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Calmodulin inhibitory activity of the malbrancheamides and various analogs

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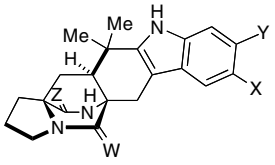
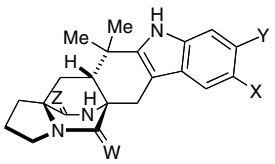
ABSTRACT

The preparation and biological activity of various structural analogs of the malbrancheamides are disclosed. The impact of indole chlorination, C-12a relative stereochemistry, and bicyclo[2.2.2]diazaoctane core oxidation state on the ability of these analogs to inhibit calmodulin dependent phosphodiesterase (PDE1) was studied, and a number of potent compounds were identified.



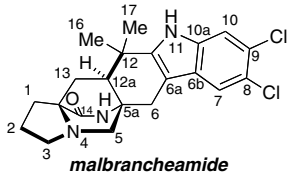
X = Y = Cl, W = Z = O: IC_{50} = 18.57 μM



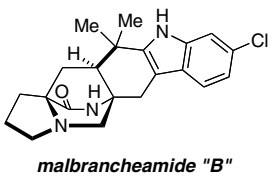
X, Y = Cl; Z = H_2; W = O: IC_{50} = 11.95 μM

The synthesis, biosynthetic investigations, and biological activities of a number of unique prenylated indole alkaloids containing a characteristic bicyclo[2.2.2]diazaoctane core has been the focus of vigorous research in our laboratory[1] and that of other research groups[2] for a number of years. This class of highly biologically active fungal metabolites includes the paraherquamides,[3] brevianamides,[4] notamides,[5] stephacidins,[6] and malbrancheamides[7] (Fig. 1) among others, and the range of biological activities exhibited by a number of representative members of this class is quite striking. We have previously disclosed that malbrancheamide is a calmodulin (CaM) antagonist that inhibits the activity of CaM-dependent phosphodiesterase (PDE1) in a concentration-dependent manner.[7]

Calmodulin is an important drug target for the development of naturally and synthetic therapeutically useful agents due to its involvement in a variety of cell functions throughout the regulation of more than 50 enzymes and ion channels. Such proteins include several kinases, PDE1, calcineurin, the nitric oxide synthases, adenylate cyclases 1 and 8, several ion channels, caldesmon, spectrin, and adducin, among others. Indeed, certain anti-psychotic, anti-tumoral, smooth muscle relaxants, α-adrenergic blocking, immunostimulant and cytoprotective drugs exert their therapeutic action by inhibiting CaM.[8]



malbrancheamide



malbrancheamide "B"

Figure 1. Structures of malbrancheamide and malbrancheamide B.

The bicyclo[2.2.2]diazaoctane core common to these natural products is proposed to arise biosynthetically by an intramolecular hetero-Diels-Alder reaction of a 5-hydroxypyrazin-2(1*H*)-one,[2] and work from this laboratory has provided a provocative body of experimental evidence to support such a hypothesis.[1] Indeed, we have applied biomimetic hetero-Diels-Alder cycloaddition strategies to prepare several prenylated indole alkaloids, including stephacidin A,[9] brevianamide B,[10] marcfortine C,[11] notoamide B,[9b] VM55599,[12] and most recently, malbrancheamide and malbrancheamide B.[13] Due to the significant biological activity of both malbrancheamide and malbrancheamide B, a program aimed at determining the effect of various structural features of the malbrancheamides on their biological activities was initiated.

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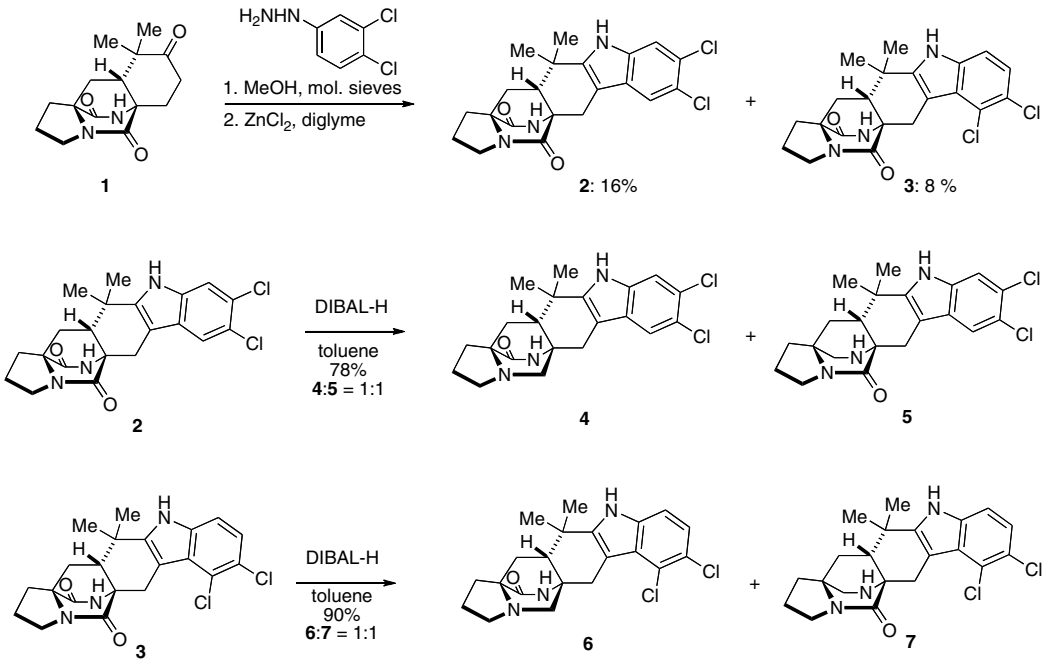


Scheme 1. Fischer indole synthesis.

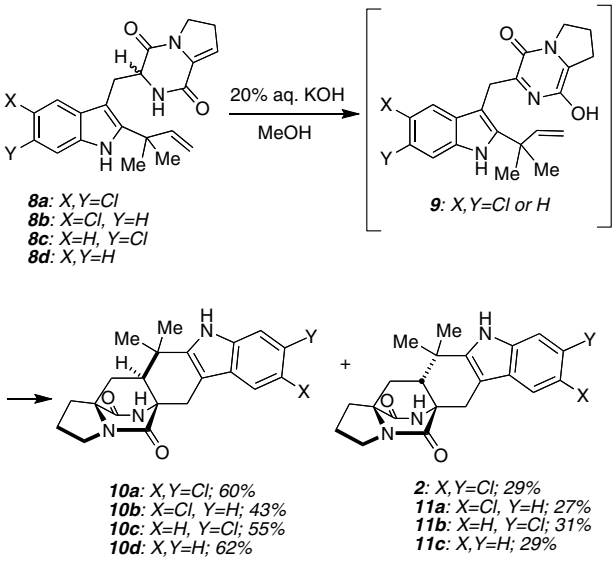
Specifically, we endeavored to explore how indole chlorination, relative stereochemistry, and bicyclo[2.2.2]diazaoctane core oxidation state altered the ability of these analogs to inhibit CaM throughout the analysis of their effect on PDE1 activity. We chose these structural parameters since the malbrancheamides were the first members of this family of alkaloids to be identified with a halogenated indole ring. The brevity and high overall yield of our synthetic approach to these substances has allowed us to readily access a number of malbrancheamide analogs with the desired structural and stereochemical variability.

A Fischer indole strategy was first devised to prepare a number of malbrancheamide analogs containing the unnatural relative stereochemistry at C-12a.[14] Ketone **1** was condensed with 3,4-dichlorophenylhydrazine followed by Fischer indole synthesis to give a mixture of regioisomers **2** and **3** in modest yield (Scheme 1). Treatment of **2** or **3** with DIBAL-H led to a 1:1 mixture of the reduced tertiary lactam, **4** or **5**, respectively, and the reduced secondary lactam, **6** or **7**, respectively, in good yield. While this route proved useful in generating an array of structural diversity, the low yield of the Fischer indole sequence prompted investigation of other synthetic strategies.

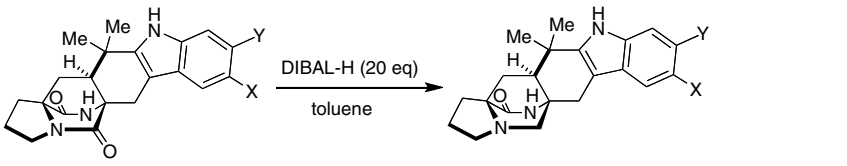
A more efficient synthetic strategy involved a biomimetic hetero-Diels-Alder reaction as the key step (Scheme 2).[13] Enamides **8a**–**8d** could be easily prepared,[13] and treatment with basic methanol resulted in tautomerization followed by cycloaddition to give a mixture of diastereomeric cycloadducts **10a**–**d** as well as **2** and **11a**–**c** with the *syn*-products **10a**–**d** predominating. The ready availability of the Diels-Alder precursors **8a**–**d** allowed the preparation of analogs **10a**–**c**, **2**, and **11a**–**d** with a variety of chlorination substitution patterns.

Next, the effect of the bicyclo[2.2.2]diazaoctane core oxidation state was interrogated. Thus, treatment of and **10a**–**d** with DIBAL-H led to selective reduction of the tertiary amide functionality to provide the tertiary amines **12**–**15** in good yields (Scheme 3).

Anti-CaM activity was measured as previously described by an enzymatic functional assay using PDE1 as monitoring enzyme,[15] and the data are summarized in Table 1. Upon examination, some surprising results are apparent. Indole chlorine substitution does have a distinct effect on PDE1 activity, however an overall structure–activity relationship with regard to chlorine substitution is mostly absent. For example, the *syn*-dioxopiperazines **10a**–**d** exhibit potencies from 0.9 to 0.1, with the dichloro substituted species **10a** displaying the highest potency (0.9) and the monochloro substituted analogs **10b** and **10c** displaying roughly equivalent potencies (0.4 and 0.2, respectively). Compound **10d**, which lacks chlorine substitution, was virtually inactive. Comparison of these results with the potencies of the *anti*-dioxopiperazines **2**, **3**, and **11a**–**c** revealed that compound **11c**, which completely lacks chlorine substitution, was the most active substance of this group studied, with a potency 0.7 times that of the positive control. The analogs **11a**, **11b**, **2**, and **3** exhibited moderate potencies. Among the monooxopiperazines **4**–**7** and **12**–**15**, synthetic D,L-malbrancheamide (**12**) and **5** were the most potent whereas C-12a-*epi*-malbrancheamides (**4** and **6**) were the least potent. It is particularly



Scheme 2. Hetero-IMDA reactions.



***10a**: X,Y=Cl*
***10b**: X=Cl, Y=H*
***10c**: X=H, Y=Cl*
***10d**: X,Y=H*

***12**: X,Y=Cl; 80% (D,L-malbrancheamide)*
***13**: X=Cl, Y=H; 68%*
***14** X=H, Y=Cl; 74% (D,L-malbrancheamide B)*
***15**: X,Y=H; 90%*

Scheme 3. Amide reductions.

Table 1
Inhibition of CaM–PDE1 by compounds **2**–**7** and **10**–**15.**

Compound	IC_{50} (μM)	Chlorpromazine	Potency
2	33.92 ± 4.64	16.78 ± 3.99	0.5
3	45.41 ± 2.39	10.76 ± 0.41	0.2
4	134.47 ± 12.63	10.76 ± 0.41	0.1
5	11.95 ± 1.05	10.76 ± 0.41	0.9
6	81.24 ± 4.46	10.76 ± 0.41	0.1
7	27.51 ± 3.13	10.76 ± 0.41	0.4
10a	18.57 ± 2.87	16.78 ± 3.99	0.9
10b	31.49 ± 2.52	14.11 ± 1.75	0.4
10c	61.79 ± 5.48	14.11 ± 1.75	0.2
10d	150.66 ± 26.79	16.78 ± 3.99	0.1
11a	62.34 ± 4.58	14.11 ± 1.75	0.2
11b	42.77 ± 1.66	14.11 ± 1.75	0.3
11c	23.61 ± 4.21	16.78 ± 3.99	0.7
12	15.99 ± 0.87	16.78 ± 3.99	1.1
nat. 12	19.33 ± 1.40	16.78 ± 3.99	0.9
13	60.33 ± 6.84	14.11 ± 1.75	0.2
14	42.85 ± 4.22	14.11 ± 1.75	0.3
nat. 14	183.28 ± 37.58	14.11 ± 1.75	0.1
15	35.73 ± 3.01	16.78 ± 3.99	0.5

Potency was obtained by the formula: IC_{50} (chlorpromazine)/IC_{50} (compound), assuming a value of 1.00 for chlorpromazine.

striking that the monooxopiperazine compound **4** (C12a-*epi*-malbrancheamide) was essentially inactive (potency of 0.1) whereas the corresponding C5-oxo-isomer **5** was very potent (0.9). The relative lack of activity of compounds **4** and **6** reveals that the relative stereochemistry at C-12a is quite important and also reveals the significance of the C5 and C14 amide carbonyl residues. Varying indole chlorine substitution on the 7-, 8-, and 9-positions led to potencies ranging from 0.1 to 0.9. The relative potencies of synthetic, racemic malbrancheamide (**12**) and malbrancheamide B (**14**) are particularly notable, as it appears to be slightly more active than naturals, optically pure (+)-malbrancheamide and malbrancheamide B relative to chlorpromazine, the control inhibitor.

In conclusion, a number of malbrancheamide analogs have been prepared with differing indole chlorine substitution, C-12a relative stereochemistry, and bicyclo[2.2.2]diazaoctane core oxidation level. Phosphodiesterase activity for each analog was measured alongside chlorpromazine, and a number of active compounds were identified (**5**, **10a**, **11c**, and **12**). The overall results revealed that natural malbrancheamide, either the natural (+)-enantiomer or as a racemate, are the most active CaM–PDE1 complex inhibitors. The unnatural enantiomers of malbrancheamide (**12**) and malbrancheamide B (**14**) must be more active than the naturally occurring enantiomers. Considering the increased relative potency of racemic malbrancheamides compared to that of the natural enantiomerically pure substances, efforts are underway to prepare their enantiomerically pure versions to interrogate the absolute stereochemical issue in more detail. The broad synthetic technology platform our laboratory has developed to synthesize both racemic as well as optically pure versions of this family of prenylated indole alkaloids is being exploited to prepare a number of additional analogs of the malbrancheamides for biological evaluation.

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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.2008.10.057.

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