Asymmetric synthesis of anticancer β-lactams via Staudinger reaction

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Abstract. This study describes the symmetric synthesis of novel β-lactams derived from chrysene directed towards their SAR, as well as their biological activities against several cancer cell lines *in vitro*. To our knowledge, this is the first report on the synthesis and biological evaluation of optically active anticancer β-lactams. That these anticancer effects are not uniform against all tumor lines suggests that the target of the action of these compounds is highly specific.

Introduction

Many of the currently available anticancer drugs are cytotoxic to normal as well as to neoplastic cells. There is therefore a need for new anticancer agents with a high degree of potency against cancerous cells, low toxicity in non-cancerous cells and unique targets of action (1). Towards this goal, the synthesis of β-lactams as new and novel anticancer agents is potentially extremely significant and timely. We have reported synthetic methods for the preparation of β-lactams (2) and anticancer agents derived from polyaromatic amines (3). The stereo-controlled synthesis of novel racemic β-lactams starting from poly-aromatic imines and their biological evaluation as anti-cancer agents has also been reported (4). In cell cycle analysis, these anticancer β-lactams demonstrated a G2 blockade against sensitive tumor cell lines (4). This study describes the asymmetric synthesis of β-lactams derived from chrysene, with the focus on examining their structure-activity relationships and their biological activities against several cancer cell lines *in vitro*. An optically active β-lactam, highly active *in vitro* against a number of cancer cell lines, was identified. To our knowledge, this is the first report on the synthesis and biological evaluation of optically active anticancer β-lactams.

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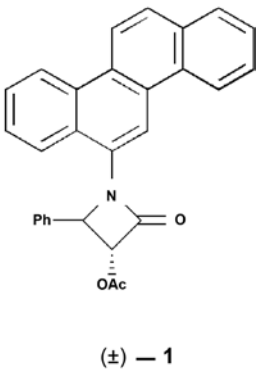
Materials and methods

Reagents. A number of organic and inorganic reagents and solvents were used for the preparation of optically active anticancer β-lactams, including acetobromoglucose, indium powder, hydrogen gas, N-methyl-2-chloro pyridiniumiodide, hydrochloric acid, sodium sulfate, acetic anhydride, ethyl acetate, hexane and silica gel. β-glycoside was prepared and was used for the cycloaddition reaction with imine. Acid-induced cleavage and acetylation were performed. The determination of the *in vitro* cytotoxicity of organic compounds was performed using the MTT assay following a previously described protocol (4).

Results

The anticancer racemic β-lactams 1 used have an acetoxy group at the C_3 position of the rings. A carbohydrate-based approach was therefore employed, as it is the best method for the preparation of these types of compounds, making it easy to cleave a glycosidic bond without affecting the β-lactam ring (Fig. 1). The reaction of acyloxy, alkoxy and nitrogen-containing acid chloride with diaryl imines produces *cis*-β-lactams under Staudinger reaction conditions (5). A similar reaction of polyaromatic imines was used in the present study to produce *trans*-β-lactams (4). The asymmetric synthesis of *cis*-hydroxy β-lactam (70%) using α-O-glycoside as the ketene component with a diaryl imine has been reported (6). The data in the literature indicate that chiral induction may depend on the stereochemistry of the anomeric carbon of the glycoside. The absolute stereochemistry of the anomeric center in the ketene component may dictate the absolute stereochemistry at the C_3 center of the β-lactam ring. In contrast to the success achieved by the cycloaddition of optically active amino ketene with imines, the analogous reaction of imines with chiral hydroxyketene equivalents has not been systematically explored (7). Due to the ready availability and stability of multiple stereogenic centers, the carbohydrate-based approach was capable of producing both enantiomers of the anticancer β-lactams. The availability of both enantiomeric forms of the acetoxy compounds was necessary for examining their anticancer activity in detail. The majority of the investigations aimed at achieving this goal have dealt with the reaction of achiral alkoxy ketenes with



(±) — 1

Figure 1. Racemic β-lactam compound.

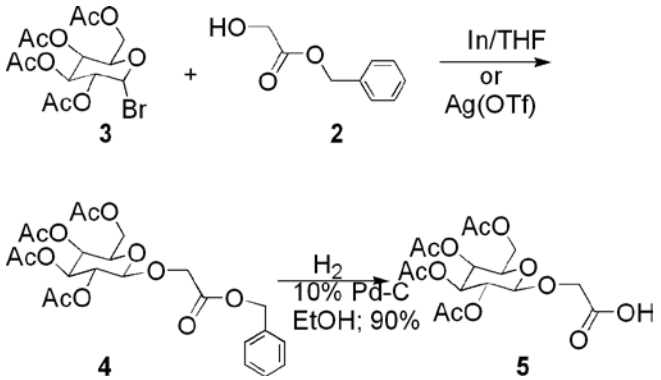


Figure 2. Preparation of β-glycosides.

Table I: *In vitro* cytotoxicity of β-lactams compounds on human cancer cell lines (μM).

Compounds	BRO	MDA-231	SKOV-3	PC-3	HL-60	K-562	HT-29
Cisplatin	7.66	12.33	5.99	4.66	1.66	2.33	16.99
(±)-1	15.70	11.98	3.90	16.32	3.64	4.33	5.66
(+)-10	22.00	8.50	8.50	15.50	5.40	6.00	8.30
(-)-12	6.10	1.80	6.80	1.40	0.70	1.10	0.70

The *in vitro* cytotoxicity assays were performed at the Pharmacology and Analytic Core Laboratory of the University of Texas M.D. Anderson Cancer Center. An MTT assay was carried out using the seven human cancer cell lines.

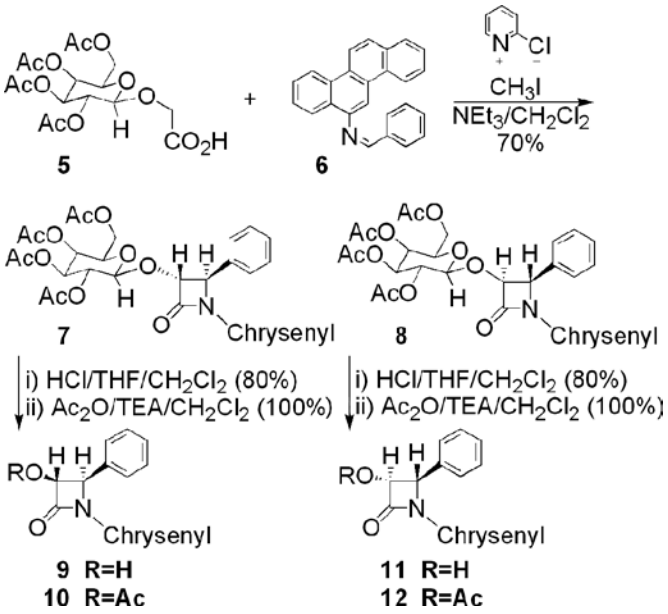


Figure 3. Synthesis of chiral β-lactams.

chiral imines derived from optically active α-oxy and α-amino carbonyl compounds. The synthesis of an acid with a β-glycosidic linkage was prepared according to our own method (8). The indium-induced reaction of acetobromoglucose 3 with 2 afforded 4 in moderately good yield. Upon hydrogenolysis, the benzyl group was cleaved to the β-glycoside 5 in good yield (Fig. 2).

The cycloaddition of the acid 5 with imine 6 was performed using imine in the presence of N-methyl-2-chloropyridiniumiodide and triethylamine at room temperature. NMR analyses of the crude reaction mixture indicated the presence of two diasteromeric *trans*-β-lactams, 7 and 8, in a ratio of 60:40 (9). These were separated using column chromatography (20% ethyl acetate in hexanes) over silica gel. The diastereomeric O-glycosidic bond was cleaved with mild aqueous acid to the hydroxy compounds 9 and 11. These were then converted to acetates. The absolute stereochemistry of the *trans*-acetoxy-β-lactam 10 and 12 was confirmed by a direct comparison with a known *trans*-β-lactam, previously described with respect to optical rotation and NMR data in the presence of a chiral shift reagent (10).

In vitro cytotoxicity of the β-lactams. The β-lactams (+)-10 and (-)-12 were tested using seven human cancer cell lines, with cisplatin and racemic 1 as controls. The results are presented in Table I.

Selected spectroscopic data. (-)-*trans*-N-(6-Chrysenyl)-3-acetoxy-4-phenyl-2-azetidine-2-one (10): mp 174-176˚C; -149˚ (c 1.0, CH_2Cl_2); ν_{max}/cm^{-1} 1755, 1595, 1515, 1486, 1456, 1440, 1394, 1373, 1314, 1283, 1219; ^{1}H NMR ($CDCl_3$) δ (ppm) 2.36 (s, 3 H), 5.54-5.55 (d, *J*=1.91 Hz, 1 H), 5.77-5.78 (d, *J*=1.94 Hz, 1 H), 7.26-7.31 (m, 3 H), 7.46-7.49 (m, 2 H), 7.60-7.71 (m, 2 H), 7.77-7.80 (m, 2 H), 7.95-7.99 (m, 2 H), 8.38-8.41 (m, 1 H), 8.45 (s, 1 H), 8.50-8.53 (d, 1 H), 8.63-8.66 (d, 1 H), 8.79-8.82 (m, 1 H). Found: C, 80.69; H, 4.89; N, 3.19. Calc. for $C_{29}H_{21}NO_3$: C, 80.72;

H, 4.91; N, 3.25.12. (+)-*trans*-N-(6-Chrysenyl)-3-acetoxy-4-phenyl-2-azetidine-2-one (12): +150˚ (c 1.0, CH_2Cl_2).

In conclusion, the cell growth inhibition data (IC_{50}) suggests that one of the optically active β-lactams, (-)-12, demonstrates significantly increased activity against 6/7 human tumor lines, while the activity of the other isomer, (+)-10, is reduced in 6/7 tumor lines compared to the racemic β-lactam (±)-1. These effects are not uniform against all tumor lines, which suggests that the target of the action of these compounds is highly specific.

The optically active β-lactam (-)-12 described herein is unique since it demonstrates superior *in vitro* antitumor cytotoxicity compared to the racemic (±)-1 and its chiral isomer (+)-10. These results suggest that other glycosides may be used for the preparation of anticancer β-lactams in chiral forms with novel mechanisms of action. Such compounds are the conformationally restricted analogs of our open-chain compounds (3,11).

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