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Original article

Synthesis and cytotoxicities of 7-aza rebeccamycin analogues bearing various substituents on the sugar moiety, on the imide nitrogen and on the carbazole framework

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Abstract

The synthesis of a family of rebeccamycin analogues in which one indole unit has been replaced by a 7-azaindole moiety is described. Substitutions have been carried out on the imide nitrogen, on the carbazole framework and on the sugar part. Compounds with a lactam upper heterocycle have also been prepared. The cytotoxicities of the newly synthesized compounds toward four tumor cell lines, one murine leukemia (L1210) and three human tumor cell lines (prostate carcinoma DU145, colon carcinoma HT29, and non-small cell lung carcinoma A549) have been evaluated and compared to those of rebeccamycin and parent non-aza and aza compounds.

Keywords: Rebeccamycin; 7-Azaindole; Antitumor compounds; Topoisomerase I inhibitors

1. Introduction

A large number of indolocarbazoles of biological interest are provided by bacteria [1]. Among them, rebeccamycin **1** (Fig. 1) is a microbial metabolite isolated from cultures of *Saccharothrix aerocolonigenes*. Its antitumor activity is linked to its capacity to induce topoisomerase I-mediated DNA cleavage [2,3]. Large structure–activity relationship studies on rebeccamycin have been carried out by several academic and industrial groups. Various families of rebeccamycin analogues have been prepared either by total synthesis or by semi-synthesis from the bacterial metabolite, and their biological properties have been evaluated [4,5].

It has been shown that if topoisomerase I remains the first target for most of the analogues, there are very likely other targets for these indolocarbazole compounds and preliminary studies have shown that kinases involved in the progression of the cell cycle could also be responsible for their antiproliferative activities [6]. Results with compounds bearing substitutions at the imide nitrogen suggested that, in the ternary complex DNA-topoisomerase I-drug, this moiety could be located in a pocket of the enzyme allowing bulky functional groups [3]. Substitutions performed at the 6′-position on the carbohydrate part of rebeccamycin showed that these substitutions could modify the biological targets. For example, compared with rebeccamycin, 6′-amino derivatives exhibit an enhanced capacity to interact with DNA but they do not behave as topoisomerase I inhibitors showing that topoisomerase I inhibition and DNA interaction correspond to two separate mechanisms. DNA and topoisomerase I can be distinct targets for indolocarbazole compounds [7], as already observed with anthracycline derivatives, which, according to the substitutions, can be either topoisomerase II poisons or only DNA intercalators [8].

3,9-Substituents on the indolocarbazole framework can enhance or abolish the cytotoxicity but also induce selectivity toward the tumor cell lines tested. 3,9-Substituted compounds can behave as DNA intercalators and topoisomerase I inhibitors (dihydroxymethyl or diamino and dihydroxy substituents), some of them can behave as topoisomerase I inhibi-

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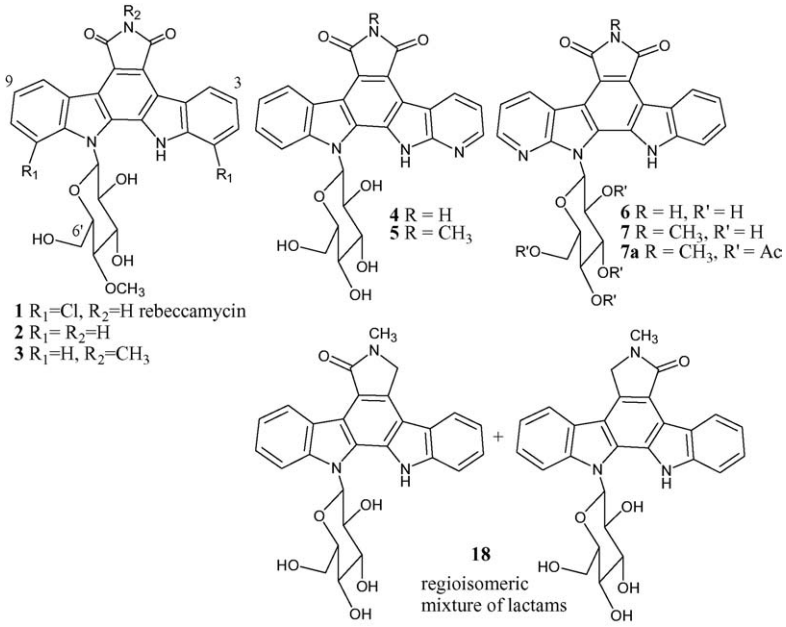

Fig. 1. Rebeccamycin and aza analogues.

tors but without intercalating properties (diformyl and dinitro substituents) [6]. 7-Aza rebeccamycins have been recently synthesized and studied [9,10]. In contrast with rebeccamycins possessing two indole moieties, most of the 7-aza rebeccamycins exhibit a very interesting profile of cytotoxicity with a high selectivity for some tumor cell lines with IC_{50} values in the nanomolar range. DNA binding experiments showed major differences between the compounds bearing the carbohydrate unit either on the azaindole moiety or on the indole part. Compounds bearing the sugar unit on the indole exhibited higher affinity for DNA than non-aza analogues, but compounds with the sugar linked to the azaindole have lost their DNA binding properties. Compounds bearing the sugar unit on the indole are much more potent topoisomerase I inhibitors than compounds with the sugar linked to the azaindole. In these series, there is a closed correlation between DNA binding and topoisomerase I poisoning. In this paper, the synthesis of new 7-azaindolyl rebeccamycins **8**–**20** are reported together with their in vitro antiproliferative activities against four tumor cell lines (one murine L1210 leukemia, and three human tumor cell lines: DU145 prostate carcinoma, A549 non-small cell lung carcinoma, and HT29 colon carcinoma).

2. Results and discussion

2.1. Chemistry

In the series of compounds in which the carbohydrate unit is linked to the 7-azaindole moiety, anhydride **8** was prepared from compound **A** [7] (Scheme 1) in two steps. Reaction of **A** with aqueous sodium hydroxide in THF led to the formation of anhydride **B** with concomitant removal of the acetyl protective groups on the sugar moiety. Oxidative photocyclization of **B** in the presence of iodine afforded anhydride **8**.

Reaction of anhydride **8** with hydrazine hydrate, hydroxylamine and diethylaminoethylamine gave compounds **9**, **10** and **11**, respectively (Scheme 1). Substitution with a chlorine atom selectively at 6′ position on the sugar part (compound **12**) was performed from compound **7** [7], by reaction with triphenylphosphine and CCl_4 in pyridine [11] (Scheme 2).

Nitration at 3-position on the carbazole moiety was carried out from **7a** [9] tetraacetylated on the sugar part using DMSO/Ac_2O affording **13a** (Scheme 2). This technique was initially chosen to induce nitration in 10 position according to nucleophilic nitrations reported on dihydrodipyridopyrazines and isoquinolines [12,13]. In these compounds, an electrophilic intermediate is formed which reacts with the heterocyclic nitrogen atom to form a carbonium ion in ortho position with respect to the nitrogen atom. A nucleophilic attack by NO_2^- leads to ortho nitration. In our case, the nitration did not occur on the azaindole moiety but in the 3-position on the indole unit. A possible mechanism could be an electrophilic substitution at the 3-position, as usually observed with non-aza rebeccamycin analogues [6,14,15], by NO_2^+ formed from an electrophilic intermediate as shown on Scheme 3.

The position of the nitro group in **13a** was assigned from NMR experiments (Fig. 2). A NOESY 2D showed a NOE effect between the indolic NH proton and the proton shifted at 7.66 ppm. ^1H–^1H COSY showed a coupling between the proton (d) at 7.66 ppm and the proton (dd) at 8.41 ppm (coupling constant J = 9.0 Hz) as well as a coupling of the proton at 8.41 ppm with the proton at 10,01 ppm (coupling constant J_{meta} = 2,0 Hz). Deacetylation of **13a** was carried out with NH_3/MeOH.

Compound **14** was prepared from **5** using the method described for the synthesis of **12**. However, in this case, the