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Progress Towards a Norcantharidin-based Scaffold for Inhibition of the Protein Phosphatase 5C (PP5C) Enzyme

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Abstract

Recent studies have shown that the overexpression of PP5C is associated with invasive ductal carcinoma of the breast, cancer cell proliferation and resistance to apoptosis. Screening and computational efforts revealed a series of potentially selective and potent small molecule chemical probes containing a 5-(3-amino)-propoxymethyl-(7-oxabicyclo[2.2.1]heptane) scaffold for PP5C. In attempt to further characterize the biological and pathological roles of PP5C in the antitumor activity of breast cancer, a multi-step synthesis scheme was devised to construct the scaffold. This work details the unsuccessful experimental procedures developed for the construction of the 5-(3-amino)-propoxymethyl-(7-oxabicyclo[2.2.1]heptane) scaffold, as well as a discussion on other possible synthesis methods.

Keywords: Norcantharidin, PP5C, Synthesis

1. Introduction

PP5C (encoded by PPP5C) is a member of the PPP-family of serine/threonine protein phosphatases, which is comprised of PP1C, PP2AC, PP2BC, PP4C, PP5C, PP6C, and PP7C. Recent research has revealed a positive correlation between overexpression of serine/threonine PP5C and ductal carcinoma, invasive ductal carcinoma, and metastatic invasive ductal carcinoma of the breast.[1] Similarly, in mouse xenograft models of tumor growth, a modest increase in PP5C protein levels significantly enhanced the growth rate of estrogen-dependent MCF-7 cell tumors.[2] Furthermore, PP5C suppresses a hypoxia-induced signaling network that promotes apoptosis, which suggests PP5C may play an important role in cancer cell survival and apoptosis resistance.[3] Therefore, a potent and highly-selective PP5C inhibitor may not only help elucidate the biochemical and pathological roles of PP5C in breast cancer activity but could also result in the development of a novel antitumor drug the medical management of advanced breast cancers.[4,5,6]

The crystal structure of PP5C's catalytic pocket reveal a highly-conserved residue sequence, allowing for the identification of specific inhibitors.[7] Consequently, a small-molecule library was screened in an ultra-high-throughput screening (uHTS) campaign to detect compounds that inhibit the catalytic activity of PP5C. More than 30 compounds are confirmed as PP5C-selective inhibitors, the strongest of which contain a common 7-oxabicyclo[2.2.1]heptane core, also known as norcantharidin (Figure 1**B**).[8] Norcantharidin is a demethylated synthetic analog of cantharidin (Figure 1**A**), a natural but universally cytotoxic phosphatase inhibitor produced by *Lytta vesicatoria*, the blister beetle.[9] The current understanding is that cantharidin's cytotoxicity is due to its PP1C inhibition activity, while it exhibits antitumor activity because of its PP5C inhibition activity. The PP5C co-crystal structures reveal matching binding orientations of cantharidin and norcantharidin within the catalytic pocket.[10] The proximal amino acids within the PP1C pocket are identical to that of PP5C; consequently, cantharidin inhibits PP1C and PP5C comparably. Norcantharidin should also

have the same binding contacts within PP1C, but it nevertheless displays a preference for PP5C over PP1C, possibly due to very small differences in the positions of the contacting residues. As a result of its 9-fold deselection of PP1C, norcantharidin and its PP5C-active adducts should have inhibitory properties and antitumor activity like that of cantharidin, without exhibiting cantharidin's universal cytotoxicity.[8]

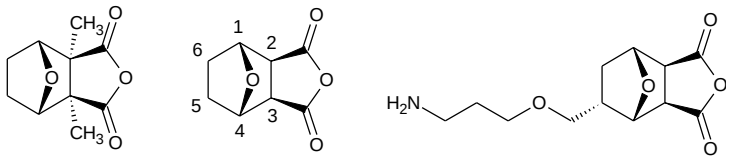


Figure 1. Structures of cantharidin (**A**), norcantharidin (**B**), and the endo-5-(3-amino)-propoxymethyl norcantharidin scaffold (**C**).

Based on the co-crystal structure of PP5C's catalytic pocket and the norcantharidin core, the molecule would be best augmented on the *endo*-C5 position to increase its binding affinity.[8] Elongation from the C5 position may increase PP5C potency and selectivity by allowing the compound to reach into a cavity bounded by the non-conserved, PP5C-specific residues Lys^{399}, Glu^{428}, Lys^{430}, and Ala^{437}. Several norcantharidin analogs have been synthesized previously, revealing that the addition of a propoxymethyl substituent to the *endo*-C5 position retained 5-fold selectivity of PP5C over PP1C as well as a 2.8-fold decrease in IC_{50} values compared to that of norcantharidin.[8] Quantum chemical modeling suggested a series of analogs based upon an *endo*-5-propoxymethyl norcantharidin scaffold (Figure 1**C**) would approach PP5C's Lys^{399} and Glu^{428} for favorable hydrogen bonding, which may amplify the compound's potency and selectivity further.[8]

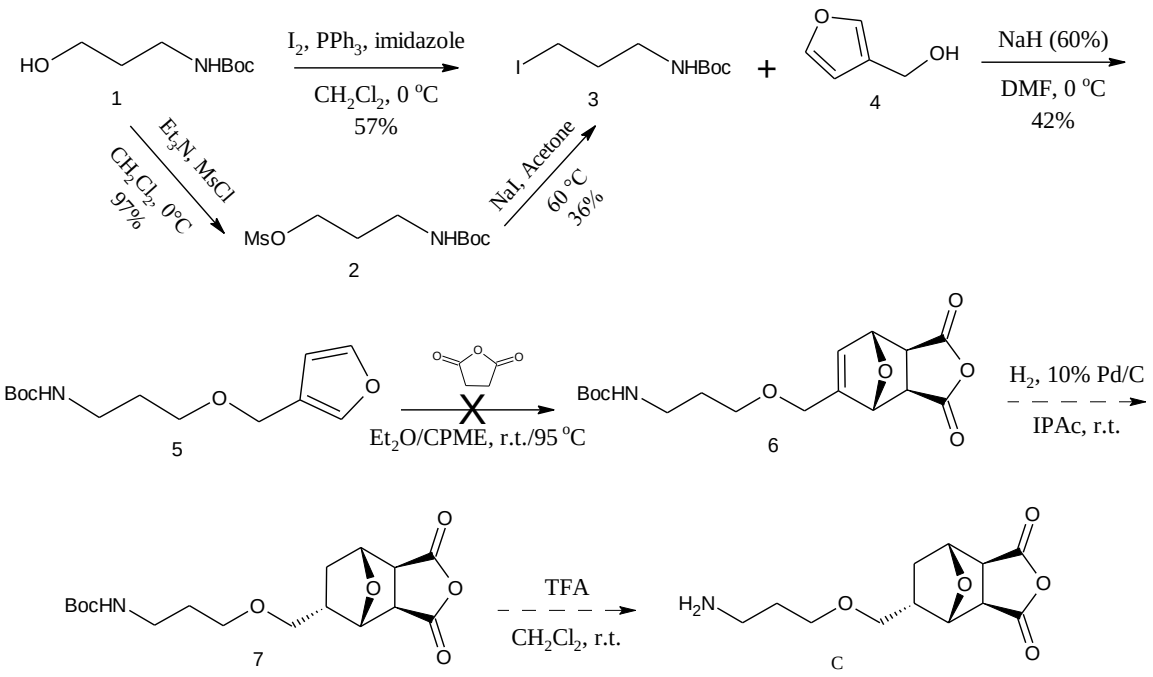
From the above findings, a general synthesis has been designed for the development of a PP5C inhibitor scaffold that combines the inhibitory properties of the norcantharidin core and the binding affinity of a propoxymethyl substituent. In this experiment, a multi-step synthetic scheme was devised for the *endo*-5-(3-amino)-propoxymethyl norcantharidin scaffold from which a series of analogous inhibitors can be synthesized. The scheme was designed by merging current organic synthesis techniques, such as the Finkelstein reaction and Williamson ether synthesis, with the established procedure for the synthesis of the norcantharidin core. Based upon the percent yield and NMR analysis of each intermediate, the potential of the scheme to yield the desired scaffold compound is determined.

2. Results and Discussion

Many norcantharidin analogs have been prepared previously by our group.[8] However, no publications have reported the synthesis of functionalized propoxymethyl norcantharidin analogs or the *endo*-5-(3-amino)-propoxymethyl norcantharidin scaffold. If synthesized, the scaffold is a key intermediate in the formation of amidized analogs, which quantum chemical modeling suggest may have promising inhibitory activity against PP5 based on a 3-methylamido adduct.

Here, the multi-step synthesis that was devised for the production of the norcantharidin scaffold and its analogs was experimentally tested, but failed to yield the desired scaffold. In Scheme 1, the Boc-protected alcohol **1** was directly halogenated with PPh_3, I_2, and imidazole to form iodo **3**; however, the triphenylphosphine oxide impurity was difficult to remove by silica column chromatography. Therefore, alcohol **1** underwent mesylation to form mesylate **2**, which then participated in a Finkelstein reaction to produce iodo **3**. Reaction with furanylmethanol **4** and iodo **3** as part of a Williamson ether synthesis gave ether **5**. Finally, the synthesis of compound **6** by Diels-Alder [4+2] cycloaddition was attempted with ether **5** and maleic anhydride in either Et_2O at room temperature or in cyclopentyl methyl ether at 95 °C, but no reaction occurred. Without this crucial cycloaddition, compounds **7** and **C** could not be synthesized.

A model synthesis with 3-[(propoxy)methyl]furan and maleic anhydride[8] suggest that a successful cycloaddition is dependent on either the nonexistence of the Boc-protected amino group or the reaction solvent. Additional reactions utilizing non-protected or other nitrogenous groups and further solvent-dependent studies are necessary to investigate the Diels-Alder cycloaddition failure.



Scheme 1. The proposed chemical synthesis of the *endo*-5-(3-amino)-propoxymethyl-norcantharidin scaffold (**C**)

3. Conclusions

Scheme 1 did not produce the desired product. Based on the percent yields and NMR analysis of each intermediate, Scheme 1 has potential to produce the *endo*-5-(3-amino)-propoxymethyl norcantharidin scaffold; however, because the Diels-Alder cycloaddition failed to occur, new reaction conditions or a new synthetic scheme need to be investigated for viability.

4. Experimental

4.1 General Experimental Conditions

NMR spectra were obtained as solutions in $CDCl_3$ unless otherwise stated. Chemical shifts were reported in parts per million (ppm, δ) and referenced (δ 7.24 (^{1}H NMR) when using $CDCl_3$). TLC analyses were performed on Whatman flexible aluminum backed TLC plates with a fluorescent indicator. Detection was conducted by UV absorption (254 nm) and charring with 10% $KMnO_4$ in water unless otherwise stated. High-purity grade silica gel (Merck Grade 7734), pore size 60 Å, 70-230 mesh was used for all chromatographic separations. All chemicals used for synthetic procedures were reagent grade or better. Solutions were concentrated *in vacuo* with a rotary evaporator and the residue was purified as stated.

4.2 Scaffold Synthesis

***tert*-Butyl (3-hydroxypropyl)carbamate** (**1**). To a solution of 3-aminopropan-1-ol (6.0 g, 0.080 mol) in CH_2Cl_2 (100 mL) was added Et_3N (11.2 mL, 0.080 mol). The reaction mixture was stirred under nitrogen for 30 min before a solution of Boc_2O (19.2 g, 0.088 mol) in CH_2Cl_2 (100 mL) was added dropwise. The resulting colorless solution was refluxed overnight, quenched with sat. NH_3Cl solution, and extracted with CH_2Cl_2 (3x). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo* to yield compound **1** (13.0 g, 93%) as a clear oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.12 (br s, 1H), 3.73 (br s, 1H), 3.55 (t, 2H), 3.15 (q, 2H), 1.63-1.55 (m, 2H), 1.37 (s, 9H).

***tert*-Butyl (3-iodopropyl)carbamate** (**3**)**.** To a chilled (0 °C) solution of 1*H*-imidazole (0.70 g, 10.3 mmol) and PPh_3 (2.69 g, 10.3 mmol) in CH_2Cl_2 (50 mL) was added I_2 (2.6 g, 10.3 mmol) in small portions. The reaction mixture was allowed to warm to room temperature before compound **1** (1.5 g, 8.6 mmol) in CH_2Cl_2 (10 mL) was added dropwise. The reaction mixture was stirred under nitrogen overnight. The resulting mixture was filtered over Celite and washed with a 5% $Na_2S_2O_3$ solution (2x). The washings were extracted with CH_2Cl_2 (3x) and the organic layers were dried over $MgSO_4$. The solution was concentrated *in vacuo* and purified by column chromatography (1:19 EtOAc:Hex) to give **3** (1.44 g, 57%) as a yellow oil contaminated with triphenylphosphine oxide: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.62 (br. s, 1H), 3.19 (t, 4H), 2.01 (t, 2H), 1.44 (s, 9H).

***N*-[3-[(methylsulfonyl)oxy]propyl]-1,1-dimethylethyl ester** (**2**). To a chilled (0 °C) solution of compound **1** (2.0 g, 0.012 mol) in CH_2Cl_2 (80 mL) was added Et_3N (1.9 mL, 0.014 mol) followed by MsCl (1.4 g, 0.014 mol) dropwise. The reaction mixture was stirred under nitrogen for 2 h, quenched with water and extracted with CH_2Cl_2 (3x), dried over $MgSO_4$, and concentrated *in vacuo* to yield compound **2** (3.0 g, 97%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.04 (s, 1H), 4.23 (t, 2H), 3.23-3.19 (m, 2H), 3.00 (s, 3H), 1.90-1.87 (m, 2H), 1.43 (s, 9H).

***tert*-Butyl (3-iodopropyl)carbamate** (**3**). A suspension of mesylate **2** (3.0 g, 0.012 mol) and NaI (5.3 g, 0.035 mol) in acetone (71 mL) was refluxed and stirred under nitrogen overnight. The reaction mixture was concentrated, quenched with water, and extracted with CH_2Cl_2 (3x). The combined organic layers were washed with sat. $Na_2S_2O_3$ solution, dried over $MgSO_4$, and concentrated *in vacuo* to give **3** (0.42 g, 36%) as a brown oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.62 (br. s, 1H), 3.19 (m, 4H), 2.01 (t, 2H), 1.44 (s, 9H).

Furan-3-ylmethanol (**4**). To a chilled (0 °C) mixture of 3-furaldehyde (5.0 g, 0.052 mol) in MeOH (250 mL) was added $NaBH_4$ (1.97 g, 0.052 mol). The reaction mixture was stirred under nitrogen overnight, concentrated, quenched with water, and extracted with EtOAc (3x). The combined organic layers were dried over $MgSO_4$ and concentrated in *vacuo* to yield alcohol **4** (4.7 g, 92%) as a yellow oil: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, 2H), 6.43 (s, 1H), 4.53 (s, 2H), 3.66 (t, 2H), 2.06 (d, 1H).

***tert*-Butyl [3-(furan-3-ylmethoxy)propyl]carbamate** (**5**). To a chilled (0 °C) solution of alcohol **4** (413 mg, 4.21 mmol) in DMF (20 mL) was added NaH (185 mg, 60% in mineral oil, 4.63 mmol). The mixture was stirred for 30 min at room temperature and a solution of iodo **3** (1.2 g, 4.21 mmol) in DMF (14.1 mL) was added dropwise. The reaction mixture was stirred under nitrogen overnight, quenched with water, and extracted with EtOAc (3x). The combined organic layers were washed with 5% LiCl solution (5x), dried over $MgSO_4$, and concentrated to yield **5** (0.453 g, 42%) as a brown oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.39 (d, 2H), 6.39 (s, 1H), 4.36 (s, 2H), 3.49 (t, 2H), 3.25-3.15 (m, 2H), 1.77 (t, 2H), 1.44 (s, 9H).

***tert*-Butyl(3-{[(1*R*,2*S*,6*R*,7*R*)-3,5-dioxo-4,10-dioxatricyclo[5.2.1.02,6]dec-8-en-8-yl]methoxy}propyl)carbamate** (**6**). To a solution of ether **5** (100 mg, 0.391 mmol) in Et_2O/CPME (3.13 mL) was added maleic anhydride (38 mg, 0.391 mmol). The reaction mixture was stirred at room temperature/refluxed (95 °C) under nitrogen overnight. The resulting mixture was concentrated in *vacuo* and purified by gradient column chromatography (1:4 EtOAc:Hex), but did not yield compound **6**.

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