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Synthesis of Intervenolin, an Antitumor Natural Quinolone with Unusual Substituents

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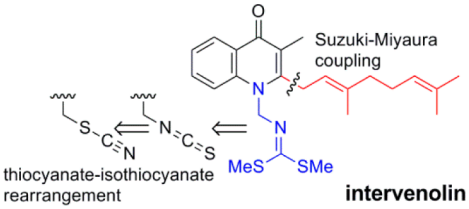
ABSTRACT



Synthesis of intervenolin, a natural quinolone discovered by screening for selective growth inhibitors of cancer cells cocultured with stromal cells over monocultured cells, was achieved. The synthesis utilized a thiocyanate−isothiocyanate rearrangement and Suzuki−Miyaura coupling to furnish the characteristic substituents, the iminodithiocarbonate moiety, and the geranyl side chain, respectively. In vivo studies showed that intervenolin inhibited tumor tissue growth in model mice.

Tumor tissues comprise not only tumor cells but also surrounding stroma.[1] The stroma consists of normal cells, including endothelial cells, fibroblast-like cells (termed stromal cells), and extracellular matrix.[2] Recent studies revealed that stromal cells regulate the growth of adjacent tumor cells positively or negatively via direct or indirect communications influenced by cell adhesion or secreted factors.[2,3]

As part of our continuous search for antitumor agents that are highly selective toward tumor cells over normal cells, we became interested in these types of communications between tumor and stromal cells as a screening target. Signals transmitted from stromal cells are particularly interesting because the machineries come from normal cells wherein the participating molecules are believed to be barely mutated. To search for modulators of tumor−stroma interactions, we constructed an assay system in which molecules inhibiting the growth of tumor cells cocultured with stromal cells more potently than in their absence are picked. Some natural products exhibited the desired selectivity.[4,5]

Among these natural products was an unprecedented second metabolite of microorganisms, intervenolin (**1**), that also has selective inhibitory activity on the proliferation of human gastric and colon cancer cell lines. Moreover, intervenolin exhibited antitumor effects in a xenograft model of human colon cancer cell in vivo and exerted a selective anti-*Helicobacter pylori* effect. To further elucidate

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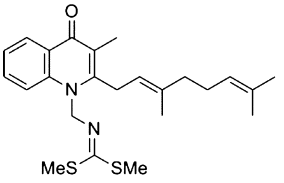
the details of its biological activity, especially its in vivo antitumor effects against a variety of tumor subtypes, a large quantity of intervenolin was required. The production of this compound by fermentation, however, was as low as 3.9 mg/10 L, which is not sufficient to meet the demand.[5] To address this supply issue, we developed a practical synthetic route to intervenolin and report our results in the present paper.



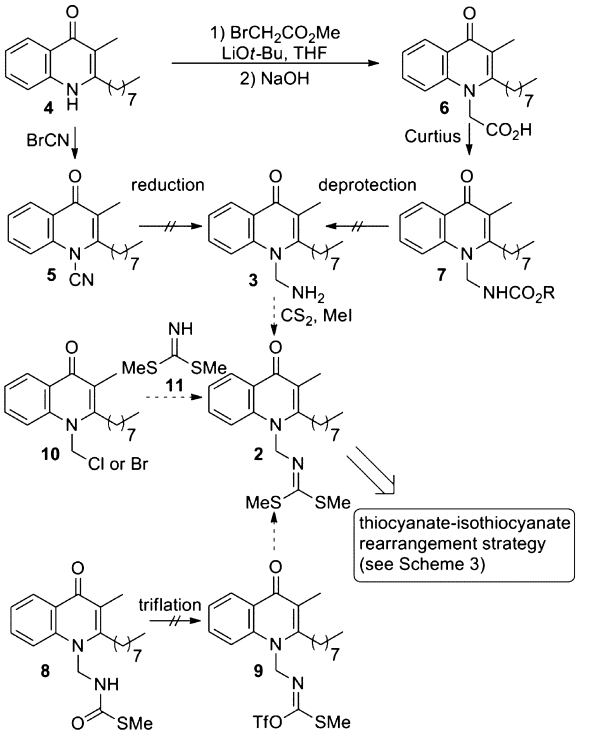
1: intervenolin

Intervenolin has a unique structural feature: a 4-quinolone skeleton substituted with a bis(methylthio)methyleneaminomethyl group and a geranyl side chain at the 1- and 2-positions, respectively. Quinolone is a privileged substructure of pharmaceutical leads,[6] and various compounds with this framework can be found in the literature. Indeed, vast numbers of quinolone-based antibiotics have been approved for clinical use to treat infectious diseases.[7] Whereas the core structure is familiar to synthetic chemists, the two characteristic substituents of intervenolin are rarely seen among the compounds of this class.

The bis(methylthio)methylidene group is a well-known protecting group for primary amines, which is typically formed by treatment of amine with CS_2 and MeI,[8] and is easily removed oxidatively[8] or by acid hydrolysis.[8,9] Intriguing applications of this protecting group are seen in the auxiliary-based asymmetric alkylation of glycine to afford optically active amino acids including unnatural ones developed by Katsuki and Yamaguchi's group[10] and Oppolzer's group.[9,11] On the other hand, this uncommon functionality has rarely been witnessed as a partial structure of natural products, which has rendered a weak repertoire to furnish this pendant structure. In Scheme 1, the approaches, including unsuccessful ones, examined in this study using model compounds to form this structural unit are summarized.

Initially, the strategy in which an aminomethyl group was introduced at the 1-position prior to the usual "protection protocol" discussed above (CS_2 and MeI) was attempted. Toward this end, *N*-aminomethylated

Scheme 1. Approaches To Form the Dithioiminocarbonate Moiety



quinolones such as **3** were needed, but this type of compound also belongs to a rare chemical entity, presumably because of its feature of vinylogous monoamide of methanediamine that appears to be sensitive to acidic conditions. Actually, scanty precedents containing this substructure are reported as natural products,[12] and no information on preparative methods has been provided. In the present study, two branched pathways starting from **4** were investigated, which commenced with cyanation by BrCN[13] to give **5** and introduction of the acetate unit to afford **6**, respectively. Disappointingly, the cyanation route led to difficulties in the reduction of the cyano group of **5**. In the alternative reaction sequence, Curtius rearrangement of **6** worked uneventfully to provide the corresponding carbamate **7** (Boc and Fmoc) upon trapping the isocyanate intermediate by alcohol. The following deprotection conditions gave rise to a considerable amount of decomposed byproduct to afford **3** in a trace amount. By changing the trapping reagent from alcohol to NaSMe in the Curtius route, we obtained the corresponding thiocarbamate **8**. We envisioned that triflation of **8** would lead to **9**, which in turn would be affected by methyl thiolate to give **2** by the addition–elimination process. The first triflation, however, did not proceed at all. We then turned our attention to the use of a commercially available iminodithiocarbonate **11** in a nucleophilic substitution using a suitably halogenated substrate **10**. The substrate **10**,

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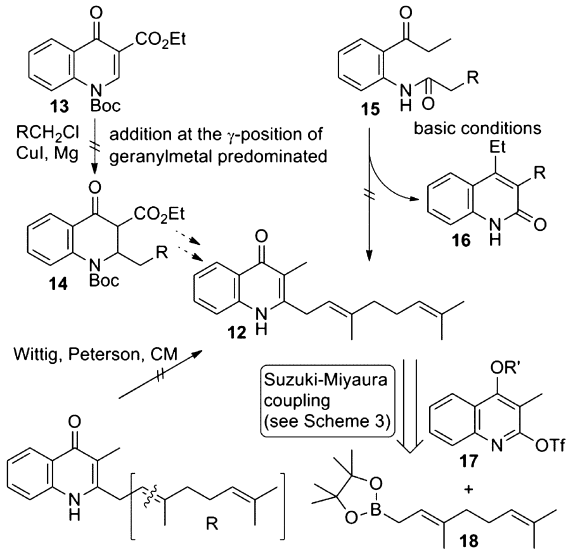
however, was not available by the halomethylation of **4** using dihalomethanes as electrophiles. Finally, a rearrangement of thiocyanate to isothiocyanate was found to be a key transformation to address the difficulty discussed above (see Scheme 3).

It is noteworthy that the choice of the base was important for chemoselectivity upon introduction of the acetate unit to the 4-quinolone core.[14] When **4** was treated with bases with Na or K (NaH, K_2CO_3, KO-*t*-Bu) as countercations before addition of $BrCH_2CO_2Me$, *O*-attack occurred almost exclusively. On the other hand, LiO-*t*-Bu gave the *N*-adduct as the main product (88% combined yield, *N*-adduct:*O*-adduct = 6.4:1),[15] which was utilized in the successful synthetic route to intervenolin (vide infra).

Scheme 2. Approaches To Install the Geranyl Side Chain



Installation of a geranyl side chain, another characteristic structural feature of intervenolin, onto the quinolone core was an unexpectedly challenging task, as summarized in Scheme 2. Limited numbers of examples of geranylated and prenylated 4-quinolone are available in the literature: only five geranylated compounds are reported as natural products of microorganism origin.[16] Likewise, one prenylquinolone derivative was disclosed as a synthetic compound;[17] introduction of the prenyl chain was attained by conjugate addition of the corresponding cuprate reagent to highly activated acceptors such as **13**.[18] At first, geranylation in our system was attempted using this conjugate addition protocol, but the reaction occurred preferencially at the γ-position of the geranylmetal species rather than at the α-position. Condensation of ketoamide such as **15** under basic conditions, another conventional method (Camps quinolone synthesis)[19] to obtain 4-quinolone derivatives with a saturated alkyl side chain at the 2-position, also failed. In our particular case, the highly acidic α-proton of the amide carbonyl was readily abstracted to afford 2-quinolone product **16**. Alternative strategies, disconnection at the internal olefin of the side chain by Wittig and its related chemistry, and cross-metathesis (CM), also provided no fruitful results. Eventually, cross-coupling using a triflate derivative of quinoline as the substrate with concomitant skeletal isomerization fulfilled the construction of this key structure as demonstrated in Scheme 3.

The successful synthesis of intevenolin departed from a Friedel–Crafts reaction of **19**[20] via mixed anhydride generated by Eaton's reagent (P_2O_5/MsOH = 1/10).[21] Then, the 4-hydroxy-2-quinolone **20** was protected as a TBS ether. The resultant 2-quinolone **21** was treated with Tf_2O in the presence of 2,6-lutidine to reveal a quinolone skeleton to afford a triflate **22**. Subsequently, the geranyl side chain was introduced by Suzuki–Miyaura coupling using the corresponding boronic acid pinacol ester **18** as a counterpart.[22] Thorough optimization studies of the reaction conditions revealed that $NaHCO_3$ was the most preferable base: Na_2CO_3 and K_3PO_4 resulted in extensive decomposition of the substrate. A mixed-solvent system comprising EtOH/toluene = 1/2.5 gave the best results in terms of conversion, whereas THF and 1,4-dioxane led to a decreased yield. Use of 10 mol % of $Pd(PPh_3)_2Cl_2$ as a precatalyst slightly improved the isolated yield from 65% to 70% compared to the use of 6 mol % of $Pd(PPh_3)_4$ as a catalyst. Under the weakly basic conditions upon heating at 90 °C, the TBS group was expelled to tautomerize the quinoline core to the 4-quinolone skeleton affording a 2-geranylquinolone intermediate **23**. The stage was then set for the construction of the pendant structure, i.e., the bis(methylthio)methyleneaminomethyl moiety, namely. Thiocyanomethylation of **23** with $ClCH_2SCN$ was accomplished using LiO-*t*-Bu as a base. So far, this is the only base of choice; NaH and K_2CO_3 gave *O*-adduct as a sole product, no reaction occurred with LiH, and substantial decomposition of the substrate was observed when $Mg(O\text{-}t\text{-}Bu)_2$ was used. During the course of the reaction, the thiocyanate species **24** spontaneously underwent a pivotal rearrangement to the corresponding isothiocyanate **25**.[23] The intrinsic lability of the product required extremely careful handling. Indeed, the reaction was quenched

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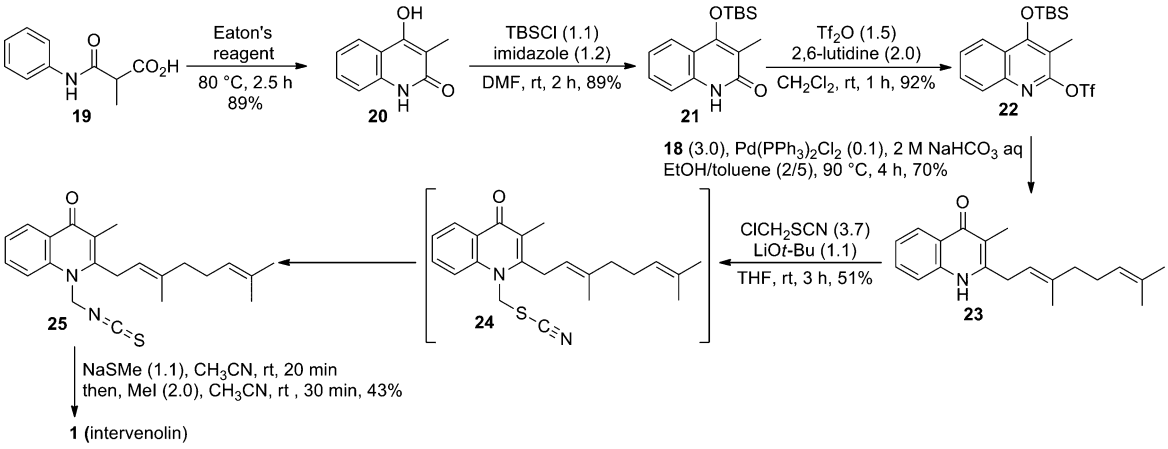
Scheme 3. Synthesis of Intervenolin (**1**)



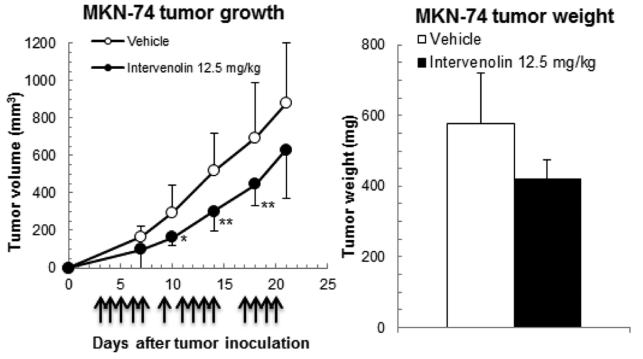


Figure 1. Effect of intervenolin on tumor growth of MKN-74 cells in vivo. MKN-74 human gastric cancer cells were inoculated subcutaneously into female nude mice. Intervenolin was administered intravenously at 12.5 mg/kg on days indicated by arrows. The mice were sacrificed on day 21 after the tumor inoculation, and the tumors were excised. The values are means ± SD of 5 mice. *$P < 0.05$ and **$P < 0.01$ versus the values with vehicle.

after 3 h of stirring, and subsequent quick chromatographic purification afforded an isothiocyanate **25** in fair yield (51%). A longer reaction time led to extensive decomposition of the product, and no product was detected after a 12-h reaction. Formation of the *O*-adduct was not detected, whereas dimerization via an intermediary of iminium generated by competing elimination of the thiocyano group to which another equivalent of **23** attacked. In the next step, the isothiocyanate moiety of **25** was readily captured by a methyl thiolate. Then, the resultant carbonimidodithioate monoanion was methylated by MeI to afford intervenolin **1** in 43% yield. The physicochemical properties and inhibitory activities toward gastric cancer MKN-74 cells cocultured with Hs738 human gastric stromal cells (Figure S1, Supporting Information) of the synthetic intervenolin were indistinguishable from those of a natural sample.

With an adequate amount of the synthetic sample in hand, we next examined the in vivo antitumor properties of intervenolin: intervenolin inhibited the growth of MKN-74 cells at a dose of 12.5 mg/kg (Figure 1). Importantly, intervenolin had no noticeable toxicity in model mice at 50 mg/kg. These findings suggest that intervenolin is an attractive lead for further structure–activity relationship studies for the development of anticancer agents with high clinical efficacy and safety profile.

In summary, we successfully achieved a practical synthesis of intervenolin by taking advantage of Suzuki–Miyaura coupling and rearrangement of thiocyanate to isothiocyanate to furnish the geranyl side chain and the iminodithiocarbonate moiety, respectively. The whole process could be performed on > 0.5 g scale, which allowed for in vivo study of the antitumor effects of intervenolin. More intensive biological studies of the antitumor effects toward different types of cancer, and the anti-*Helicobacter pylori* activities of intervenolin and its derivatives prepared by the present synthetic route will be reported in due course.

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Supporting Information Available. Characterization of new compounds, biological data, and experimental procedures. This material is available free of charge via the Internet at http://pubs.acs.org

The authors declare no competing financial interest.