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Biomimetic semi-synthesis of fradcarbazole A and its analogues

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ABSTRACT

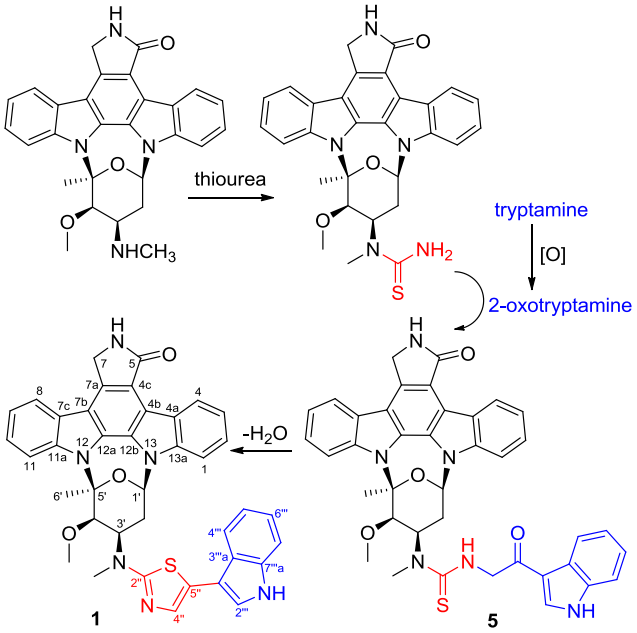
The first synthesis of fradcarbazole A (**1**) has been accomplished by using a biomimetic intramolecular cyclization/dehydration to construct the staurosporine-thiazole-indole skeleton. The phenyl and oxazole analogues of fradcarbazole A (**2**–**4**) were also synthesized using the same strategy. Compounds **1**–**4** displayed cytotoxicity against A549 cell line with IC_{50} values of 0.4–3.6 μM, induction of G_0/G_1 arrest of A549 cell cycle at 10 μM, and inhibition of PKC-β kinase with IC_{50} values of 0.5–0.9 μM.

1. Introduction

Fradcarbazole A is a novel alkaloid isolated from a mutant strain of the marine-derived *Streptomyces fradiae* 007M135.[1] This alkaloid belongs to indolocarbazoles (ICZs) that have received great attention for their unusual structures and important biological activities.[2–4] Staurosporine (ST) is the first example of ICZs isolated in 1977.[5] Since then more than 130 ICZs have been identified from the nature.[6] Although ST has not been used in anti-tumor applications for its low selectivity and high toxicity, its analogues, CEP-701 has been granted orphan drug status for acute myeloid leukemia by FDA.[7] In addition, PKC-412, UCN-01 and CEP-2563 are currently in clinical trials as anti-cancer drugs.[7] The unique structure and interesting bioactivity of fradcarbazole A[1] drives us to synthesize this ICZ alkaloid and its new analogues.

2. Results and discussion

The biosynthetic strategy that we proposed in the previous paper was used to construct the special ST-thiazole-indole skeleton (Scheme 1).[1] The thiourea intermediate (**5**) was synthesized from ST and 2-oxotryptamine resulted from the oxidation of tryptamine (Trp). Compound **5** further underwent intramolecular cyclization/dehydration to yield fradcarbazole A (**1**). The common method for preparing thiourea derivatives is the nucleophilic reaction of isothiocyanates with amines.[8–10] To avoiding the use of the dangerous $CSCl_2$ or CS_2, the thiocarbamoylimidazolium salt[11] was used to synthesize the thiourea intermediate **5**.



Scheme 1. Plausible biosynthetic pathway of fradcarbazole A (**1**).[1]

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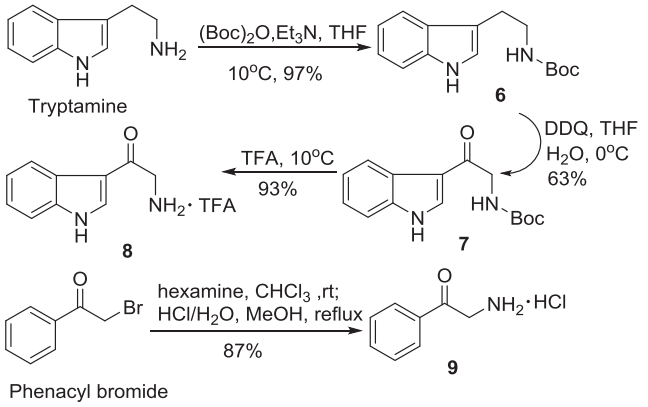
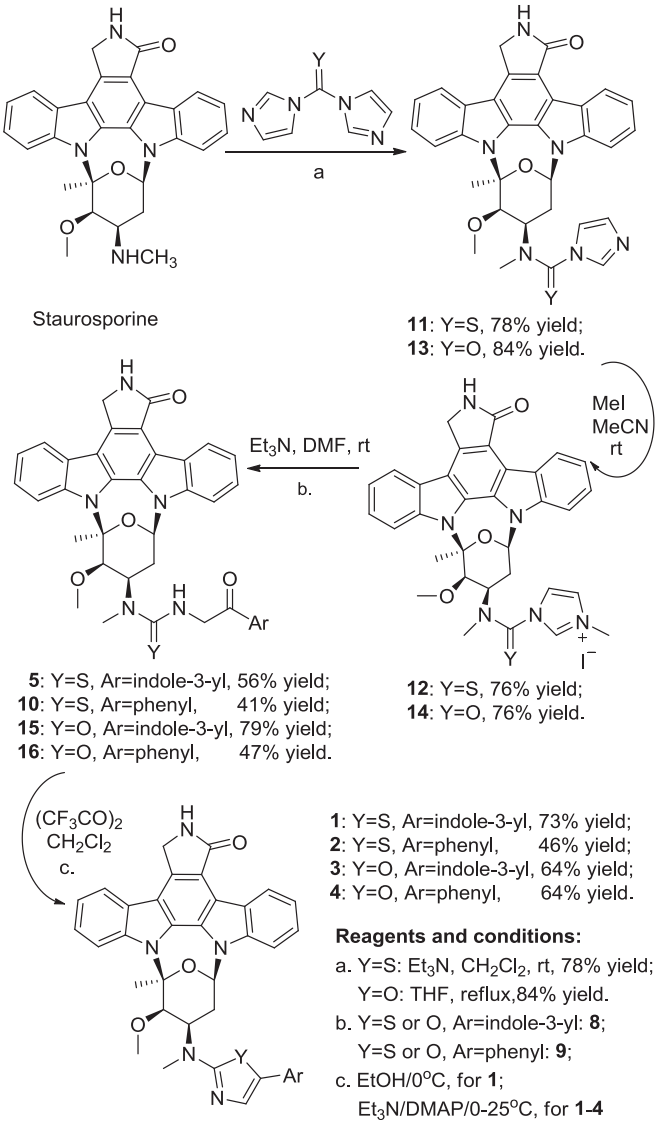
Taking into account the use of trifluoroacetic acid (TFA) to cleave the Boc protecting group, we started to synthesize the trifluoroacetate (**8**) other than the chloride of 2-(1*H*-indol-3-yl)-2-oxoethan-1-aminium. Compound **8** was synthesized from tryptamine by selective protection of the primary amine, installation of the keto-carbonyl through the oxidation of DDQ in aqueous THF, and then exposed of the resultant to TFA to concomitantly cleave the Boc protecting group in 57% yield for three steps (Scheme 2).[12]



Scheme 2. Synthesis of the trifluoroacetate (**8**) and chloride (**9**) of the corresponding 2-oxoethan-1-aminium.

Reaction of ST with thiocarbonyldiimidazole gave 3′-*N*-(1*H*-imidazole-1-carbamothioyl)staurosporine (**11**). Alkylation of **11** with MeI in MeCN gave the crude product **12** that was purified by washing with petroleum ether (PE)-CH_2Cl_2 (1:1) (Scheme 3). The nucleophilic substitution of **8** with the thiocarbamoyl imidazolium iodide (**12**) afforded the thiourea intermediate **5** in 56% yield. However, the attempts to synthesize fradcarbazole A (**1**) were failed by the intramolecular cyclization/dehydration of **5** with the reported Ac_2O–EtOH,[13] Ac_2O–H_3PO_4,[14] and $POCl_3$-pyridine.[15] When using the stronger $(CF_3CO)_2O$ to replace Ac_2O, the cyclization/dehydration was successfully accomplished to form **1** in 73% yield at 0 °C (Scheme 3). Compound **1** was identified by ^1H and ^{13}C NMR data that were consistent with the natural-**1** (Table 1). Furthermore, compound **1** displayed almost the same CD Cotton effects (Fig. 1) to those of the natural-**1**.[1] The specific rotation ($[\alpha]_D^{18}$ +242 (*c* 0.3, $CHCl_3$), +307 (*c* 0.01, $CHCl_3$), +195 (*c* 0.01, MeOH)) was not accorded with the reported natural-**1**.[1] Thus, the specific rotation of the natural-**1** was re-measured and the value was $[\alpha]_D^{18}$ +279 (*c* 0.01, $CHCl_3$), and +183 (*c* 0.01, MeOH).[16]

After the success in synthesis of fradcarbazole A (**1**), this strategy was used to synthesize its new analogues, 3′-*N*-(5-phenylthiazole-2-yl)staurosporine (**2**), 3′-*N*-(5-(1*H*-indole-3-yl) oxazole-2-yl) staurosporine (**3**), and 3′-*N*-(5-phenyloxazole-2-yl) staurosporine (**4**). The 2-phenyl-2-oxoethan-1-aminium chloride (**9**) was synthesized from phenacyl bromide[17] in 87% yield (Scheme 2). Then coupling of **9** with **12** gave the intermediate **10** in 41% yield. The carbamoylimidazole **13** was produced from the nucleophilic reaction of ST with carbonyldiimidazole, whose alkylation with MeI afforded the carbamoylimidazolium iodide (**14**). The further nucleophilic reactions of compound **14** with **8** and **9** gave 3′-*N*-(2-(1*H*-indol-3-yl)-2-oxoethylaminocarbonyl) staurosporine (**15**) and 3′-*N*-(2-phenyl-2-oxoethyl aminocarbonyl) staurosporine (**16**), respectively. However, the cyclization/dehydration of compounds **10**, **15** and **16** could not be accomplished by the same method for **1**. Raising the reaction temperature was also useless. This can be concluded that the weaker nucleophilicity of carbonyl oxygen than carbonyl sulfur and the less electron-donating effect of phenyl than



Scheme 3. Synthesis of fradcarbazole A (**1**) and its analogues (**2**–**4**).

indolyl decrease the corresponding nucleophilic cyclization/dehydration. The speed-limited steps of the cyclization/dehydration are nucleophilic attacking of the amide-carbonyl sulfur or oxygen to the ketone carbonyl carbon and the elimination of H^+ to form the C=C double bond (Scheme 4).

Thus, we tried to use the basic $(CF_3CO)_2O$–Et_3N–DMAP system to help the elimination of H^+. The result showed that compounds **10**, **15** and **16** smoothly underwent intramolecular cyclization/dehydration to form the analogues **2**–**4** in 46%, 64% and 64% yields, respectively. Compound **1** was also produced in 49% yield from **5** under the same condition. The structures of compounds **2**–**4** were identified by comparison of ^1H and ^{13}C NMR (Table 1, Figs. S1–S10) and CD spectra (Fig. S33) with those of **1**. All the new compounds including new intermediates **5** and **10**–**16** were identified by NMR data (Figs. S11, S12 and S19–S32) and HR-ESIMS (Figs. S34–S45).

Compounds **1**–**4** were assayed for their cytotoxic effects on MCF-7, MD-MAB-231, MCF/3K and A549 cell lines by the MTT[18] and SRB method,[19] respectively. The inhibitory effects on the cell cycle[20] and the kinase PKC-β[21] were also evaluated. The results show that both analogues **3** and **4** with a substitution of oxazole for thiazole are effective on A549 and MCF/3K tumor cells. And the analogue **2**