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N-Glycosyl-thiophene-2-carboxamides: synthesis, structure and effects on the growth of diverse cell types

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Abstract—A range of *N*-glycosyl-thiophene-2-carboxamides, including a 6*H*-thieno[2,3-*c*]pyridin-7-one and a bivalent compound, have been synthesised and assayed for their effects on DNA synthesis in bovine aortic endothelial cells or on the growth of synoviocytes. Per-O-acetylated analogues of the glycoconjugates were significantly more effective inhibitors when compared to their corresponding non-acetylated analogues, indicating that the lower potency observed for hydroxylated derivatives is due to less efficient transport of these compounds across the cell membrane. Thiophene-2-carboxamide was inactive as an inhibitor of bFGF induced proliferation, confirming the requirement of the carbohydrate residue for the observed biological properties. Glucose, mannose, galactose and 2-amino-2-deoxy-glucose analogues were active as were a variety of substituted thiophene derivatives; the 6*H*-thieno[2,3-*c*]pyridin-7-one conjugate was inactive. Conformational analysis of the title compounds was investigated. X-ray crystal structural analysis of four *N*-glucosyl-thiophene-2-carboxamides showed that the pyranose rings adopted the expected $^4C_1$ conformations and that *Z-anti* structures were predominant (H1–C1–N–H anomeric torsion angle varied from −168.2° to −175.0°) and that the carbonyl oxygen and sulfur of the thiophene adopted an s-cis conformation in three of the isomers. In a crystal structure of a 3-alkynyl derivative, the hydrogen atom of the NH group was directed toward the acetylene group. The distance between the hydrogen atom and acetylene carbons and angles between nitrogen, hydrogen and carbon atoms were consistent with hydrogen bonding and this was supported by IR and NMR spectroscopic studies. The geometries of thiophene-2-carboxamides were explored by density functional theory (DFT) and Møller-Plesset (MP2) calculations and the s-cis conformer of thiophene-2-carboxamide was found to be more stable than its s-trans isomer by 0.83 kcal mol^{-1}. The s-cis conformer of 3-ethynyl-thiophene-2-carboxamide was 5.32 kcal mol^{-1} more stable than the s-trans isomer. The larger stabilisation for the s-cis conformer in the 3-alkynyl derivatives is explained to be due to a moderate hydrogen bonding interaction between the alkyne and NH group.

Keywords: Signal transduction; Angiogenesis; Rheumatoid arthritis; Glycosyl amides; Thiophene-2-carboxamides; Endothelial cells; Synoviocytes; Inhibitors; Proliferation; Growth; DNA synthesis

1. Introduction

The signal transduction processes that modulate cellular behaviour are important biological events. For example, angiogenesis[1] provides new blood vessels to growing and developing tissues including tumours, and it relies on the up-regulation of endothelial cell proliferation. Up-regulated angiogenesis is characteristic in rheumatoid arthritis and diabetic retinopathy during tumour growth and metastasis.[2] The tumour angiogenesis process results from the production of the pro-angiogenic factors basic

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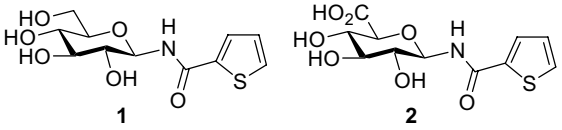
fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) in a signalling cascade, and the down-regulation of negative modulators, like angiostatin, in tissues with a quiescent vasculature. Angiogenesis is also a major factor affecting the metastatic spread of malignant cells. Thus the development of angiogenic inhibitors may allow a new therapeutic strategy against malignant tumours. Consequently, inhibitors of endothelial cell proliferation and growth are of interest and a number of strategies are being considered for the development of anti-proliferative agents.[3] Additionally, signalling pathways up-regulated by bFGF are also important in arthritis. The normal synovium is a delicate tissue lining the joint capsule; however, in inflammatory joint diseases including rheumatoid arthritis (RA), the synovium transforms into an aggressive, tumour-like structure called pannus. Synoviocyte cells in the pannus tissue are targeted by many signals including cytokines (IL-1β and TNF-α) and growth factors (bFGF and TGB-β) to promote proliferative and invasive capacity, increased cell adhesion molecule expression and guided migration.[4] Previously, efforts to discover compounds reduced in carbohydrate character (monosaccharide conjugates) that had potential as modulators of bFGF induced endothelial cell growth[5] led to the identification of *N*-(β-D-glucopyranosyl)-thiophene-2-carboxamide **1** and the glucuronic acid analogue **2** as inhibitors of bovine aortic endothelial cell (BAEC) growth.[6] The development of more potent analogues than **1** could ultimately be helpful for the determination of the biological mechanism of these glycoconjugates, which is unknown and could lead to the identification of novel targets in signalling pathways. The synthesis of novel analogues of **1**, the evaluation of their effects on the proliferation and growth of both endothelial and synovial cells[7] and a structural study of these bioactive compounds is described herein.

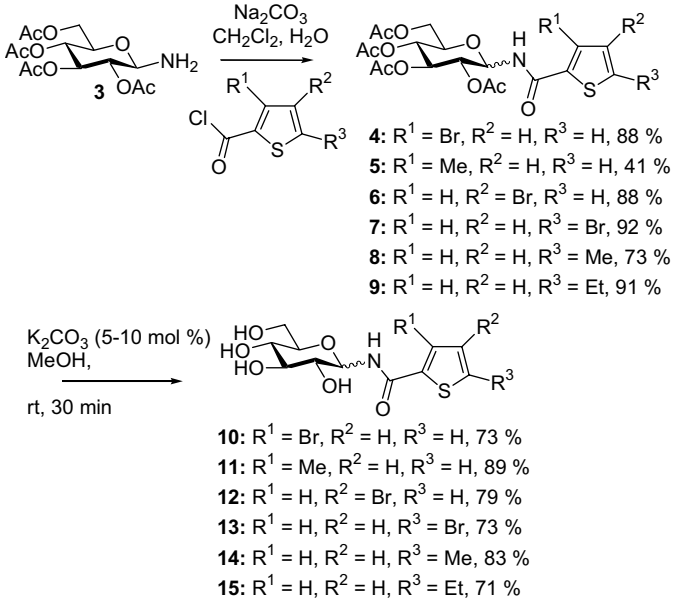


2. Results and discussion

2.1. Synthesis of *N*-glycosyl-thiophene-2-carboxamides

N-Glucosyl-thiophene-2-carboxamides were prepared from the protected glucopyranosylamine **3** (Scheme 1). The reactions of **3**, in the presence of sodium carbonate, with acyl chlorides derived from thiophene-2-carboxylic acids afforded **4–9**. Anomerisation occurred during the coupling reactions and led to mixtures of anomers (α:β = 1:18–1:16 by ^{1}H NMR). The reaction of acid chlorides with **3** in the presence of pyridine in dichloromethane or coupling of the appropriate thiophene-2-carboxylic acid using *N*,*N*′-dicyclohexylcarbodiimide (DCC) and 1-hydroxybenzotriazole (HOBt) with **3** also resulted in mixtures (α:β = 1:16–1:3 by ^{1}H NMR). Deacetylation of per-O-acetylated derivatives **4–9** gave the unprotected *N*-glucosyl-thiophene-2-carboxamides **10–15**. Mixtures of anomers were separated by reverse phase HPLC to obtain pure β-anomers for biological evaluation.

The acetylene derivatives **16/17** were prepared from **4/6** by a Sonogashira coupling with ethynyltrimethylsilane (Scheme 2), which was carried out in a sealed reaction



Scheme 1. Synthesis of *N*-glycosyl-thiophene-2-carboxamides.