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New antitumor 6-chloropurine nucleosides inducing apoptosis and G2/M cell cycle arrest

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ABSTRACT

Treating cancer has been challenging for decades, following countless approaches and attempts. Nucleosides, alone or as part of nucleotides, are vital elements of living systems and have shown pharmacological effects, *e.g.* as antibiotic or antiviral agents. We investigated the antitumor potential on human melanoma, lung and ovarian carcinomas, and on colon adenocarcinoma of a new series of purine nucleosides based on a 6-chloropurine or a 2-acetamido-6-chloropurine scaffold linked to perbenzylated hexosyl (glucosyl, galactosyl and mannosyl) residues. All compounds were tested in a sulforhodamine B (SRB) assay for their cytotoxicity and provided micromolar GI_{50} values with order of magnitude comparable to structurally similar chemotherapeutics, namely 2-chloro-2′-deoxyadenosine (cladribine). Furthermore, the induction of apoptosis was established and cell cycle analysis was accomplished demonstrating a G2/M cell cycle arrest.

1. Introduction

Purine nucleosides and nucleotides have been major targets of anticancer research for several decades. One of the first investigations was accomplished by the group of Noell in 1962 synthesizing a series of thioguanines and 2-amino-6-alkylthiopurine derivatives [1]. Studies dealing with compounds containing purine moieties, *e.g.* in gold (III) complexes [2] or adenine derivatives [3–5] have also been reported and effective camptothecin purine derivatives possessing GI_{50} values in micromolar range were synthesized by Li et al. [6]. Caba et al. [7] reported on an antiproliferative agent against the MCF-7 adenocarcinoma with micromolar GI_{50} value embodying tetrahydrobenzoxazepine N^9-linked to a 6-chloropurine while a coumarin N^9-linked to a 2-amino-6-chloropurine showed moderate activity (25–35 μM) against the HeLa, HepG2 and SW620 cell lines [9]. Voller et al. accomplished substitutions at different positions of a 6-aminopurine scaffold and have shown that some of the ribosides exhibited micromolar anticancer activity while their N^7- or N^9-linked glucoside analogues were not active [8]. In addition, a 9-norbornyl-6-chloropurine was recently reported as a novel antileukemic compound [10]. The previously reported anticancer potential of 6-chloropurine derived compounds encouraged us to investigate, for the first time, a series of purines embodying a 6-chloro substitution (CP) or both 6-chloro- and 2-acetamido groups (ACP), linked at N^7 or N^9 to perbenzylated d-*glucosyl*, D-*mannosyl* and D-*galactosyl* residues. The reaction conditions described by Marcelo et al. [11] using a silylated base was optimized for this type of nucleosides. The anticancer activity was determined using a sulforhodamine B (SRB) assay to yield GI_{50} values for human melanoma, lung and ovarian carcinoma, and colon adenocarcinoma cancer cell lines. Furthermore, the substances were tested on murine embryonic fibroblasts (NiH 3T3) to investigate their tumor cell-to-control-specifity. In addition, acridine orange/propidium iodide assays, DNA laddering experiments and cell cycle analyses were performed for the most active compound to gain some information about the mode of action of this family of new anticancer molecular entities.

2. Results

2.1. Chemistry

Nucleoside synthesis can be performed by a two steps procedure, starting with the acetylation [12] or halogenation [13,14] of

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Table 1
Experimental conditions for the reaction of methyl 2,3,4,6-tetra-*O*-benzyl-α-D-glycoside with the purine (CP or ACP). Yields and product ratios were determined by ^{1}H NMR experiments. 1 Equivalent of the monosaccharide and 1.5 equivalents of the silylated purine in dry acetonitrile were used, conventional heating at 65 °C was performed.

Entry	Purine	Eq. TMSOTf	Time	β-N^7/N^9	Overall yield
1	CP	1	24 h		n.d.[a]
2	CP	2	2 h	1/1.9	43%
3	CP	4	2 h	1/4.5	62%
4	CP	8	2 h	1/3.8	65%
5	ACP	1	24 h		n.d.
6	ACP	2	2 h	1/2.4	1%
7	ACP	4	2 h	1/1	62%
8	ACP	8	2 h	1/1.3	63%

[a] n.d: no product detected.

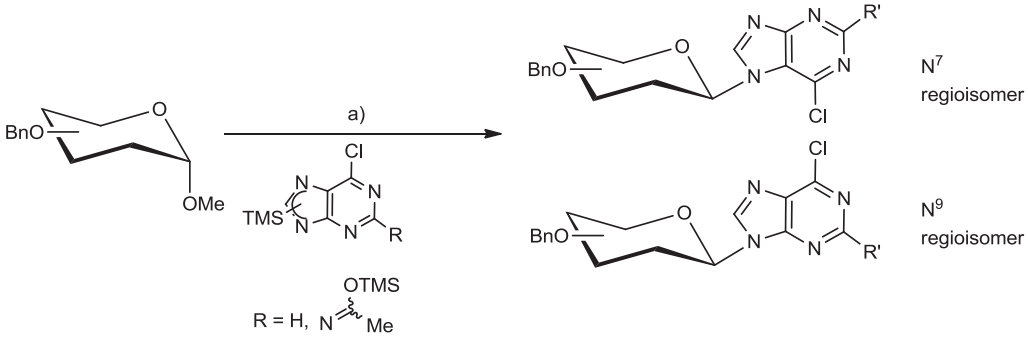
suitable precursors, followed by reaction with the heterocyclic base. A direct access to nucleosides can be gained by Lewis acid activation with tin chloride [15,16] or TMSOTf [17] of methyl glycosides in a reaction employing a persilylated purine. Marcelo et al. applied TMSOTf in acetonitrile to link regioselectively bicyclic pyranosides to purine scaffolds at their N^7 position with β-stereoselectivity [11]. These conditions were investigated in this study for this nucleoside series. Therefore, monosaccharidyl donors were prepared according to established procedures [18]. In the next step, the molarity of TMSOTf was investigated regarding its impact on the reaction yield and N^7/N^9 ratio. The proportions of the reaction products formed, determined by ^{1}H NMR, are compiled in Table 1, showing that the use of eight equivalents of TMSOTf worked best regarding the overall yields, while the N^7/N^9 ratio did not change significantly by increasing the concentration of TMSOTf. However, the expected N^7 regioselectivity obtained by Marcelo et al. [11] in the presence of TMSOTf in acetonitrile occurred only for the β-mannosylation and the β-galactosylation of 2-acetamido-6-chloropurine, while the thermodynamically controlled N^9 nucleoside was the major β-anomer resulting from the N-glucosylation and the N-galactosylation of 6-chloropurine. All four isomers (α/β, N^7/N^9) could be detected using these reaction conditions [19]. Nevertheless, only the β-anomers (Scheme 1) were subjected to biological testings, in as much as they were the predominant species when introducing the glycosyl or galactosyl moieties and the α-anomers appeared in very low amount. Therefore, also the minor β-mannosyl nucleosides were isolated and tested in order to receive comparable biological data to correlate structure with bioactivity in this family of compounds. The hydroxy groups of the glycosyl moities remained benzylated, since the presence of benzyl groups enhances the cytotoxicity of glycosylated compounds [20,21]. Moreover, benzyl groups represent a suitable metabolic protection.

The configuration of the anomeric center as well as the purine substitution pattern could be confirmed by the observed chemical shifts in the ^{1}H and ^{13}C NMR spectra and the $^3J_{H\text{-}1,H\text{-}2}$ coupling constant of the anomeric proton. Chemical shifts of $\delta = 5.4$ ppm to $\delta = 5.7$ ppm and coupling constants of $J = 7$ to $J = 9$ Hz were obtained for D-*gluco* and D-*galacto* configurated derivatives proving an axial position of the anomeric proton, while coupling constants of approx. $J = 1$ Hz were determined for D-*manno* configurated compounds. In the ^{13}C NMR spectra, the chemical shifts of $\delta = 83$ to $\delta = 85$ ppm for the anomeric carbon are in full agreement with data given in the literature for the anomeric carbon of other β-hexopyranosyl nucleosides [22–24]. The distinction between N^7 or N^9 substitution at the purine scaffold can be achieved by the chemical shift of purine carbon 5. In CP and ACP nucleosides the resonance of C-5 ranges from $\delta = 131$ ppm to $\delta = 128$ ppm indicating N^9 substitution while N^7 substitution was characterized by C-5 chemical shifts of about $\delta = 122$ ppm for CP and about $\delta = 118$ ppm for ACP nucleosides. These assignments were also confirmed with NMR HMBC experiments.

2.2. Biology

The cytotoxic activities of all synthesized compounds are represented by their GI$_{50}$ values given in Table 2. The values were determined in photometric SRB assays, using four different human tumor cell lines as well as murine embryonic fibroblasts (NiH 3T3). Some general tendencies could be observed within the bounds of our study. While the purines CP and ACP did not show any activity below 30 μM (cut-off), their N-glycosylation, increased considerably their cytotoxic activity. In general, the ACP nucleosides showed higher activities than the corresponding CP analogues. Furthermore, N^7 derivatives showed lower GI$_{50}$ values when compared to their N^9 regioisomers.

The influence of the glycosyl moiety was, however, less significant. The introduction of glucosyl and galactosyl groups showed similar results, *e.g.* GI$_{50}$ values from 18 to above 30 μM were determined for compounds **2** and **4**, both N^9 CP regioisomers,



1 R' = H, Glc-N^7-CP (19%) **7** R' = NHAc, Glc-N^7-ACP (25%)
2 R' = H, Glc-N^9-CP (30%) **8** R' = NHAc, Glc-N^9-ACP (35%)
3 R' = H, Gal-N^7-CP (15%) **9** R' = NHAc, Gal-N^7-ACP (47%)
4 R' = H, Gal-N^9-CP (44%) **10** R' = NHAc, Gal-N^9-ACP (18%)
5 R' = H, Man-N^7-CP (16%) **11** R' = NHAc, Man-N^7-ACP (26%)
6 R' = H, Man-N^9-CP (13%) **12** R' = NHAc, Man-N^9-ACP (3%)

Scheme 1. Synthesis of the nucleosides. CP: 6-chloropurine; ACP: 2-acetamido-6-chloropurine. Reagents and conditions: (a) TMSOTf, CH_3CN, 65 °C, 2 h.