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Synthesis and biological evaluation of simplified mycothiazole analogues

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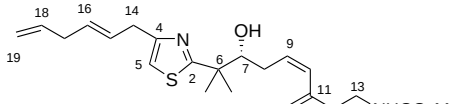
Abstract—Thiazoline and oxazoline analogues of the natural product mycothiazole were synthesized from a common intermediate and evaluated in vitro against HCT-15 colon cancer cells and L_4 larvae of nematode *Nippostrongylus brasiliensis*. The nature of the heterocyclic moiety seems to modulate the cytotoxic or anthelmintic activity.

The natural product mycothiazole (**1**) is a marine secondary metabolite isolated from *Spongia mycofijiensis*[1a] and *Dactylospongia*.[1b] This compound has in vitro anthelmintic activity against *Nippostrongylus brasiliensis*[1a] as well as toxicity throughout the 60 NCI human tumor cell line panel.[2]



(-)-Mycothiazole (**1**)

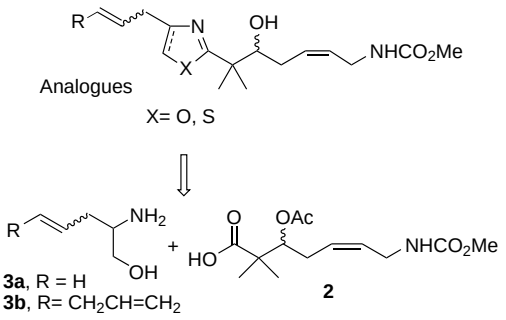
Mycothiazole was first synthesized by Shioiri and coworkers[3a] who later presented the assignation of the chiral carbon present at the molecule in 2003. They reported that the synthetically pure material is rather labile as well as the natural product.[3b] Recently, a new formal approach has been published by Cossy and coworkers.[3c]

The use of marine natural products as a target for the development of new bioactive compounds is broadly described in the literature.[4]

Within the context of synthesizing new bioactive compounds based on natural products, we were interested in preparing stable and simplified analogues of mycothiazole in order to evaluate their biological activities as cytotoxic and anthelmintic.[5]

For the design of a series of first-generation analogues, we considered to evaluate the absence of the conjugated *Z*-diene moiety at the C_2 side chain of mycothiazole in order to increase the stability and the substitution of the thiazole ring by related heterocycles like thiazoline and oxazoline (Scheme 1).[6]

Access to the analogues can be envisioned by the coupling of aminoalcohols like **3** and alkenyl acid **2** via a



Scheme 1. Retrosynthetic analogue approach.

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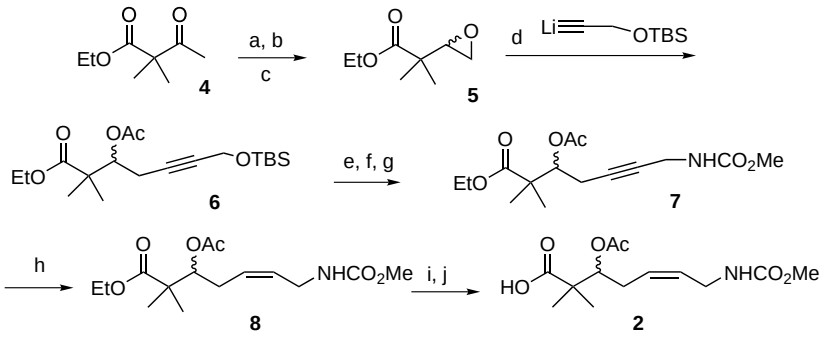


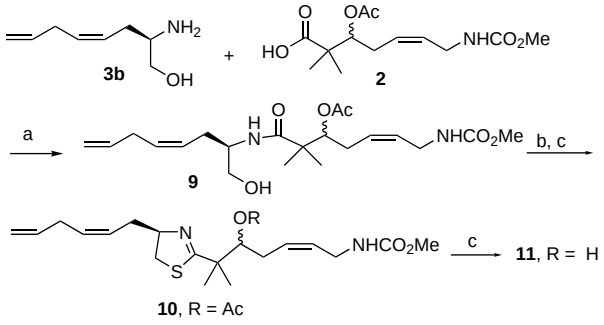
Scheme 2. Synthesis of acid **2**. Reagents and conditions: (a) Br_2, AcOH, 55 °C, 22 h, 88%; (b) $NaBH_4$, MeOH, 2 h; (c) K_2CO_3, 2 h, 82%; (d) $BF_3{\cdot}Et_2O$, THF, −80 °C; Ac_2O, Py, 1 h, 65%; (e) TBAF 1 M, 2 h, 96%; (f) CCl_4, Ph_3P, PhH, reflux, 6 h, 97%; (g) KOCN, MeOH, DMF, 100 °C, 1 h, 65%; (h) H_2, Lindlar, AcOEt, rt, 1 h, 70%; (i) LiOH, THF/MeOH/H_2O, rt, 1 h; (j)Ac_2O, Py, rt, 12 h, 70%.

cyclodehydration and oxidation sequence. This methodology allows the preparation of different types of heterocycles from the same intermediates.

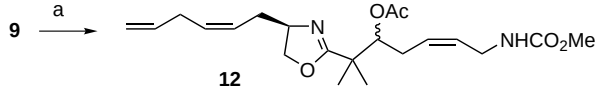
Aminoalcohol **3b** was synthesized using the methodology developed in our group by a Wittig coupling of homoallyltriphenylphosphonium bromide with the homologated Garner's aldehyde.[5a]

Acid **2** was prepared as depicted in Scheme 2. The sequence started with the bromination of ethyl 2,2-dimethylacetoacetate **4** using Br_2 in acetic acid at 55 °C to afford the corresponding bromoketone (88%). This intermediate was converted into the epoxide **5** via a bromohydrine using $NaBH_4$ in basic media (82%). Alkylation of epoxide **5** was carried out using lithio–propargylic–OTBS and $BF_3{\cdot}OEt_2$ as a catalyst. A solution of lithio acetylide (1 equiv) was added dropwise to a solution of epoxide **5** (1 equiv) and $BF_3{\cdot}OEt_2$ (1 equiv) in THF at −80 °C. Using this procedure, we obtained only the desired alkynyl compound **6** (65% yield). Interestingly, the protocol described by Yamaguchi and Hirao (1.5 equiv of the acetylide and $BF_3{\cdot}OEt_2$)[7] provided compound **6** (50%) along with the product resulting from dialkylation at the epoxide and at the ester group (10%). The synthesis of carbamate **7** was achieved by conversion of the OTBS-group into chloride followed by reaction with potassium cyanate in MeOH. This allows the direct substitution of the chloride by the carbamate group via an isocyanate to afford compound **7** (65% yield).[8] Hydrogenation of alkynyl compound **7** using Lindlar catalyst afforded the corresponding *Z*-alkene **8** (70% yield). Acid **2** was obtained by hydrolysis of compound **8** with LiOH followed by acetylation of the secondary alcohol (70% yield).

With the two fragments in hand, the target compounds were assembled using our planned strategy: segment condensation and cyclodehydration to form the heterocycle core. The thiazoline analogue was synthesized using the sequence shown in Scheme 3. The coupling of acid **2** and aminoalcohol **3b** with EDCI (1-(3-dimethylamino-propyl)-3-ethyl-carbodiimide hydrochloride) provided the amide **9** in 60% yield. Protection of compound **9** followed by treatment with Lawesson's reagent provided thiazoline **10** (40% yield). The formation of **10**



Scheme 3. Synthesis of thiazoline analogues **10** and **11**. Reagents and conditions: (a) EDCl, CH_2Cl_2, 0 °C to rt, 6 h, 60%; (b) TBSCl, Et_3N, CH_2Cl_2, 100%; (c) Lawesson's reagent, PhH, reflux, 40%; (d) LiOH·H_2O, THF/H_2O/MeOH, 100%.



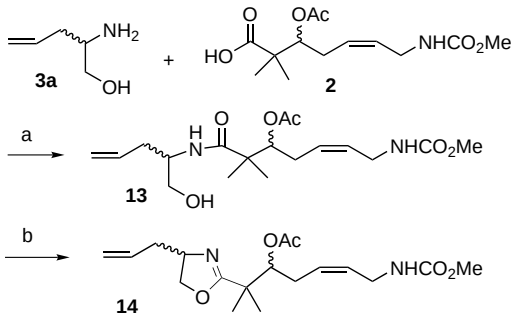
Scheme 4. Synthesis of oxazoline **12**. Reagents and conditions: (a) Deoxo-Fluor, CH_2Cl_2, −20 °C, 1 h, 60%.

can be explained by spontaneous cyclodehydration of the β-hydroxythioamide. This type of reaction was not expected, because usually β-hydroxythioamide cyclodehydration needs an activating reagent to give the thiazoline, even though we found one precedent in the literature.[9] Deprotection of compound **10** with LiOH led to the analogue **11** (100% yield).

The first oxazoline analogue, **12**, was prepared from amide **9**, using Deoxo-Fluor cyclodehydrative reagent, in a clean fashion (60%), see Scheme 4.[10]

Analogue **14** contains an oxazoline moiety, while the C_{17}–C_{19} side chain present in mycothiazole is absent. The product was prepared using the same strategy used for compound **12** (Scheme 5). Starting with 2-amino-pent-4-en-1-ol (**3a**),[11] oxazoline **14** was obtained as a diasteromeric mixture in 70% yield.

Attempts to aromatize the compounds **10**, **12**, and **14**, using MnO_2 or NiO_2, were unsuccessful, and the current strategy has not granted the access to analogues of



Scheme 5. Synthesis of oxazoline **14**. Reagents and conditions: (a) EDCl, CH_2Cl_2, 0 °C to rt, 6 h, 50%; (b) Deoxo-Fluor, CH_2Cl_2, −20 °C, 1 h, 70%.

Table 1. Cytotoxicity and anthelmintic assay results for compounds **10**, **11**, **12**, and **14**

Entry	Compound	*N. brasiliensis* LC_{50} (μM)	Cytotoxicity HCT-15 IG_{50} (μM)
1	Albendazole	0.34	—
2	Mytomycin C	—	1.6
3	**10**	>300	nt[a]
4	**11**	nt[a]	13
5	**12**	40	150
6	**14**	40	430
7	Mycothiazole	120[b]	44[c]

[a] nt = not tested.
[b] EC_{100} reported by Crews et al.[1a]
[c] NCI database (NSC 647640).[2]

mycothiazole containing an aromatic heterocyclic moiety.[12]

The analogues were evaluated against HCT-15 cells (colon cancer) using sulfurhodamine B (SRB)-assay and mytomycin C as standard.[13] The anthelmintic assay was performed on the L_4 larvae of the nematode *N. brasiliensis* using albendazole as a standard according with the described protocol.[14]

While the overall activities are moderate, there are some differences among the assayed compounds (Table 1). Thiazoline analogue **11** is the most active on HCT-15 cells showing the same cytotoxicity as mycothiazole. The acetylated thiazoline **10** shows poor anthelmintic activity.

Oxazoline containing compounds **12** and **14** show similar behaviors, both being better as anthelmintic than cytotoxic on a HCT-15 cell line. The anthelmintic activity of oxazoline **12** is better than that of its parent thiazoline **10**. The nature of the heterocyclic moiety appears to play a role in determining the activity of these compounds.

In conclusion, we prepared the first series of simplified and stable thiazoline and oxazoline analogues to mycothiazole. The different cytotoxic properties resulting from their biological evaluation will serve as the basis for further design of structurally modified and potentially bioselective new analogues.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2005.11.072.

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