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Design and synthesis of indole derivatives of adenophostin A. A entry into subtype-selective IP_3 receptor ligands

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ABSTRACT

Indole derivatives **3a** and **3b** of adenophostin A (**2**) in which the adenine of **2** was replaced with indole or 4-fluoroindole was designed as potential inositol trisphosphate receptor ligands. These target compounds were successfully synthesized from the key disaccharide unit **6**. Biological evaluation showed that **3b** selectively activates IP_3R1, a subtype of IP_3 receptors.

Adenophostin A (Fig. 1, **2**), isolated from *Penicillium brevicompactum*,[1] is a very potent *myo*-inositol 1,4,5-trisphosphate (IP_3, **1**) receptor agonist, which is 10–100 times more potent than the endogenous ligand IP_3 in stimulating Ca^{2+} release and in binding to IP_3 receptors.[1b,1c] Due to the high potency, its total synthesis[2] and also structure–activity relationship studies[3,4] have been extensively investigated. These studies have demonstrated that the adenine or the corresponding aromatic ring at the 1′β-position is essential for the high potency as an IP_3 receptor ligand.[3,4]

As a second messenger, IP_3 functions generally in many organs,[5] and at least three types of IP_3 receptor subtypes, those are, IP_3R1, IP_3R2, and IP_3R3, are known.[5b] IP_3 receptor ligand, which is selectively active to one of the receptor subtypes, can be effectively used as a biological tool for investigating cellular signal transduction via IP_3. However, none of the subtype-selective IP_3 receptor ligands have been known so far. Thus, we focused our attention on the development of adenophostin analogs with IP_3 receptor subtype selectivity.

Recently, Potter and co-workers investigated the binding mode of **2** to the IP_3 receptor by molecular modeling using the X-ray crystal structure of IP_3R1-binding core to suggest that the adenine of adenophostin A seems to associate with the guanidium side chain of Arg504 via a cation–π interaction in the binding pocket of IP_3R1.[3d] Based on these modeling results, we designed an indole derivative **3a** of adenophostin A in which the adenine of adenophostin A is replaced by indole, as shown in Figure 1. The shape of indole is similar to that of adenine as a 5/6-fused aromatic ring system, and it has efficient electron-donating feature,[6] which might be suitable for the cation–π interaction speculated by the modeling. In addition, the electron-donating potency of the indole

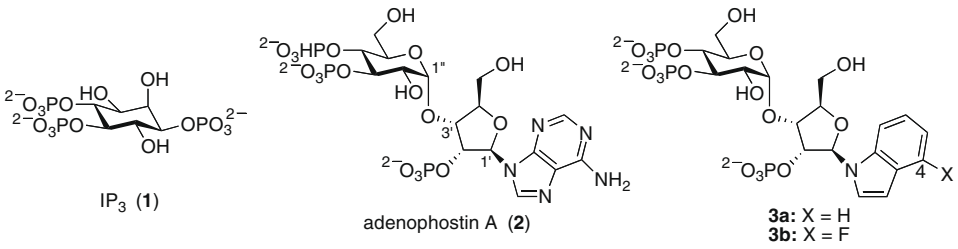

Figure 1. IP_3 (**1**), adenophostin A (**2**), and the target indole derivatives **3a** and **3b**.

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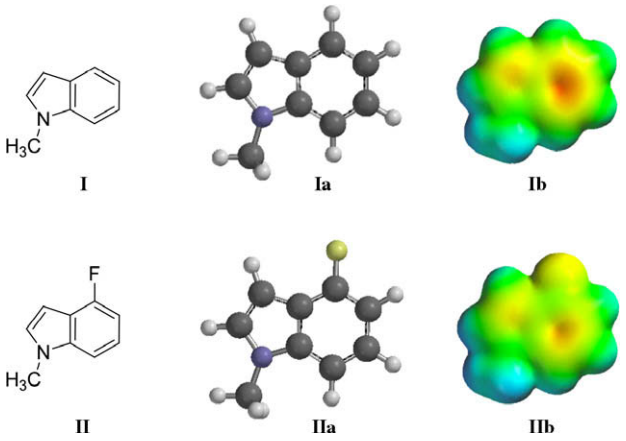


Figure 2. The optimized structures (**Ia** and **IIa**) and their electronic potential surfaces (**Ib** and **IIb**) of the model compounds **I** and **II** by DFT calculation.

ring may be controlled by introducing an electron-donating substituent or electron-withdrawing substituent.

Based on these findings and considerations, we also designed another indole derivative **3b** in which an electron-withdrawing fluoro group is substituted at the indole 4-position. We thought that if the cation–π interaction is indeed important for the binding of adenophostin A to the receptor, affinity for the receptor might be changed depending on the electron-donating feature or electron-withdrawing feature of the substituent introduced at the indole moiety.

To understand the electronic properties of indole derivatives, quantum chemical calculation was carried out using 1-methylindole (**I**) and 4-fluoro-1-methylindole (**II**) as model compounds (Fig. 2).[7] The model compounds **I** and **II** were fully optimized based on density functional theory (DFT) giving the stable structures **Ia** and **IIa**, respectively. Electrostatic potential surface of the optimized structures **Ia** and **IIa** showed that 1-methylindole is electron-richer at the center of the aromatic ring than the corresponding 4-fluoro derivative, which is shown as **Ib** and **IIb**, respectively, in Figure 2. Furthermore, ionization energies of **Ia** and **IIa** were calculated at 7.0725 eV and 7.1424 eV, respectively. These calculation data indicated that 1-carbon-substituted indole is more electron-donating than the corresponding 4-fluoro congener, as we expected.

As shown in Scheme 1, we planned to synthesize the target compounds **3a** and **3b** via the α-disaccharide **6**, which can be provided by the α-selective glycosidation with a sulfoxide donor **4** and an acceptor **5**.[4d] The α-disaccharide **6** has been used previously as an effective synthetic intermediate for synthesizing various derivatives of adenophostin A.[4f]

Synthesis of the target compound **3a** from **6** was accomplished as summarized in Scheme 2. After the removal of the 5-*O*-TBS group of **6**, the 5-hydroxyl was re-protected with an allyl group[8] to give **7**. Acidic removal of the ketal-protecting groups of **7** followed by acetylation of the resulting free hydroxyls afforded the 1,2,3′,4′-tetra-*O*-acetate **8**.

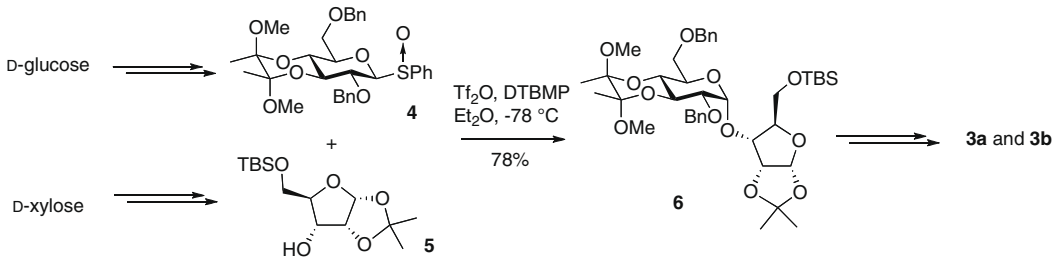
Indole nucleosides have been synthesized via glycosidation with indoline as a glycosyl acceptor and subsequent oxidative aromatization of indoline ring into indole ring.[9] Thus, using the tetra-*O*-acetate **8** as a glycosyl donor, when a solution of **8** and indoline (3 equiv) was heated in AcOH/EtOH (1:10) under reflux, the β-selective glycosidation occurred to give glycosidation product **9a**. Subsequent oxidative treatment of **9a** with MnO_2 in toluene yielded the desired β-indole nucleoside derivative **10a**[10] in 69% yield from **8**.

The 5′-*O*-allyl-protecting group was replaced with a TBS group at this stage.[11] Thus, long time heating of **10a** with $(PPh_3)_3RhCl$ in toluene/EtOH under reflux and subsequent treatment of the resulting product with TBSCl/DMAP in pyridine gave the 5′-*O*-silyl ether **12a**. The three *O*-acetyl groups of **12a** were removed simultaneously with NaOMe/MeOH to give **13a**. The phosphate units were introduced into the 2′-, 3″-, and 4″-hydroxyls using the phosphoramidite method with *o*-xylene *N,N*-diethylphosphoramidite (XEPA).[12] Thus, **13a** was treated with XEPA in the presence of tetrazole as a promoter in CH_2Cl_2, followed by oxidation with *m*-CPBA to form the desired 2′,3″,4″-trisphosphate derivative **14a** in 97% yield. After the removal of the 5′-*O*-TBS group with TBAF/THF, the protecting groups of the phosphate moieties were finally removed under hydrogen atom transfer conditions with $Pd(OH)_2$ and cyclohexene in MeOH to furnish the target trisphosphate **3a** as a sodium salt, after treatment with ion-exchange resin.

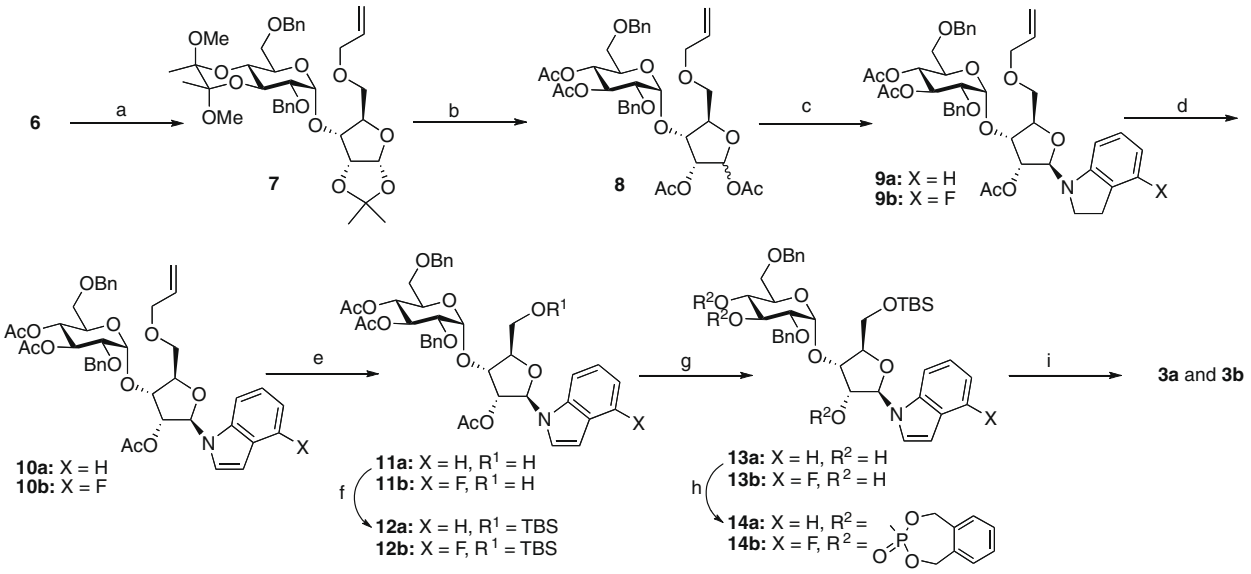
Another target compound **3b** was synthesized by employing 4-fluoroindoline as an acceptor instead of indoline in the glycosidation step with the donor **8**, as shown in Scheme 2.

We have previously developed a method for the identification of ligands of IP_3Rs using fluorescent biosensors,[13,14] and established subtype-specific IP_3 biosensors LIBRAvI and LIBRAvII for investigating binding affinity of IP_3 receptor ligands for IP_3R1 and IP_3R2, respectively.[15] Thus, the binding abilities of adenophostin A and the target compounds **3a** and **3b** were examined with these biosensors. Figure 3 indicates the increase in emission ratios of LIBRAvI and LIBRAvII with a same concentration (1 μM) of these compounds. Effect of adenophostin A, **3a**, and **3b** on LIBRAvI was ~80%, ~40%, and ~35% of the maximal response, respectively. These results indicate that the potency of adenophostin A for the activation of IP_3R1 is higher than that of compounds **3a** and **3b**. Although similar effects of adenophostin A and **3a** were observed on the emission ratio of LIBRAvII compared to those on LIBRAvI, the effect of target compounds **3b** on LIBRAvII was significantly small. Thus, their selectivity index (LIBRAvI/LIBRAvII) is 0.97 for adenophostin A, 1.1 for **3a**, and 8.4 for **3b**, respectively. These results indicate that 4-fluoroindole derivative **3b** has a higher selectivity for IP_3R1 over IP_3R2. To our knowledge, this kind of subtype-selective IP_3 receptor ligands has not been reported so far.

These results suggest that the indole ring in **3b** might not effectively work for the expected cation–π interaction in its binding to the receptor. However, significantly weak binding of **3b** to the IP_3R2 compared with that to the IP_3R1 suggested that the subtype



Scheme 1.



Scheme 2. Reagents and conditions: (a) (1) TBAF, THF, rt; (2) $CH_2{=}CHCH_2Br$, $Ca(OH)_2$, CaO, DMF, rt, 97%; (b) (1) 90% aq TFA, rt; (2) Ac_2O, DMAP, Et_3N, MeCN, rt, 77%; (c) indoline, AcOH, EtOH, reflux; (d) MnO_2, toluene, rt, 69% (**10a**) from **8**, 77% (**10b**) from **8**; (e) $(PPh_3)_3RhCl$, toluene/EtOH, reflux, 68% (**11a**), 72% (**11b**); (f) TBSCl, DMAP, pyridine, rt, 64% (**12a**), 79% (**12b**); (g) NaOMe, MeOH, rt, quant (**13a**), quant (**13b**); (h) XEPA, tetrazole, CH2Cl2, −40 °C to rt, then *m*-CPBA, −40 °C, 97% (**14a**), 71% (**14b**); (i) (1) TBAF, THF, rt; (2) $Pd(OH)_2$, cyclohexene, MeOH, reflux, 97% (**3a**), 97% (**3b**).

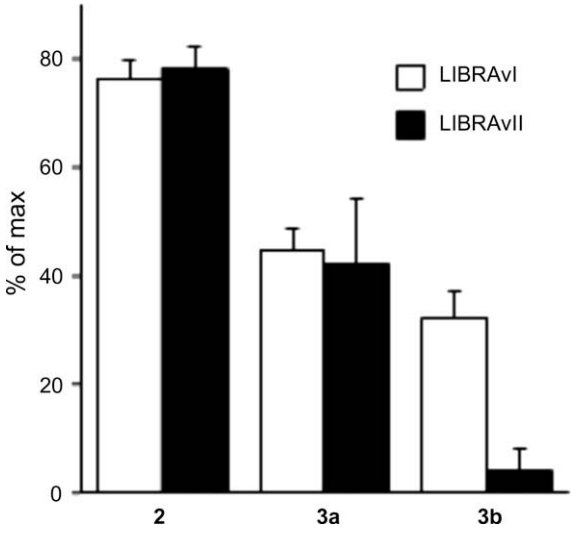


Figure 3. Binding affinities of adenophostin A (**2**) and its indole derivatives **3a** and **3b** for LIBRAvI and LIBRAvII. Effects of compounds on the emission ratio (480/535 nm) of LIBRAvI and LIBRAvII are expressed as % of maximal response. The maximal response was determined by the application of 30 μM IP_3 at the end of each experiment.

selectivity may be manipulated by changing the substituent at the indole 4-position in indole derivatives of adenophostin A.

In conclusion, the indole derivatives **3a** and **3b** designed as a novel IP_3 receptor ligands were successfully synthesized from the key disaccharide unit **6**. While binding affinities of **3a** and **3b** for IP_3R1 and IP_3R2 are weaker than adenophostin A, the 4-fluoroindole derivative **3b** was shown to be an IP_3R1-selective ligand. Therefore, **3a** may be an effective lead for developing subtype-selective IP_3 receptor ligands, which can be useful biological tools.

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