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Natural product-based synthesis of novel anti-infective isothiocyanate- and isoselenocyanate-functionalized amphilectane diterpenes

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ABSTRACT

The marine natural product (−)-8,15-diisocyano-11(20)-amphilectene (**1**), isolated from the Caribbean sponge *Svenzea flava*, was used as scaffold to synthetize five new products, all of which were tested against laboratory strains of *Plasmodium falciparum* and *Mycobacterium tuberculosis* $H_{37}Rv$. The scaffold contains two isocyanide units that are amenable to chemical manipulation, enabling them to be elaborated into a small library of sulfur and selenium compounds. Although most of the analogs prepared were less potent than the parent compound, **5** was nearly equipotent showing IC_{50} values of 0.0066 μM and 0.0025 μM, respectively, against two strains (Dd2 and 3D7) of the malaria parasite. On the other hand, when assayed against the tuberculosis bacterium, analogs **5** and **6** were found to be more potent than **1**.

Tuberculosis and Malaria are two of the world's deadliest diseases, with more than 2 million deaths worldwide in 2013, most of them in sub-Saharan Africa, South-East Asia and Western Pacific regions.[1] *Plasmodium falciparum* has for some time been developing resistance against known antimalarial drugs, and therefore new drugs are urgently needed.[2] Chloroquine was the first drug produced on a large scale for treatment and prevention of malaria infection. Chloroquine has activity against the blood stages of *Plasmodium ovale*, *Plasmodium malariae*, and susceptible strains of *Plasmodium vivax* and *Plasmodium falciparum*.[3] Widespread resistance in most malaria-endemic countries has led to a continual decline in its use for the treatment of *P. falciparum*, although it remains effective for treatment of *P. ovale*, *P. malariae*, and, in most regions, *P. vivax*.[4]

Tuberculosis (TB) is second only to HIV/AIDS as the greatest killer worldwide due to a single infectious agent, *Mycobacterium tuberculosis* (*Mtb*).[5] Standard antimycobacterial drugs (isoniazid, rifampicin, pyrazidamide, ethambutol, streptomycin) have been used for decades, and resistance to the medicines is also widespread. If a patient is unable to tolerate isoniazid, or if isoniazid-resistant TB is present, rifampicin, ethambutol, and pyrazidamide are usually used for 18 months. If rifampicin-resistant TB is present, the regimen usually consists of isonizaid, ethambutol, and pyrazidamide for 18 months. If there is resistance to both isoniazid and rifampicin, the disease is very difficult to treat.[6] Disease strains that are resistant to a single anti-TB drug have been documented in every country surveyed. In some cases more severe drug resistance can develop. Extensively drug-resistant TB, XDR-TB, is a form of multi-drug resistant tuberculosis (MDR-TB) that responds to even fewer available medicines, including the most effective second-line anti-TB drugs. About 480,000 people developed MDR-TB in the world in 2013. More than half of these cases were in India, China and the Russian Federation. It is estimated that about 9.6% of MDR-TB cases had XDR-TB.[5,7] Hence, the search for new antitubercular drugs is a priority so as to overcome the problem of drug resistance and to finally eradicate TB.

The marine sponge metabolite (−)-8,15-diisocyano-11(20)-amphilectene (**1**) was first reported by Faulkner and co-workers from *Hymeniacidon amphilecta* in 1978, and has been shown subsequently to exhibit potent in vitro anti-infective activity.[8,9] Several structurally related natural products as well as a small number of synthetic analogs prepared from diisocyanide **1** also exhibit antimalarial and antimycobacterial potential.[10] Whilst comparison among their activities reveals that the biological activity is generally dependent on the presence of the isocyanide functionality, the

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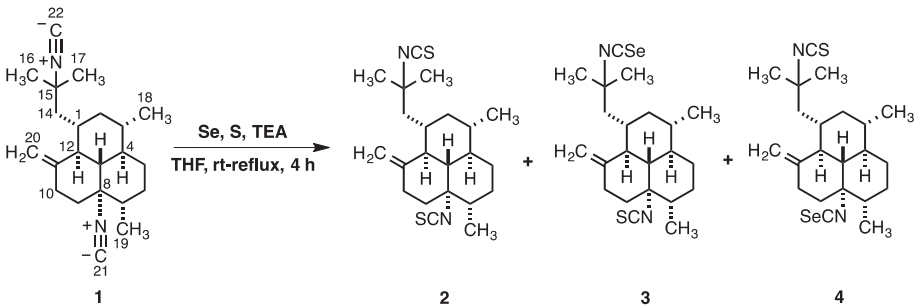
structural features of the carbon backbone and the location of the isocyanide groups also seem to play a pivotal role.[11] Notwithstanding, the observation that a plethora of sponge-derived isocyanide-, isothiocyanate-, isocyanate-, and formamide-containing diterpenoids based on amphilectane, cycloamphilectane, isocycloamphilectane, and isoneoamphilectane skeletons are often active (usually in the low nanomolar range), suggests that the biological activity does not depend strictly on the presence of the isocyanide functionality.[12] This observation implies that the metabolite's carbon skeleton can also modulate biological activity.

As part of our continued drug discovery program in search of new agents for the treatment of Malaria and Tuberculosis, we became interested in the synthesis of a limited number of amphilectane-based isothiocyanate and isoselenocyanate diterpenes for biological evaluation. Of the two classes of congeneric compounds, organic isoselenocyanates are of particular interest to us since so far they have received much less attention compared to their sulfur and oxygen analogs.[13] We targeted diisocyanide **1** as a suitable starting material, a well-known antimalarial and antimycobacterial pharmacophore accessible to us which contains both a rigid amphilectane skeleton and two isocyanide 'handles' with potential for further synthetic elaboration.[8] We anticipated that comparison among the biological activities exhibited by the strictly related amphilectane analogs with those of **1** would reveal definite structure–activity relationships. While the isothiocyanate moiety is found in many natural products only two isothiocyanate-containing amphilectane diterpenoids with antiplasmodial activity have been documented.[12a] Remarkably, no studies assessing the potential antiplasmodial or antimycobacterial properties of isoselenocyanate-containing compounds (synthetic or natural) have been reported so far.[14] In the present work, the syntheses of analogs **2**–**6** were swiftly accomplished through the isothio- and isoselenocyanation of metabolite **1**, previously isolated by us from the marine sponge *Svenzea flava*.[9] All compounds were characterized by detailed inspection of ^{1}H NMR, ^{13}C NMR, DEPT-NMR, 2D NMR (COSY, HSQC, HMBC, and NOESY), mass spectrometry, and UV and IR spectra. The purity of these compounds was ascertained by TLC, HPLC and spectroscopic analysis. All of the semisynthetic derivatives exhibited strong to potent in vitro inhibition of *Plasmodium falciparum* Dd2 and 3D7 strains with some exhibiting greater antiplasmodial activity than the standard drug chloroquine. Likewise, the new compounds have shown sub-micromolar to low micromolar in vitro antimycobacterial activity. In order to assess their microbe-specific selectivity (i.e., whether the observed antimicrobial activity was a specific or general toxic effect) the cytotoxic effects of compounds **1**–**6** using a mammalian Vero cell line were also investigated. The results obtained are further evidence of the anti-infective potential of these novel amphilectane-based chemotypes.

Since aliphatic isocyanides hardly react with elemental sulfur,[15] the desired diisothiocyanate **2** was synthesized via the isothiocyanation of **1** as outlined in Scheme 1. Thus, treatment of diisocyanide **1** with S, Et_3N, and catalytic amounts of Se in refluxing THF following a synthetic protocol previously described by Fujiwara and co-workers, afforded 8,15-diisothiocyano-11(20)-amphilectene (**2**) in 18% yield.[16,17] Surprisingly, the desired product was accompanied by large amounts of unreacted **1** along with smaller quantities of congeners **3** and **4** (53%), formed as a 2:3 mixture of regioisomers that was inseparable by chromatography (the integration of selected signals in the ^{1}H NMR spectra of the reaction products provided the isomer ratio). Addition of 2.5 mol % of S or increasing the refluxing time up to 16 h failed to afford full conversion to **2** or to preclude the formation of **3** and **4**. These results suggest that in this case the reaction might exhibit a low catalytic activity of Se (i.e., the rate determining step appears to be the reaction between **1** and elemental Se and not the Se–S exchange) and that perhaps the amount of Se catalyst to isocyanide should be increased to >10 mol % (vide infra).[18] Even though the reaction was very sluggish, we were delighted to have these compounds at hand since their biological evaluation was at this point of outmost interest to us. As the only differences between **3** an **4** were a result of the –NCS and –NCSe functionalities switching positions, these isomers have nearly identical ^{13}C NMR shifts, apart from those at C-8 and C-15 (and their substituents). Nevertheless, we were able to distinguish the terpene isothiocyanate groups from its isoselenocyanate counterparts in **3** (minor) and **4** (major) by the ^{13}C chemical shift of the –NCS (129–132 ppm) versus –NCSe (121–125) group. Although these signals are typically of low intensity in the ^{13}C NMR spectra (during 1D spectroscopic acquisition an extended delay time (>5 s) and a 90° pulse angle are usually required to enhance their intensity) their detection was easily accomplished with a 700 MHz spectrometer. These noticeable differences in ^{13}C NMR spectroscopic data, in combination with 2D NMR experiments (HSQC and HMBC spectra), allowed us to assign the structure of each isomer unambiguously.

Concomitant with these efforts, we sought to achieve the isoselenocyanation of diisocyanide **1** with elemental selenium in the presence of TEA to give 8,15-diisoselenocyano-11(20)-amphilectene (**5**) in satisfactory yield.[19] The synthesis and biological evaluation of **5** was very appealing to us since natural products bearing the isoselenocyanate moiety have never been isolated.[20] Furthermore, synthetic isoselenocyanate-containing compounds apparently have never been investigated for potential antiplasmodial or antimycobacterial activity.[21] Thus, insertion of two selenium atom equivalents at C-21 and C-22 of diisocyanide **1** via an isoselenocyanation reaction with Se using TEA in THF at 25 °C led cleanly to **5** (78% yield). Gratifyingly, when the reaction was conducted in refluxing THF diisoselenocyanate **5** (obtained in 50% yield) was accompanied by lesser quantities of isoselenocyanate **6** as a single regioisomer following purification by flash- and HPLC chromatography. In this fashion, the reaction proceeded with selective base-mediated decomposition of **5** at the more reactive



Scheme 1. Synthesis of isothiocyanate analogs **2**–**4**.