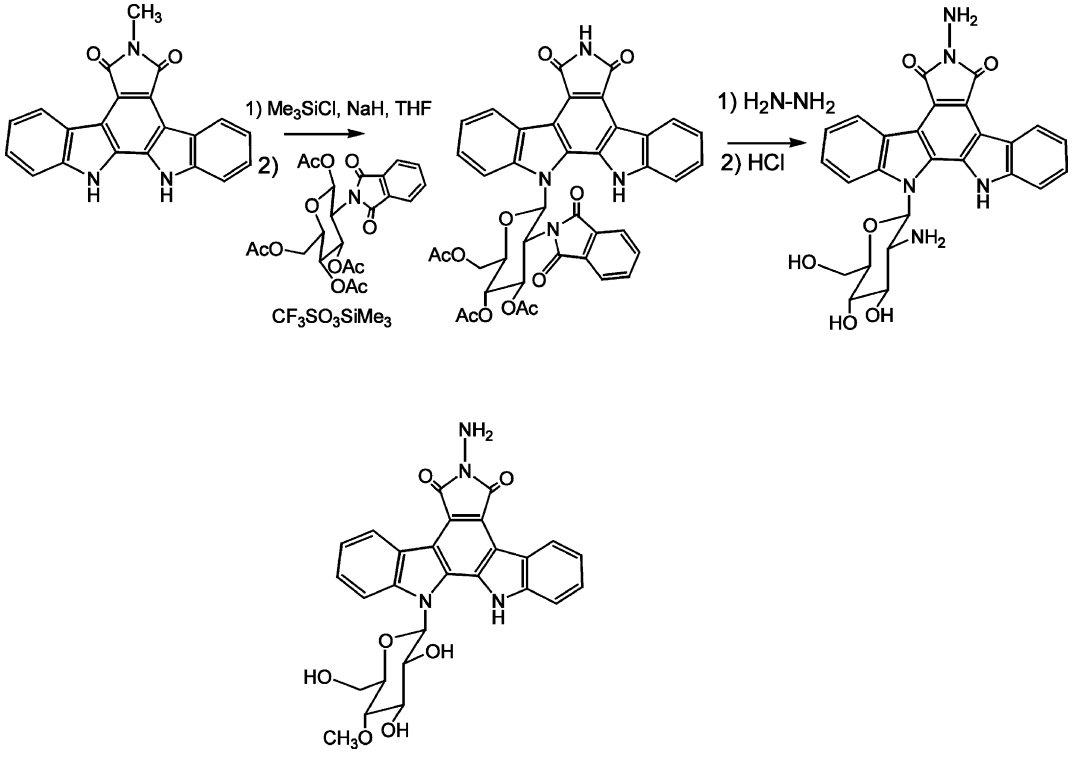


Fig. 6. Introduction of an amino function at 2′ position of the sugar unit.

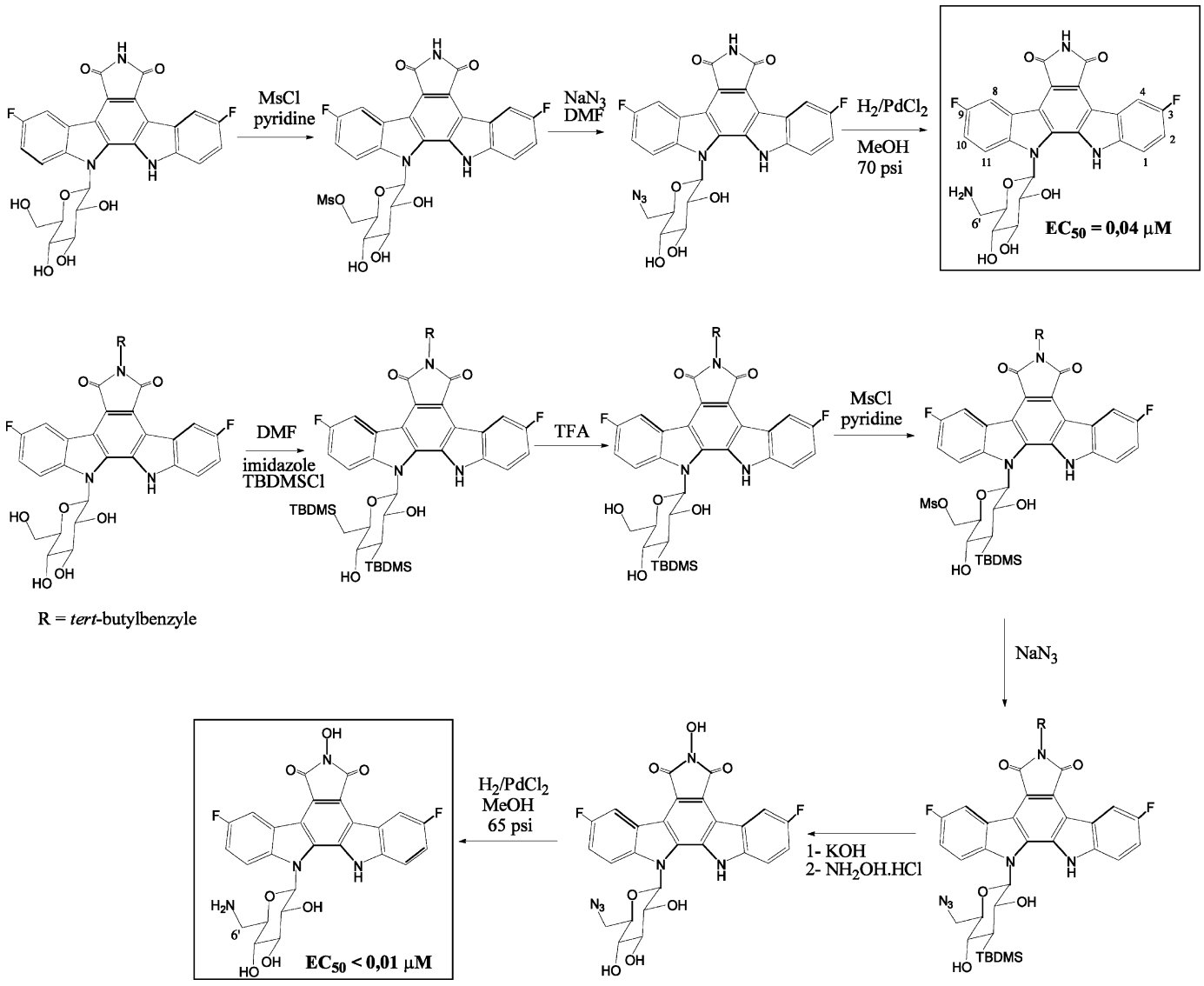


Fig. 7. Fluoro rebeccamycin analogues.

the esters or intrinsic reactivity of the alkylating moiety which is too low. Nevertheless, the drugs behave as typical topo I poisons, they are significantly more toxic toward P388 cells than toward P388/CPT5 cells resistant to camptothecin. Compound **VIII** bearing a bromoacetyl group at 3′-position was the most cytotoxic in this series.

The possibilities of improving the solubility were investigated by introducing amino acids or peptides which may also increase the capacity to bind to the target. Compounds bearing a lysine or the tripeptide GHK linked to one of the nitrogen atoms of the aglycone via a propylamino chain were synthesised, and rebeccamycin analogues with a lysine substituent on the sugar moiety at 2′ or 3′ positions were prepared by semi-synthesis (Fig. 9) [25]. As expected, compounds bearing a lysine or a GHK substituent are much more soluble than the parent compounds. The in vitro antiproliferative activities were tested against four tumour cell lines: one murine leukemia (L1210), one human leukemia (K-562) and two human solid tumours: one colon carcinoma (HT29), one non-small cell lung carcinoma (A549) (Table 3). Compared with dechlorinated rebeccamycin, compounds **XII** and **XIII**, bearing a lysine group at 2′ and 3′ positions, respectively, exhibited a reduced cytotoxicity toward L1210 leukemia cells but similar profiles of cytotoxicity toward the other tumour cell lines. Compared with *N*-methylated aglycone bearing the propylamino chain at one indole nitrogen, compound **IX** bearing a lysine had the same profile of cytotoxicity whereas **X** had a lower cytotoxicity and **XI** was almost inactive. The examination of effects on the L1210 cell cycle seems to indicate that in the absence of the sugar unit, the cells are accumulated in the G1 phase whereas compounds with the sugar part induce accumulation of the cells in the G2+M phases

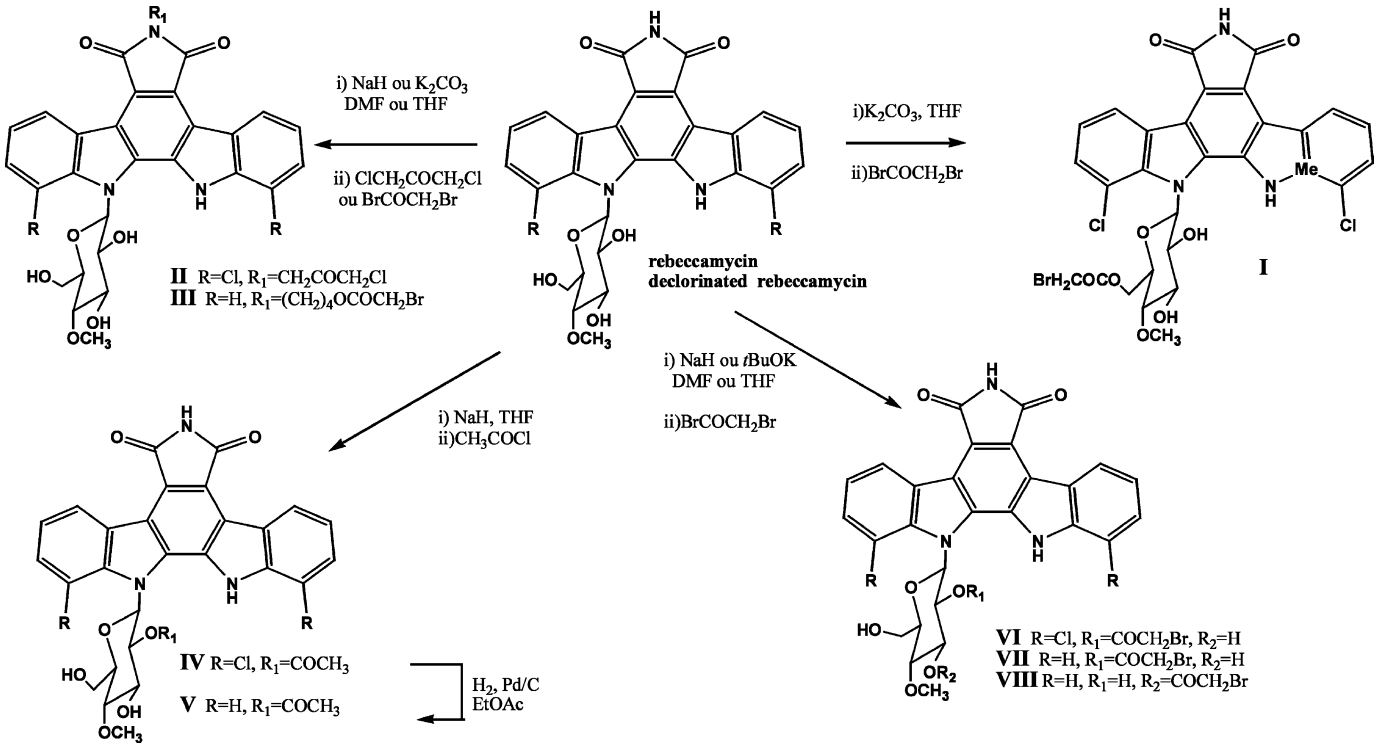


Fig. 8. Rebeccamycin analogus bearing a halogenoacetyl substituent.

Table 2
Inhibitory activities toward topo I and cytotoxicities toward murine leukemia P388 cells and P388/CPT5 cells resistant to camptothecin

Compound	Topoisomerase I [a] MIC (μM)	IC_{50} (μM)	
		P388	P388/CPT5
Rebeccamycin	1.75	1.22	10.5
Dechlorinated rebeccamycin	0.59	0.69	> 20
I	1.45	0.43	4.3
VI	> 14	0.26	0.94
VII	1.59	0.48	5.0
VIII	1.61	0.16	3.2
IV	> 16	0.48	3.3
V	18	0.55	4.6
II	1.14	0.24	3.5
III	1.44	1.94	> 14

[a] MIC, minimum drug concentration required to the detection of DNA cleavage.

which could be explained by different cellular targets. Selectivity toward the various cell lines was observed for compounds bearing the sugar part but not for compounds lacking the carbohydrate substituent. The analysis of drug–DNA interactions showed that **IX** and **X** exhibited a much higher affinity for DNA than the other compounds in this series. Compound **XI**, which exhibited the lowest antiproliferative activity, had also the lowest affinity for DNA. The introduction of a lysine residue on the propylamino chain (compound **IX**) highly increased the DNA interaction, which was not the case when the lysine was attached to the sugar moiety (compound **XII** and **XIII**). Interestingly, compared with **IX**, which tightly binds to DNA, the analogue **XI** lacking the methyl group at the imide nitrogen weakly binds to DNA. The methyl group seems to be critical to DNA affinity and cytotoxicity.

5. Dimers from dechlorinated rebeccamycin

Since a good affinity for DNA could increase the stability of the ternary complex DNA-topo I-drug, dimers were synthesised with the aim of improving DNA binding by forming bis-intercalating agents. The design of dimeric compounds represents an attractive strategy to increase both DNA binding affinities and sequence selectivity. This strategy has already been successfully used with anthracyclines [26,27]. The two chlorine atoms of rebeccamycin being detrimental to the formation of DNA intercalation complexes, two dimers were prepared by coupling the imide nitrogens of two molecules of dechlorinated rebeccamycin [28]. Two linkers were used, one flexible, $-(CH_2)_2-NH-(CH_2)_3-NH-(CH_2)_2-$, and another one more rigid, $-NH-CH_2-C_6H_4-CH_2-NH-$. The dimers were prepared by reaction of the corresponding diamines with dechlorinated anhydride obtained from rebeccamycin by treat-

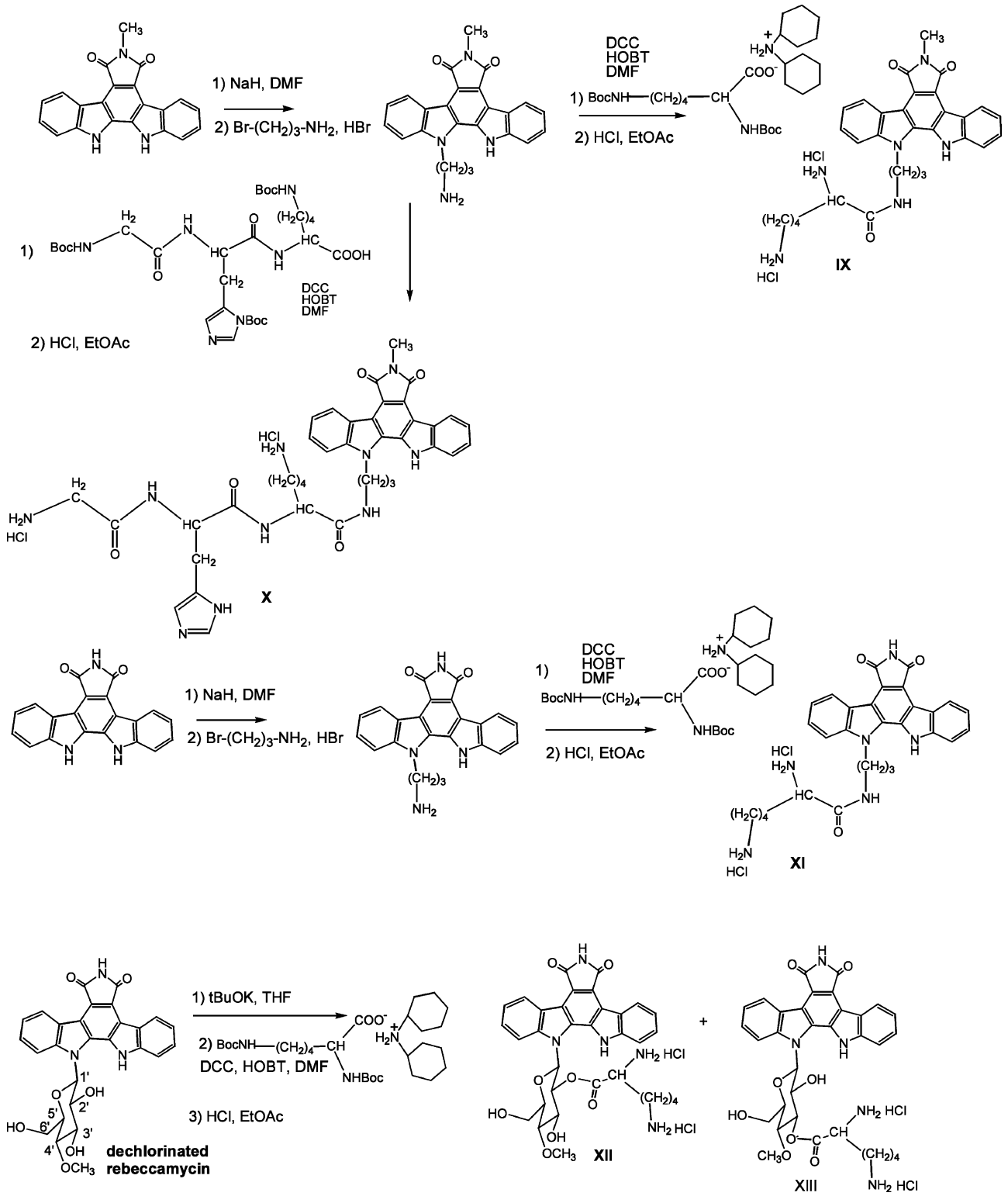


Fig. 9. Introduction of a lysine or a tripeptide substituent.

ment with Raney nickel in aqueous sodium hydroxide (Fig. 10). The interaction of the two dimers with DNA was studied. Compared with dechlorinated rebeccamycin, only dimer **A** exhibited a higher affinity. The rigid linker containing an aromatic ring is very likely too short to enable bis-intercalation. However, no enhancement of topoisomerase I inhibition was observed with the two dimers. The antiproliferative activities of the two dimers toward four tumour cell lines were examined (Table 4). Dimer **A** is the most efficient and exhibits a

Table 3
In vitro antiproliferative activities against four tumour cell lines: murine leukemia L1210, and human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukemia (IC_{50} μM)

Compound	L1210	L1210 cell cycle	HT29	A549	K-562	Binding constants K ($\times 10^5$ M^{-1})
N-methylated aglycone	5.1	G1 66% at 25 μM	ne	ne	ne	
N-methylated aglycone with the propylamino chain	2	NS, tox at 10 μM	5.1	5.5	4.5	3.8
X	3.8	G1 59% at 10 μM	15.0	8.1	5.6	15
IX	1.9	NS, tox at 10 μM	6.4	5.5	5.4	17
XI	36.4	Ne	> 10	> 10	> 10	1.7
Dechlorinated rebeccamycin	0.11	G2M 71% at 1 μM	4.0	3.4	< 0.1	2.7
XII	0.31	G2M 71% at 2 μM	2.3	2.8	< 0.1	4
XIII	1	G2M 68% at 2.5 μM	6.2	5.6	0.25	4

Effect on L1210 cell cycle: percentage of cells recovered in G1 or G2+M phases with a concentration of drug expressed in μM. Binding constants were calculated from fluorescence measurements. Twenty four percent of untreated control cells were in the G2+M phase of the cell cycle, 44% in the G1 phase and 28% in the S phase. NS, not specific, i.e. no modification of the cell cycle; ne not evaluated.

stronger cytotoxicity than dechlorinated rebeccamycin against HT29 and A549 cells.

6. Coupling the sugar to the second indole nitrogen

As reported in the introduction, staurosporine is structurally related to rebeccamycin but the sugar moiety is linked to both indole nitrogens. Whereas rebeccamycin is a topo I inhibitor, staurosporine is a non selective kinases inhibitor without anti-topo I activity. Via semi-synthesis from rebeccamycin, analogues were prepared in which the carbohydrate part was coupled to the second indole nitrogen (Fig. 11) [29]. Tosylation occurred at 2′ position on the sugar part, treatment with sodium azide led to the formation of compound **3** via deprotonation of the second indole nitrogen followed by nucleophilic substitution on C2′. The minor product of the reaction was the azide at 3′ position formed via 2′,3′-epoxide. The stereochemistry at C2′ was assigned from crystallographic data (unpublished results). Dechlorination led to **4** which was further reduced to obtain staurosporine analogues **5**–**5′** as a mixture of regioisomers.

Table 4
In vitro antiproliferative activities against four tumour cell lines: murine leukemia L1210, and human HT29 colon carcinoma, A549 non-small cell lung carcinoma and K-562 leukemia (IC_{50} μM)

Compound	L1210	HT29	A549	K-562
Dechlor reb	0.11	2.5	2	< 0.1
Anhydride	70.3	~ 10	> 10	> 10
A	0.9	0.5	0.4	~ 0.1
B	14	32	23	ne

Twenty four percent of untreated control cells were recovered in the G2+M phase of the cell cycle; ne, not evaluated.

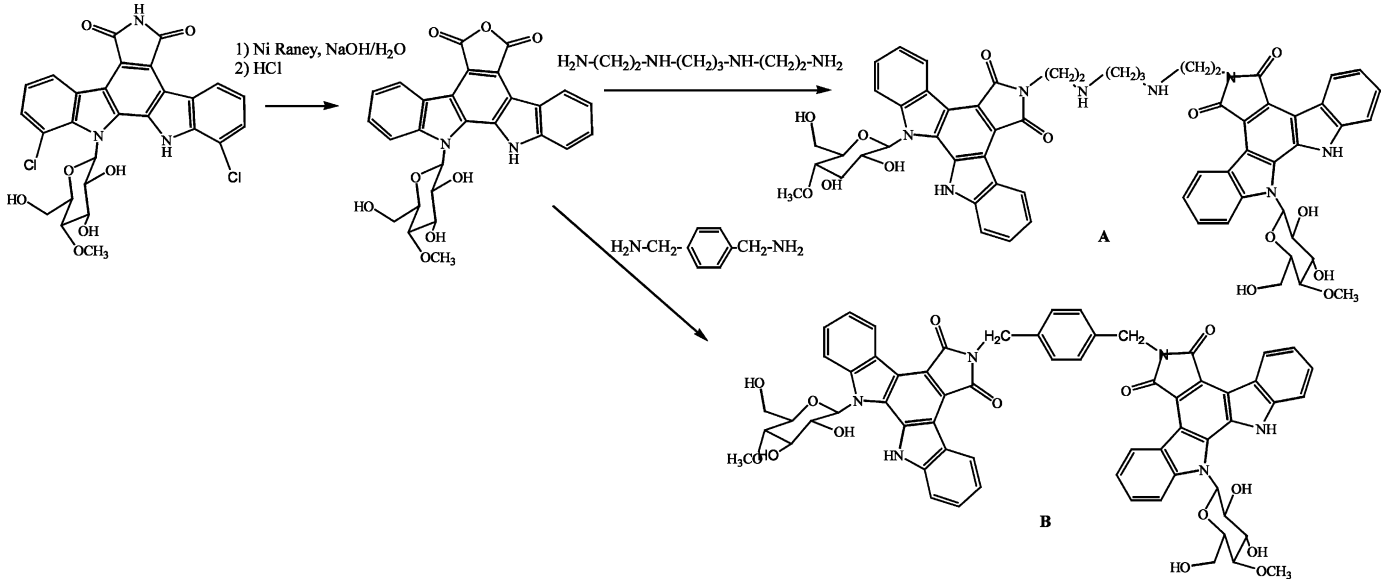


Fig. 10. Dimers from dechlorinated rebeccamycin.

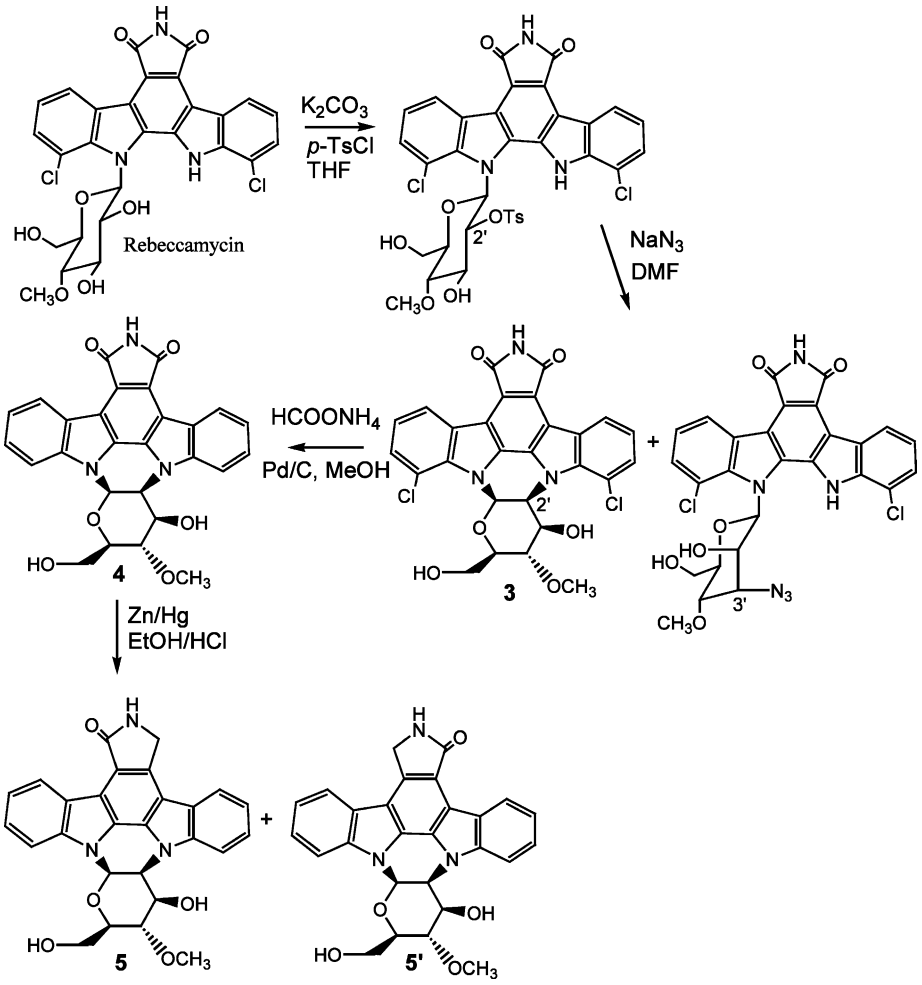


Fig. 11. Coupling sugar moiety to the second indole nitrogen.

Compound **4** was the most efficient compound of this series both as topo I inhibitor and antiproliferative agent against murine B16 melanoma and P388 leukemia cells. None of compounds **3**–**5** exhibited any activity against PKC. Compound **4** was the only drug in this series capable to stabilise DNA-topo I cleavable complexes and as a result to exhibit marked antiproliferative activities. The cytotoxic effect of compound **4** was examined toward P388 CPT cells resistant to the topo I inhibitor camptothecin. The high resistance index ($R = IC_{50}$ P388 CPT/IC_{50} P388) suggested a major contribution of topo I inhibition to the cytotoxicity of this compound. To enhance the solubility of compound **4**, substitutions were performed either on the aromatic moieties or on the imide nitrogen (Fig. 12) [30].

The in vitro antiproliferative activities of compounds **3**–**19** were tested against eight tumour cell lines: one murine leukemia (L1210), one human leukemia (K-562) and six human solid tumours: one ovarian carcinoma (IGROV1), one neuroblastoma (SK-N-MC), one colon carcinoma (HT29), one non-small cell lung carcinoma (A549), one small-cell lung carcinoma (H69) and one epidermoïd carcinoma KB-3-1. Results, expressed as IC_{50}, are reported in Table 5. The most cytotoxic compound against L1210 cells is the dinitro compound **9**. Contrary to rebeccamycin, some of the compounds in this series are highly selective toward the tumour cell lines tested. Dinitro **9** exhibit strong and selective antiproliferative activities whereas the dibromo analogue **6** is highly cytotoxic but non selective. Diphenol **18** is selective toward SK-N-MC and NCI-H69 cells, moreover, it is the only compound in this series able to inhibit the cyclin-dependent kinase CDK1. Diformylated **11**, dihydroxymethyl **12**, and anhydro **15** are also selective. Anhydride **7** is almost inactive. The effect on the L1210 cell cycle of compounds in this series was examined. Most of them induced an accumulation in the G2+M phases except for the anhydro **15** with which an accumulation of the cells in the G1 phase was observed. The biological results suggest either different targets for these compounds or different expression of the target enzymes in the various cell lines tested. The inhibitory potencies against a panel of kinases, which regulate the cell cycle are being investigated.

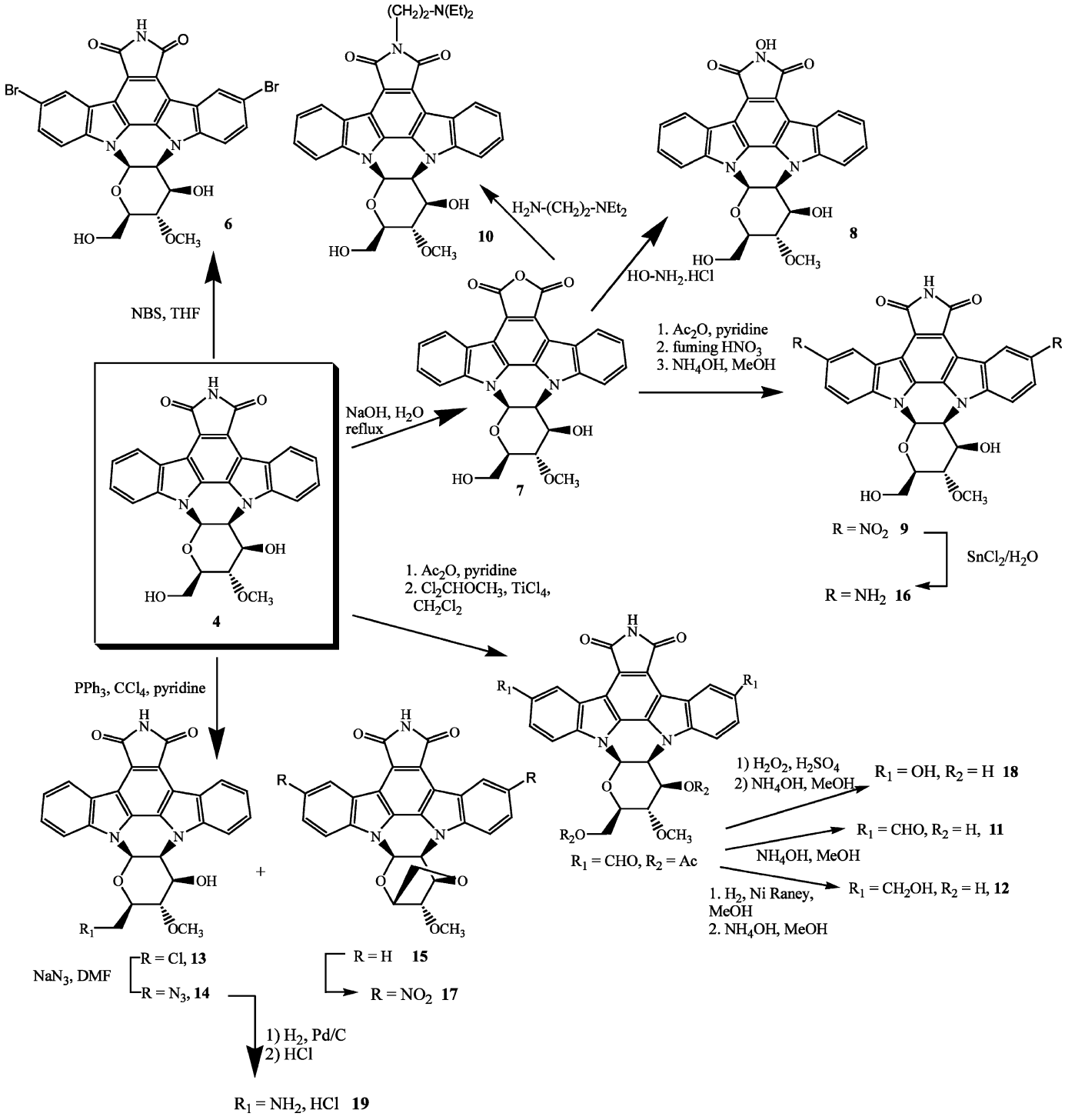


Fig. 12. Staurosporine analogues from rebeccamycin.

7. 'Inverted' rebeccamycins

To investigate whether the orientation of the sugar moiety and of the indolocarbazole framework are critical to the biological activity, 'inverted' rebeccamycins possessing an indolo[2,3-*c*]carbazole chromophore instead of the conventional indolo[2,3-*a*]carbazole unit were synthesised from isatin and indole (Fig. 13) [31].

A mono-glycosylated compound **20** and a bis-glycosylated compound **21** were prepared, their interaction with DNA, topo I inhibition and antiproliferative activities were examined. The results indicate that the mono-glycosylated analogue **20** retains the capacity to intercalate into DNA, but the structural change is detrimental to topo I inhibition and antiproliferative activities. The bis-glycosylated derivative **21** has lost its

Table 5
In vitro antiproliferative activities against nine tumour cell lines: one murine leukemia (L1210), one human leukemia (K-562) and seven human solid tumours: one ovarian carcinoma (IGROV1), one neuroblastoma (SK-N-MC), one colon carcinoma (HT29), one non-small cell lung carcinoma (A549), one small-cell lung carcinoma (H69) and one epidermoïd carcinoma KB-3-1 (IC_{50} μM)

Compound	L1210	IgROV	SK-N-MC	HT29	A549	NCI-H69	K-562	KB-3-1
Rebeccamycin	0.1	0.25	< 0.1	0.3	0.3	0.25	0.2	0.3
3	1.3	< 10	< 1	3.5	3.3	< 1	0.8	< 1
4	0.91	1	< 1	2.5	2.0	< 1	0.5	< 0.1
6	0.18	0.1	< 0.1	0.1	0.2	< 0.1	< 0.1	1
7	70.3	> 10	10	10	> 10	> 10	> 10	> 10
8	0.6	< 10	< 0.1	3.0	3.1	< 1	0.4	< 1
9	0.1	> 10	< 10	> 10	> 10	< 0.1	< 0.1	0.1
10	0.38	0.39	0.22	0.9	1.2	0.19	0.36	0.29
11	0.2	> 10	< 1	> 10	> 10	10	> 10	10
12	18.1	> 10	< 1	> 10	> 10	0.1	0.8	10
15	2.9	> 10	> 10	> 10	> 10	> 10	> 10	> 10
16	19.3	> 10	< 10	> 10	> 10	< 10	~ 10	> 10
17	0.3	< 0.1	< 0.1	< 0.1	0.1	< 0.1	0.1	< 1
18	0.7	10	< 0.1	> 10	10	< 0.1	0.1	< 10
19	0.24	0.28	0.09	0.21	0.23	0.08	0.23	0.11

ne, Not evaluated. IC_{50} values: < 1 for $0.1 < IC_{50} < 1$; < 10 for $1 < IC_{50} < 10$.

capacity to form stable complexes with DNA. The IC_{50} values of **20** and **21** toward murine L1210 and human HT29, A549 and K-562 cells were found to be > 10 μM. The naturally-occurring indolo[2,3-*a*]carbazole skeleton should be preserved to maintain anti-topo I and antiproliferative activities.

8. Replacing one or both indole moieties by azaindole units

Azaindoles, as biosteres for indoles, present considerable biological importance [32]. Replacement of one or both indole moieties by azaindole units could enhance the binding of the drugs to the target macromolecules. Aza rebeccamycin analogues were synthesised from commercially available 7-azaindole [33,34]. Compounds **22** and **23** possessing two azaindole moieties, *N*-substituted or not at the imide nitrogen with a methyl group, were synthesised from 7-azaindole and dibromomaleimide, substituted at the imide nitrogen by a methyl or a benzyloxymethyl group (Fig. 14). A Mitsunobu reaction was performed to introduce the sugar part. The key photocyclisation was achieved in the presence of iodine. When $R = CH_3$, aminolysis led to *N*-methylated compound **22** whereas when R = BOM, hydrogenolysis followed by aminolysis led to **23**.

An identical strategy was adopted for the synthesis of compounds possessing one azaindole moiety with the sugar unit linked to the azaindole nitrogen (Fig. 15). According to the substituent of the dibromomaleimide, benzyloxymethyl or methyl, compounds **24** and **25** were obtained, respectively. Bromination of the indole moiety at 9-position was performed from the more soluble tetraacetylated intermediate. Nitration at 9-position of intermediate **C** led to mononitro compound **28**. The electrophilic substitutions were not observed on the azaindole moiety.

To synthesise compound **29**, the best yield was obtained by using a phase transfer catalyst in the key glycosylation [35]. 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranosyl chloride was coupled to aglycone **D** [36] giving two coupling products, the major product of the reaction was the compound with the sugar linked to the indole unit. Its hydrogenolysis led to compound **29** (Fig. 16).

Compound **30** was prepared according to an identical scheme as presented in Fig. 10 using 7-azaindole instead of indole as the starting compound and *N*-benzyloxydibromomaleimide. The biological activities of the aza analogues were compared with rebeccamycin, dechlorinated rebeccamycin and $N\text{-}CH_3$ analogue E (Fig. 17).

DNA binding and topo I inhibitory properties of the compounds in this series were evaluated. The position of the newly incorporated nitrogen atom in the aromatic framework has a major effect on the DNA binding capacity of the drug. When the carbohydrate is linked to the indole moiety and the azaindole is unsubstituted, DNA binding is promoted. When the azaindole bears the sugar, it abolishes the DNA binding capacity. The introduction of bromo or nitro substituents has no significant effect on the binding to DNA. At first sight, topo I inhibitory potency may be correlated with DNA affinity, except for the compounds in which the indole ring bears a bromo or nitro group which markedly reinforces the anti-topo I activity. The in vitro antiproliferative activities of compounds **22**–**30** were tested against nine tumour cell lines: one murine leukemia (L1210), one human leukemia (K-562) and seven human solid tumours: one ovarian carcinoma (IGROV1), one

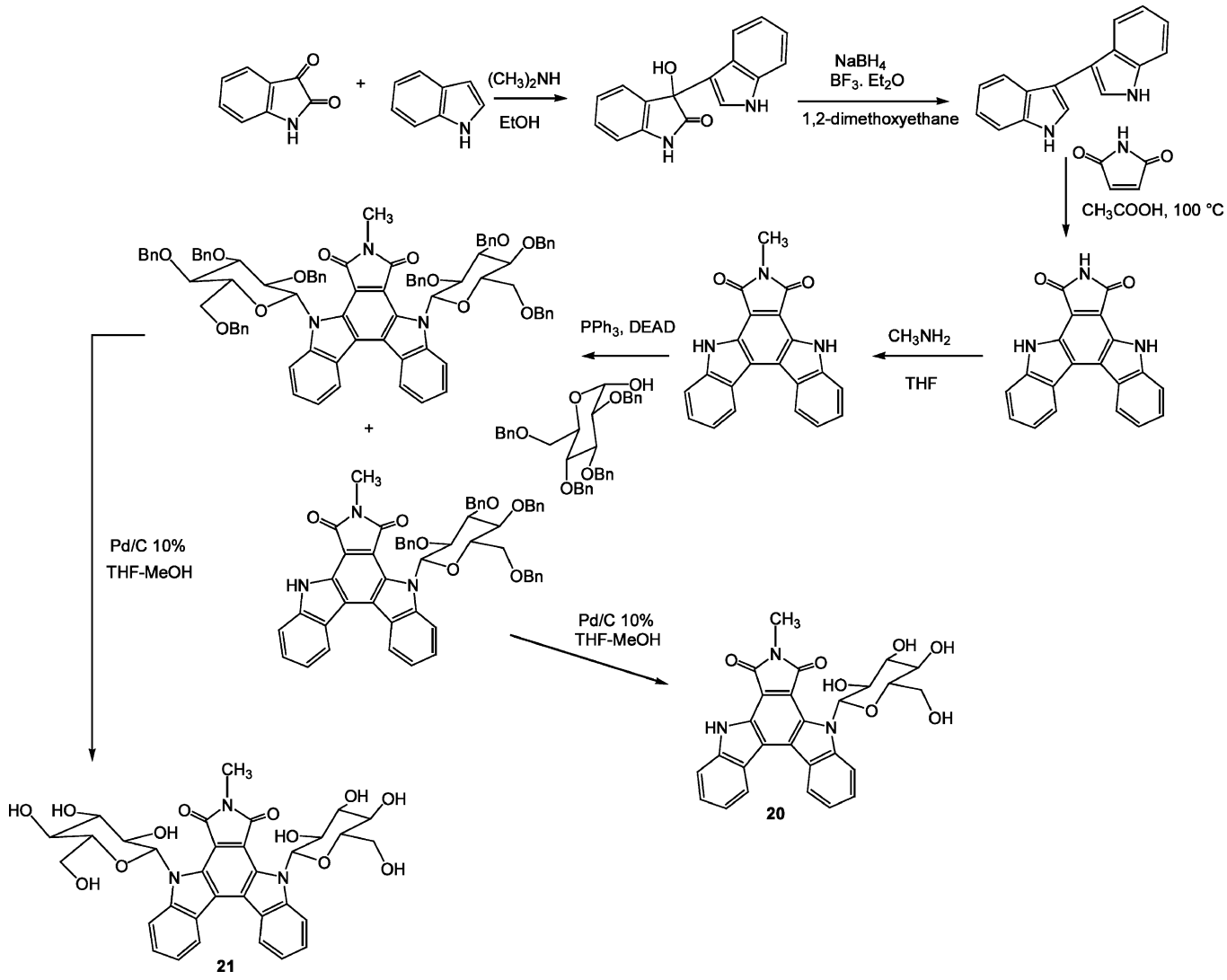


Fig. 13. Rebeccamycin analogues possessing an indolo[2,3-*c*]carbazole framework.

neuroblastoma (SK-N-MC), one colon carcinoma (HT29), one non-small cell lung carcinoma (A549), one small-cell lung carcinoma (H69) and two epidermoïd carcinomas (A431 and KB-3-1). Results, expressed as IC_{50}, are reported in Table 6. In contrast with rebeccamycin, dechlorinated rebeccamycin and *N*-methylated compound **E**, aza analogues are much more selective toward the tumour cell lines tested. The most sensitive cells are L1210, SK-N-MC, A431 and NCI-H69 cells. The parent compounds **24** and **30** in one hand, and **25** and **29** in the other hand, in which the sugar is linked to the azaindole and to the indole, respectively, exhibit no marked differences in the cytotoxicities toward L1210 in spite of their important differences in DNA binding capacities and anti-topo I activities. In aza analogues series, as in the previously described series in which the sugar moiety is linked to both indole nitrogens, the same conclusions may be deduced: very probably, either the biological targets are different or the targets are differently expressed in the various tumour cell lines. The effect of the drugs on the L1210 cell cycle was examined. The most cytotoxic compounds toward L1210 cells, **23**, **26**, **28** and **30** induced a marked accumulation of the cells in the G2+M phases (66–87% at 0.25 μM) (Table 7). The progression of G2+M phases depends on several enzymes and mostly on the cyclin-dependent kinase CDK1. Therefore, the inhibitory activities of the aza analogues toward CDK1 have to be determined.

9. Cellular uptake and interaction with purified membranes

As there were no information concerning the capacity of rebeccamycin analogues to penetrate into the cells and to reach the nuclear compartment where topoisomerase I is located, the uptake of a rebeccamycin

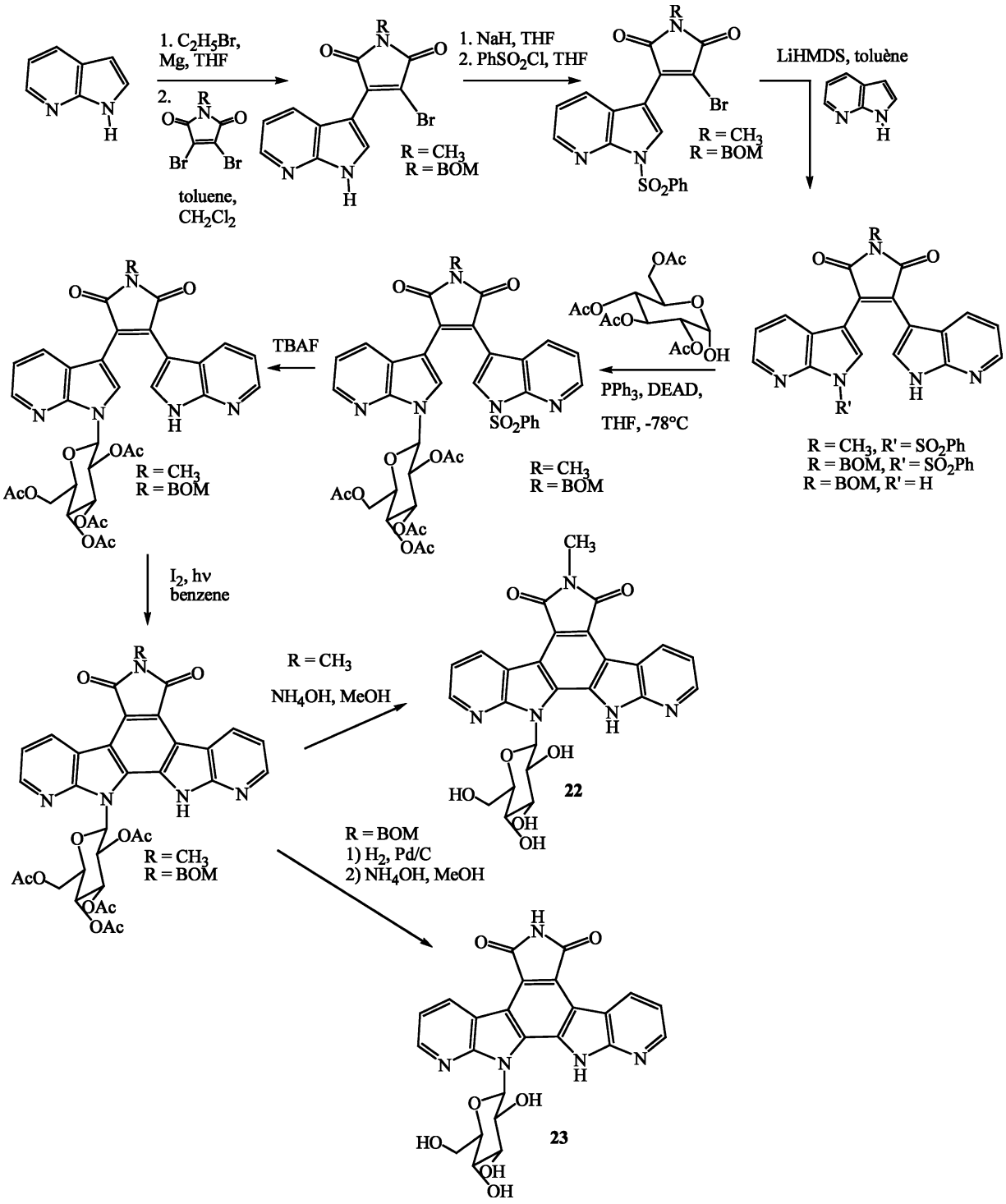


Fig. 14. Diaza rebeccamycins.

derivative by four tumour cell lines was investigated using radiolabelled [^{3}H]dechlorinated rebeccamycin obtained by hydrogenolysis of the chlorine atoms of rebeccamycin in the presence of $^3H_2$. The uptake is fast but limited to a few percent of the initial dose, suggesting a passive diffusion mechanism. The interaction of the rebeccamycin derivative **R-3** and the parent aglycone with purified membranes from HL60 leukemia cells was investigated by fluorescence studies [37]. It has been shown that the carbohydrate part contributes positively to the transfer of the drug from the membrane to the other cellular components such as DNA, whereas the highly lipophilic aglycone remains tightly bound to the membranes.

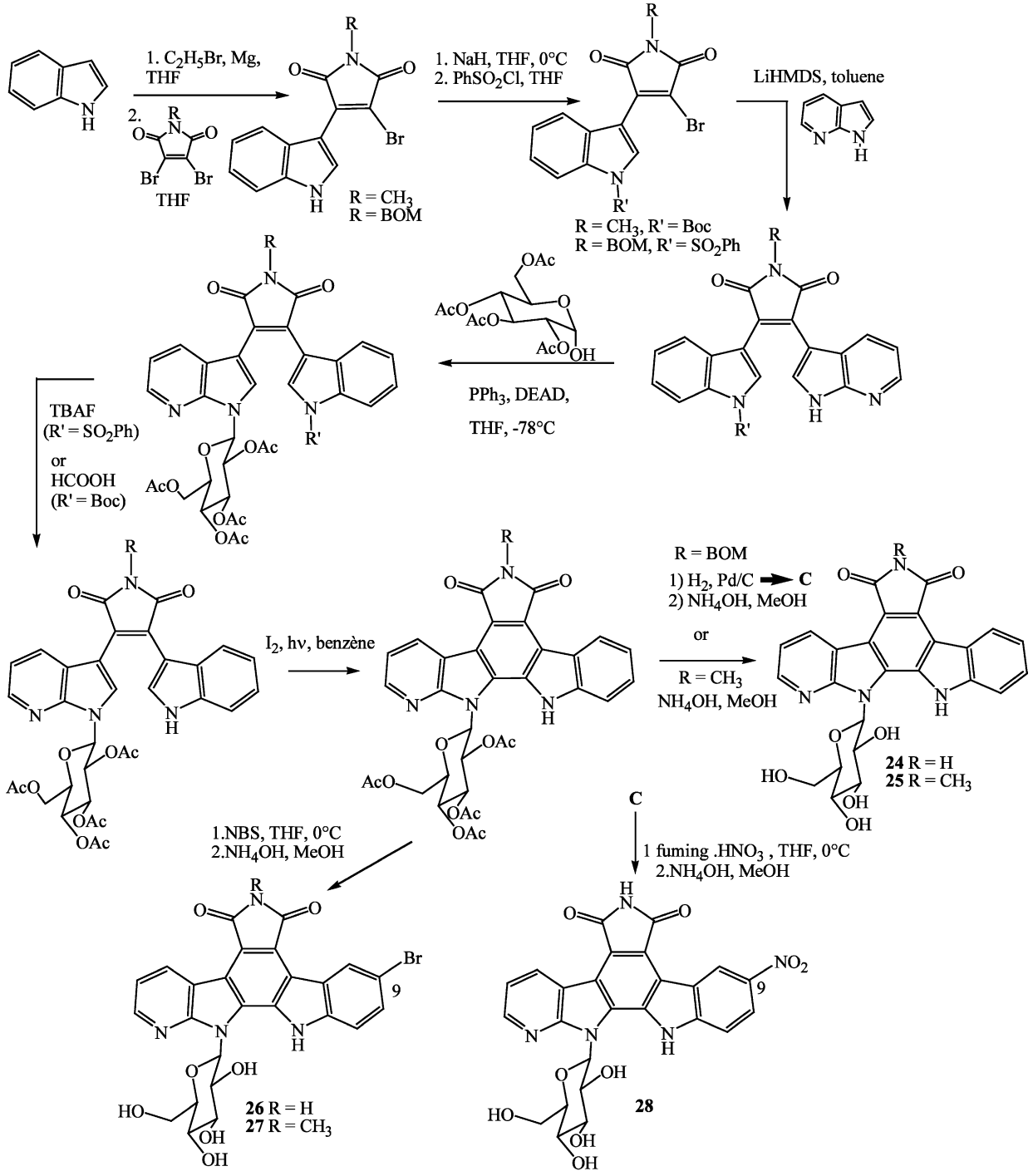


Fig. 15. Monoaza rebeccamycins with the sugar part attached to the azaindole moiety.

10. Conclusion

Rebeccamycin derivatives represent a promising class of antitumour agents. Over the last few years, more than 150 rebeccamycin derivatives have been synthesised. The structure–activity relationships are not yet fully elucidated but some important rules were established. The rebeccamycin analogues may inhibit topoisomerase I and/or kinases according to their chemical structures.

Concerning topo I inhibition (i) the two chlorine atoms on the indolocarbazole framework of rebeccamycin are detrimental to the interaction with DNA and to topo I inhibition, (ii) A variety of substituents can be added on the imide nitrogen without disrupting the

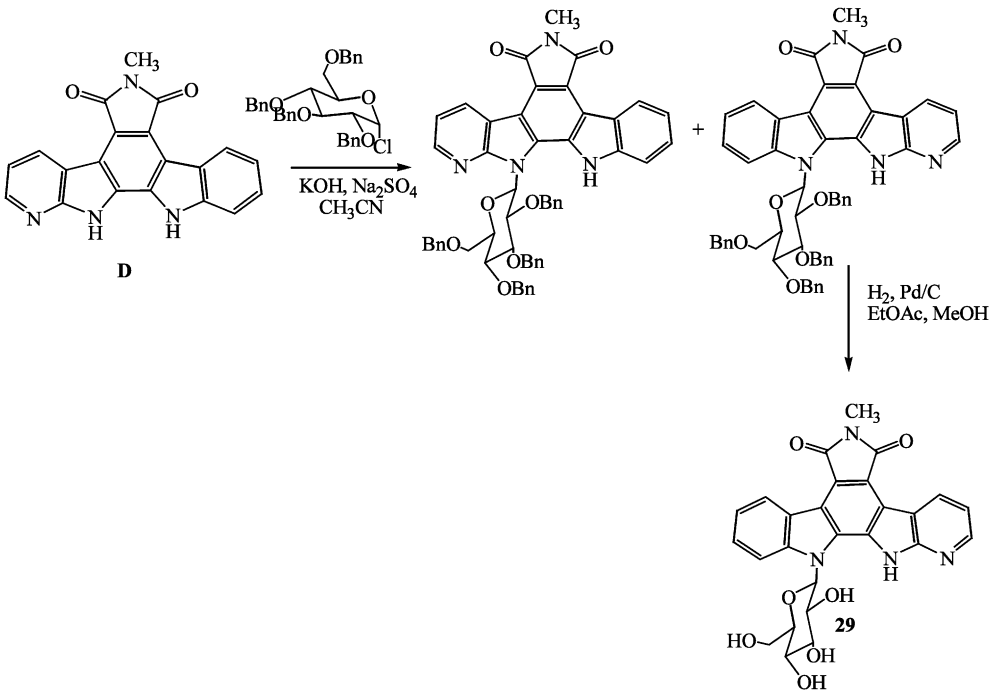


Fig. 16. Monoaza rebeccamycin with the sugar part attached to the indole moiety.

drug–DNA complex. For example, polar hydroxyl or formylamino groups as well as non polar methyl groups can be tolerated without loss of the topoisomerase I poisoning effect, (iii) the sugar residue is absolutely required to ensure tight interaction with DNA and anti-topo I activity. The aglycones without the sugar residue are inefficient toward topo I, (iv) when the sugar moiety is linked to both indole nitrogens, the inhibitory potencies toward topo I are lower than those of analogues in which the carbohydrate part is linked to only one of the indole nitrogens, but the antiproliferative activities are in the same range or even stronger, suggesting different biological targets for these series of compounds, (v) when the sugar part is linked to an indolo[2,3-*c*]carbazole, the structural change is clearly detrimental to the anti-topo I activity. The carbohydrate pointing toward the imide nitrogen may not be recognised by topo I, (vi) substitutions with halogen atoms on various positions of the sugar residue and at 3,9 positions on the indolocarbazole framework may enhance anti-topo I activity and antiproliferative activity, (vii) the aza rebeccamycin analogues are much more selective toward the various tumour cell lines tested than the non aza parent compounds. When only one indole moiety is replaced by one azaindole, the sugar residue linked to the indole part leads to compounds exhibiting anti-topo I activity, whereas the sugar residue linked to the azaindole induces no more topo I poisoning effect, but the antiproliferative activities are in the same range, suggesting also for this series of analogues different biological targets. Some aza rebeccamycin analogues exhibit very strong cytotoxicities toward several tumour cell lines with IC_{50} values in the nanomolar range.

Considering the similarity of structure of rebeccamycin analogues and staurosporine and also their effects on the L1210 cell cycle, it can be postulated that some

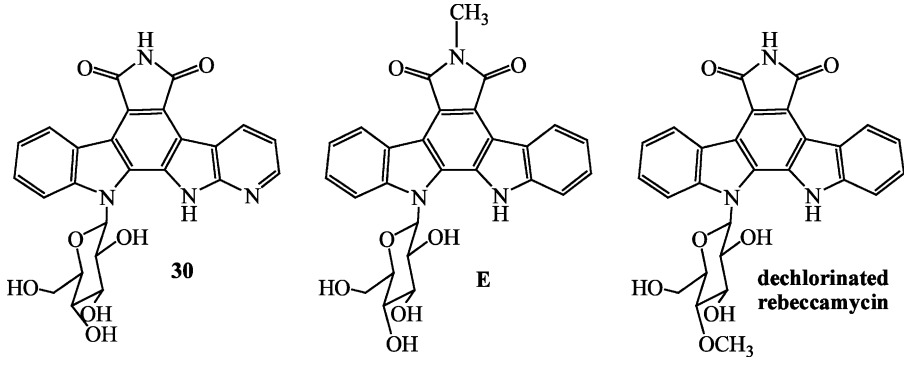


Fig. 17. Compounds **30**, **E**, and dechlorinated rebeccamycin possessing the sugar unit linked to the indole moiety.

Table 6
In vitro antiproliferative activities against nine tumour cell lines: one murine leukemia (L1210), one human leukemia (K-562) and seven human solid tumours: one ovarian carcinoma (IGROV1), one neuroblastoma (SK-N-MC), one colon carcinoma (HT29), one non-small cell lung carcinoma (A549), one small-cell lung carcinoma (H69) and two epidermoïd carcinomas (A431 and KB-3-1) (IC_{50} μM)

Compound	L1210	IgROV	SK-N-MC	HT29	A549	A431	NCI-H69	K-562	KB-3-1
Rebeccamycin	0.14	0.25	< 0.1	0.3	0.3	0.25	< 0.1	0.2	0.3
Dechlorinated rebeccamycin	0.11	2.6	< 0.1	2.5	2	3.8	< 0.1	< 0.1	0.3
E	0.67	0.88	0.25	0.86	0.94	0.84	0.33	ne	0.6
22	0.58	> 50	0.28	97	> 100	0.062	0.31	0.7	24.3
23	0.067	ne	ne	0.57	41.5	ne	ne	ne	ne
29	1.3	ne	ne	17.8	47.2	ne	ne	ne	ne
25	0.45	8.5	0.18	67.2	59.9	0.012	0.11	0.7	0.11
27	0.27	19.5	0.041	27.5	32	0.053	0.043	ne	1.2
24	0.13	68.8	0.059	> 100	> 100	0.238	0.01	ne	2.56
26	0.061	24.7	0.018	32.3	85.2	0.229	0.007	ne	0.93
28	0.07	33.4	ne	> 100	> 100	10	0.017	ne	0.552
30	0.066	ne	ne	4.8	5.3	ne	ne	ne	ne

ne, Not evaluated.

Table 7
Cytotoxicity toward murine L1210 leukemia cells (IC_{50} values μM). Effect on the cell cycle of L1210 cells

Compound	Cytotoxicity L1210	Cells in the G2+M phases (%) (drug concentration)
Control	–	24%
Rebeccamycin	0.14	69% (1 μM)
Dechlorinated Rebeccamycine	0.11	71% (1 μM)
E	0.67	77% (2.5 μM)
22	0.58	31% (5–20 μM)
23	0.067	66% (0.25 μM)
29	1.3	ne
25	0.45	26% (5–20 μM)
27	0.27	78% (5 μM)
24	0.13	79% (0.5 μM)
26	0.061	85% (0.25 μM)
28	0.07	87% (0.25 μM)
30	0.066	78% (0.25 μM)

ne, Not evaluated.

families of rebeccamycin derivatives could inhibit kinases involved in the progression of the cell cycle, e.g. cyclin-dependent kinases. First investigations seem to validate this hypothesis.

From this studies, it can be concluded that rebeccamycin analogues are a class of compounds which can possess strong antiproliferative activities and high selectivities toward tumour cell lines via several mechanisms of action, and could be considered as potential novel anti-cancer agents. In addition to their potential therapeutical applications, it has been shown that triple helix-forming oligonucleotides conjugated to rebeccamycin drugs direct topoisomerase-mediated DNA cleavage to specific sites [38–40]. Rebeccamycin derivatives were covalently attached to a triple helix-forming oligonucleotide. These conjugates bind to DNA with a higher affinity than the nucleotides without the drugs, and induce topoisomerase I-mediated DNA cleavage in a sequence-specific manner. The rational design of drug–oligonucleotide conjugates carrying a topoisomerase I poison may be exploited to improve the efficacy and selectivity of chemotherapeutic cancer treatments by targeting specific genes and reducing drug toxicity.

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