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Structure–activity relationships of antitubercular scalaranes: Heteronemin revisited*

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Abstract: A series of heteronemin-related antitubercular scalaranes, both from natural products and from chemical derivatization, were subjected to structure–activity investigations. Based on the activity profile, three main regions; i.e., the substituted groups hovering over C-19/C-18 and furan moiety, the functionalities in the vicinity of C-16 and the right-hand side of ring D, and the substituted groups on C-12, were speculated as the areas influencing the antitubercular activity of the scalaranes. The results suggested the promising possibility for the further investigation toward the modes of actions and/or target sites of the compounds.

Keywords: SARs; antitubercular; scalaranes; heteronemin; *Hyrtios*.

INTRODUCTION

Tetracarbocyclic sesterterpenes of the scalarane family are rare natural products found exclusively in marine invertebrates, distributing mainly among the Dyctioceratid sponges, especially those of the genera *Hyrtios* [1], *Hyatella* [2], *Phyllospongia* [3], *Smenospongia* [4], and *Spongia* [5]. Certain derivatives were also found in a nudibranch species, presumably via dietary accumulation [6]. Of particular interest among the scalaranes in this report is heteronemin (**1**). The compound was first isolated from the sponge *Heteronema erecta* [7], and later along with its several sesterterpene analogs from various sponge species, including those mentioned above.

Heteronemin was reported to be biologically active in various bioassay models, including cytotoxicity [1b–d,6], protein function inhibition [1e], and antitubercular activity [8]. Specifically for the latter, **1** was first reported active against *Mycobacterium tuberculosis* $H_{37}Rv$ with a minimum inhibitory concentration (MIC) of 6.25 μg/ml and an IC_{50} of 1.3 μg/ml. Despite the promising antitubercular activity, **1** and its related scalarane congeners have never been investigated extensively for the further application with regard to their biological activities. This is attributed to the cytotoxicity of **1** itself, of

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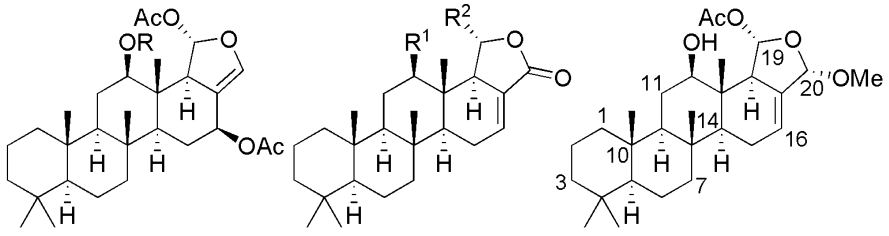
which the IC_{50}s were reported to be at least 10-fold more potent than that of its antitubercular activity. For examples, in our previous report [9], **1** was found to be cytotoxic with the IC_{50}s in a range of 0.2–0.5 μM against a panel of cancer cell lines (MCF-7, HeLa, HT-29, and KB). It has been suggested, however, that certain chemical derivatization and/or microorganism-assisted structural transformation may improve the antitubercular activity and lower the cytotoxicity of the compounds [8].

Recently, we reported the isolation of a series of scalarane-type sesterterpenes from the Thai sponge *Brachiaster* sp. The isolated sesterterpenes included **1** (as a major component), heteronemin acetate (**2**), 12-deacetyl-12-epi-19-deoxyscalarin (**3**), 12-epi-19-deoxyscalarin (**4**), and 12-deacetoxy-scalarin acetate (**5**) [9]. The biological activities of the isolated compounds were assessed to show the antitubercular activity with the MICs in a range of 10^0–10^2 μM, and the cytotoxicity against various cancer cell lines in a wider range of the IC_{50} from 0.2 μM to virtually inactive (IC_{50}s exceeding 100 fold of highest experimental concentration). Even with limited items of compounds tested, our results clearly indicated that slight changes in certain functionalities exert considerable influences on the potency of the antitubercular activities, as well as on the selectivity between the antitubercular and cytotoxic activities. Our findings also agreed well with the early suggestion made by Crews and Bescansa [1a] that, as for the biologically active scalaranes, the oxygenated functionalities in the vicinity of the furan moiety of the scalaranes, i.e., the functional groups surrounding C-19 and C-20, may contribute to the biological activities, especially those related to the chemotherapeutic ones. A wider variation in the chemical structures of the heteronemin-based scalaranes, therefore, is expected to extend the activity profile, leading to a clearer view for the structure–activity relationship (SAR) of the scalarane scaffold.



1; R = H
2; R = Ac

3; R^1 = OH, R^2 = H
4; R^1 = OAc, R^2 = H
5; R^1 = H, R^2 = OAc

6

SCALARANE DERIVATIVES

In order to extend the chemical profile of the scalaranes, our investigation relied on both sources of the compounds, i.e., the natural scalaranes obtained from the sponge collected in the wild and the chemical derivatization of the scalaranes. The chemical diversity from nature combined with a directed approach in chemical manipulation is expected to be advantageous in the variety both of chemical structures and of biological activities of compounds to be tested.

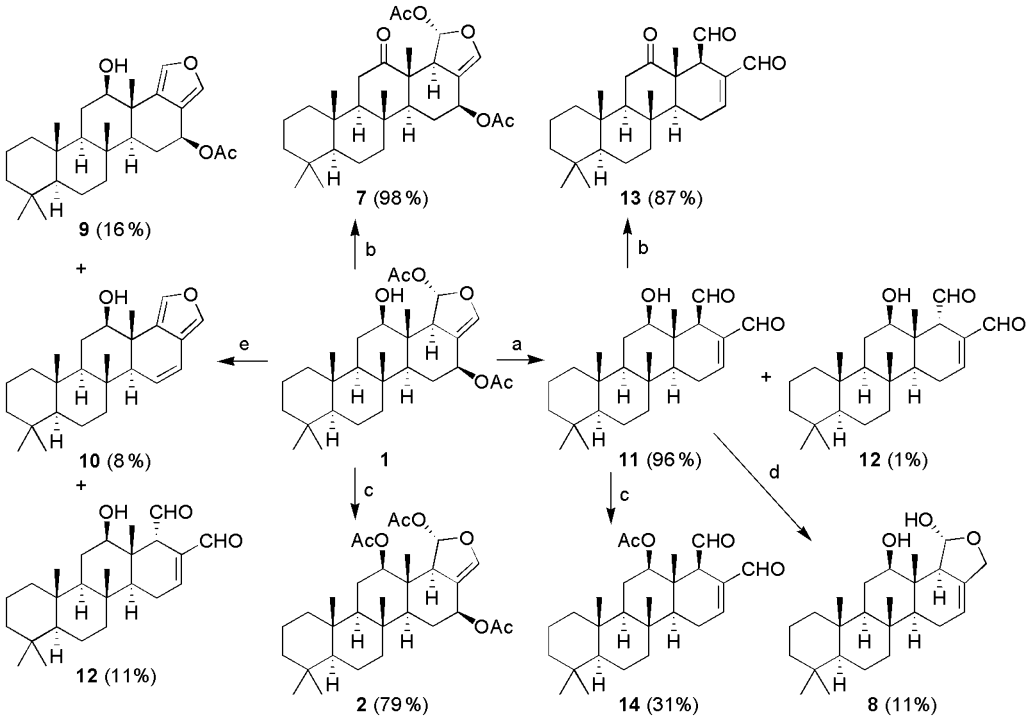
A minor scalarane derivative

Along with the isolation of **1** from the Thai sponge *Hyrtios* sp. collected from Koh-Tao, Surat-Thani Province, a minor scalarane derivative (**6**) was isolated from the hexane extract [10]. The structure determination of **6** was carried out by means of spectroscopic analysis to show that the compound, designated as 12-epideacetyl-19α-acetoxy-20α-methoxyscalaran [11], was a new member of the tetracarbocyclic sesterterpenes, possessing an attached dihydrofuran moiety with two acid-labile acetal carbons.

Such acetal functionalities were presumably responsible for the irreversible decomposition of **6** upon prolonged standing in $CDCl_3$. The relative configuration of **6** was determined with the aid of a series of nOe-ds experiments; however, we were unable to perform the more extended experiments necessary to determine the absolute configuration. The depicted configuration is thus based upon analogy with the known structures of other naturally occurring scalaranes isolated from the same sponge specimen.

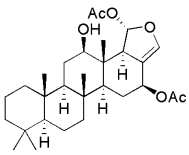
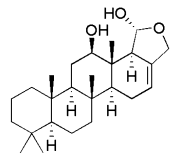
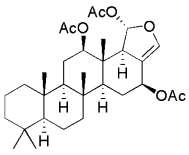
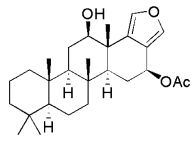
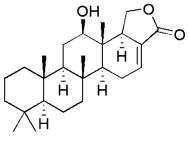
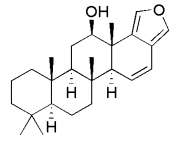
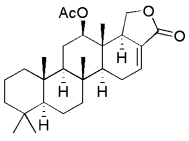
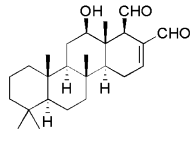
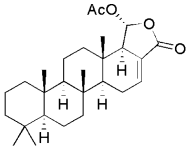
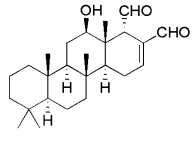
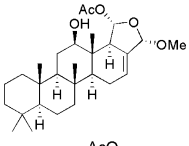
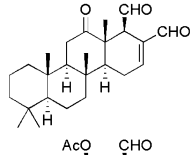
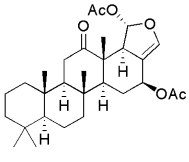
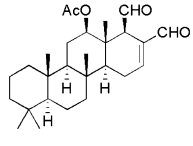
Derivatizations of the scalaranes

Similar to several previous reports, we were able to obtain **1** as a major component from the sponge *Hyrtios* sp. [10] in a very good yield (isolated yield 1.6 g from 101 g of freeze-dried sponge). This opened an opportunity to us to employ **1** as the primary starting material for the chemical derivatizations of the scalarane derivatives (Scheme 1). The scalaranes obtained here included 12-oxoheteronemin (**7**); 12-deacetyl-12-epi-20-deoxoscalarin (**8**); scalarafuran (**9**); 16-deacetoxy-15,16-dehydroscalarafuran (**10**); 12-deacetyl-12-episcalaradial (**11**); 12-deacetyl-12,18-diepiscalaradial (**12**); 12-deacetyl-12-oxoscalaradial (**13**); and 12-episcalaradial (**14**). Please note here that, in order to facilitate the discussion regarding the SARs, the order of the compound entries mentioned above was referred to the structural similarity as tabulated in Table 1.



Scheme 1 Condition; (a) $BF_3.OEt_2$, wet MeCN, 0 ºC; (b) PCC/SiO_2, CH_2Cl_2, rt; (c) Ac_2O, pyridine, rt; (d) LAH, THF, Δ; (e) neat, 220 ºC.

Table 1 Antitubercular and cytotoxic activities of **1–14**.

	Entry	Anti-TB[a] (MIC; μM)	Cytotoxicity[b] (IC_{50}; μM)		Entry	Anti-TB[a] (MIC; μM)	Cytotoxicity[b] (IC_{50}; μM)
1		3	5.25	**8**		257	inactive[c]
2		3	16.09	**9**		14	inactive[c]
3		16	NA	**10**		135	inactive[c]
4		117	NA	**11**		64	inactive[c]
5		4	NA	**12**		8	inactive[c]
6		54	inactive[c]	**13**		130	inactive[c]
7		0.23	0.91	**14**		58	1.26

[a]Referred to as isoniazid, rifampicin, and kanamycin; MICs 0.02, 0.04, and 2.58 μM, respectively.
[b]Referred to camptothecin; IC_{50} 0.45 μM.
[c]Cell viability >80 % at the highest concentration of 5 μg/ml.

Naturally, our first attempts for the structural modification were to remove the acetate functionalities, both from C-16 and from C-19. To our surprise and disappointment, **1** was too labile to most standard transformations for such acetate removal. These included saponification (methanolysis with NaOH or K_2CO_3) and transesterification (with NaCN). In addition, in most attempts, the degradation of **1** proceeded so destructively that neither starting materials nor any possible side products were recoverable. It turned out that the acetate removal was achieved in a rather acidic condition ($BF_3{\cdot}OEt_2$ in wet, cold MeCN) [12], nevertheless with a complete hydrolytic cleavage of the dihydrofuran moiety, to yield the dialdehyde **11** [1a] as a major product. The reaction generally proceeded cleanly and swiftly

within less than 5 min. Prolonged exposure to such acidic medium, as expected, led to the epimerization at C-18, yielding **12** [13] in a trace amount.

With both **1** and **11** in hand, the two compounds were conveniently used for a series of simple transformations in a paralleled manner. Chromate oxidation at 12-OH yielded the oxo derivatives **7** [7a] and **13** [13], and acetylation on the same hydroxyl group did the 12-acetate derivatives **2** and **14** [1a], respectively. It is noteworthy to mention here that, whereas the above transformations progressed smoothly with reasonable yields, O-methylations (either with NaH, with amine bases, or with Ag_2O, all followed by MeI) onto C-12 of both **1** and **11** were too slow. This presumably resulted from the steric hindrance surrounding C-12, providing an environment similar to that of a neopentyl system. On the other hand, with LAH reduction, **1**, again, was too labile, whereas **11** did proceed sluggishly under reflux [14], and instead of the expected triol, a recyclized tetrahydrofuranol **8** [6] was obtained only in a low yield.

Manipulation of **1** toward the furan functionality was carried out simply via pyrolytic cleavage. Brief exposure of **1** to a non-oxidative pyrolytic condition resulted in rapid decomposition, and three major products, **9** [7a], **10** [7a], and **12**, were isolated.

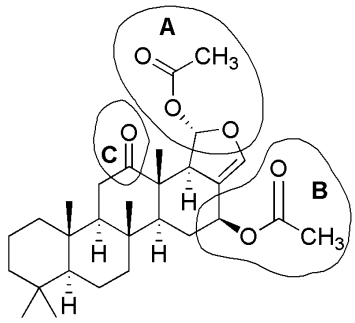
ANTITUBERCULAR AND CYTOTOXIC ACTIVITIES OF THE SCALARANES

The antitubercular activity of all the scalaranes as mentioned in the above sections was determined according to the microplate alamar blue assay protocol [15], targeting on nonvirulent strain of *M. tuberculosis* $H_{37}Ra$. The magnitudes of the potency ranged from 10^{-1} to 10^{2} μM, with **7** exhibiting the most potent antitubercular activity (Table 1). Also included in Table 1 were the MICs of the antitubercular activity of **3–5**, all of which were acquired from our previous report [9]. The potency can be confidently compared for all were determined in the same laboratory (Central Bioassay Lab, BIOTEC, Thailand). For the cytotoxicity, the toxicity against a human normal cell line (human oral fibroblasts) is emphasized here, the assay protocol was referred to the SRB microplate assay [16]. With the exception of **3–5**, which were no longer available at the time of bioassay, the cytotoxicity of all the compounds was assessed to show an even wider range in potency, from strongly toxic (e.g., **7**) to virtually inactive (Table 1).

STRUCTURE–ACTIVITY RELATIONSHIP

As mentioned earlier, it had been postulated that the oxygenated functionalities surrounding the furan/furanol moiety were responsible to the biological activities of the scalaranes, particularly those related to the chemotherapeutic ones [1a,8]. This has been confirmed here as seen in Table 1. In addition, certain features regarding the SAR of the scalaranes, extrapolated from the above postulation, are proposed below.

The SAR of the scalaranes discussed here is based on the biological activities, both antitubercular and cytotoxic ones, of 14 compounds shown in Table 1. With reference to the structure of **7**, which represents the most active scalarane in this series, it is proposed that three main regions (Fig. 1) appear to be implicated in the antitubercular activity. The most influential region lies in area **A**, in which a 19α-acetoxy group, representing either an enlarged and steric functionality or an H-bond acceptor, exerts a strong positive influence on the antitubercular activity as seen in **7**, **1**, **2**, and **5**, respectively. In addition, the conformer that can provide a precise orientation of such functionalities may play an important role. This can be seen in **12**, despite lacking such acetoxy group, yet exhibiting strong activity presumably due to the α orientation of the formyl group on C-18. On the contrary, **6**, whose less rigid furanol moiety could possibly distort the 19α-OAc from its ideal binding site, is the least active of those scalaranes possessing a 19α-OAc group. A similar effect can be seen in area **B**, of which the 16β-OAc is also found positively related to the antitubercular activity. The influence could be synergistic between



7

Fig. 1 Regions affecting SAR of **7**.

the 19α- and the 16β-OAcs, for **7**, **1**, and **2** are the three most active congeners; however, with either one substitution alone (e.g., only one acetoxy group on 19α- as in **5**; and on 16β- as in **9**), the effect from either OAcs is still adequately pronounced.

The influences within area **C**, on the other hand, were rather inconclusive. The early observations suggested that, to a certain extent, the electrostatic field within this region may affect the activity. Comparing the antitubercular activity among compounds with similar structural features, i.e., the sets of **1**, **2**, and **7**; of **3** and **4**; and of **11**, **13**, and **14**, the relatively inconsistent trends within each set, however, preclude a meaningful empirical inference about the influence of 12-substituents upon SARs. This may indicate that such functionality is either less significant, or that the nature of any electrostatic influences may be diffuse or less predictable, owing to the limited number of available structural variants of scalaranes upon which to draw conclusions.

As for the cytotoxicity against a normal cell line, the negative results from more than half of the tested compounds suggested the potentials of the scalaranes, and encouraged the further extensive investigation both on the modes and mechanisms of actions, and on the structural modification of its core chemical structures for improved activities. The paralleled tendency between the antitubercular activity and the cytotoxicity among the four active samples was disappointing, suggesting that along with its necessity for the antitubercular activity, the 19-OAc group also contributes to the toxicity of the compounds. Nonetheless, with only limited number of active compounds tested, a better conclusive remark regarding the functional groups affecting the cytotoxicity is yet to be made.

CONCLUSION

A profile of antitubercular and cytotoxic activities of 14 heteronemin-related sesterterpenes led to a tentative conclusion of the SAR of the bioactive scalaranes. The positive influence from the 19α-OAc and/or 18α-CHO moieties suggested the ideal binding sites that required either steric functionalities or H-bond acceptors crowning the scalarane skeleton and slightly leaning toward the α-side of the carbocyclic plane. A similar effect from the OAc group was seen from the right-hand side of the structure, of which a 16β-OAc moiety also exerted a positive effect on the antitubercular activity. Our preliminary studies on the 2-D and 3-D QSAR seem to agree well with such suggestion. However, with a limited number of samples, other possible pharmacophores, e.g., C-12 substituted groups, and the SAR on the cytotoxicity, remain ambiguous. The expansive search for a large variety of the scalarane derivatives is now on the way, and will lead to a more complete understanding of the SAR of the antitubercular scalaranes.

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10. The sponge was identified by Dr. Somchai Bussarawit of Phuket Marine Biology Center. The voucher specimen (PMBC 24608) is deposited at Phuket Marine Biology Center, Phuket, Thailand.
11. The sponge was extracted in MeOH, and the crude MeOH-extract was partitioned to yield a hexane-extract, which was subjected to a series of chromatographic separation (SiO_2, hexane:EtOAc:acetone 7:2:1; then SiO_2, gradient hexane:EtOAc 7:3 – 1:1) to yield **6** (5 mg). 12-epi-deacetyl-19α-acetoxy-20α-methoxyscalaran (**6**). White solid; $[\alpha]_D$ +7.1 (*c* 0.21, $CHCl_3$); UV (MeOH) λ_{max} (log ε) 208 (4.47), 218 (4.45) nm; IR (neat) ν_{max} 3444, 1734 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 6.45 (d, *J* = 3.0 Hz; H-19), 5.79 (dd, *J* = 6.5, 3.0 Hz; H-16), 5.09 (s; H-20), 3.38 (d, *J* = 12.0, 4.5 Hz; H-12), 3.32 (s, 3H; 20-OC*H*$_3$), 2.81 (t, *J* = 3.0 Hz; H-18), 2.08 (m; H-15a), 2.04 (s, 3H; 19-OCOC*H*$_3$), 1.95 (m; H-15b), 1.64 (m; H-11a), 1.56 (m; H-1a), 1.51 (m; H-7a), 1.45 (m; H-2a), 1.40 (m; H-6a), 1.33 (m; H-11b), 1.29 (m,; H-2b), 1.26 (m; H-6b), 1.24 (m; H-3a), 1.15 (dd, *J* = 11.0, 5.5 Hz; H-14), 1.07 (td, *J* = 13.0, 3.5 Hz; H-3b), 0.86 (m; H-9), 0.85 (m; H-7b), 0.83 (s, 3H; H-24), 0.81 (m; H-1b), 0.78 (s, 6H; H-21 & H-23), 0.74 (s, 3H; H-22), 0.72 (dd, *J* = 14.0, 2.0 Hz; H-5), 0.69 (s, 3H; H-26); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.0 (19-O*C*OCH$_3$), 135.8 (C-17), 121.8 (C-16), 104.6 (C-20), 99.6 (C-19), 80.5 (C-12), 58.6 (C-9), 56.8 (C-18), 56.4 (C-5), 54.6 (20-O*C*H$_3$), 53.1 (C-14), 42.0 (C-7), 41.3 (C-3), 39.9 (C-1), 39.2 (C-13), 37.5 (C-10), 37.3 (C-8), 33.2 (C-21), 33.3 (C-4), 27.6 (C-11), 22.4 (C-15), 21.3 (C-22),

21.3 (19-OCO*C*H_3), 18.5 (C-6), 18.0 (C-2), 17.3 (C-24), 16.5 (C-23), 8.8 (C-25); HR-ESIMS *m/z* $[M+Na]^+$ 483.3071 (calcd for $C_{28}H_{44}O_5Na$ 483.3087).

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