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Synthesis, characterization and cytotoxicity of new piplartine dimers

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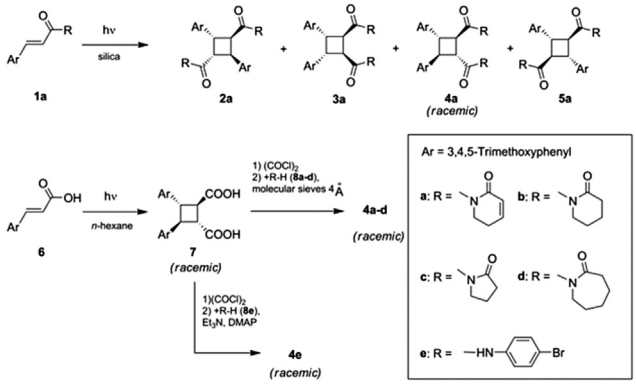
ABSTRACT

Several head-to-head and head-to-tail dimers of piplartine (**1a**) were prepared, and the configurations of the resulting truxillic and truxinic acid derivatives were established by a combination of NMR experiments and single-crystal X-ray analysis. Their cytotoxic activity was screened in photometric sulforhodamine B assays. All of these dimers showed a decreased cytotoxicity compared to **1a** except for the β-truxinic acid derivative **3a**. For this unprecedented head-to-head dimer high cytotoxic activity was established against several human tumor cell lines, and IC_{50} values as low as 1.1 μM were found.

1. Introduction

Spices and herbs have been used for many different purposes by humankind since antiquity; meals have been 'spiced up', and extracts have been used for medicinal applications. Among the herbs, peppers are the most widely used spices, and black pepper in particular was one of the first oriental spices to be introduced into the Western world. The genus *Piper* comprises about 2000 species, and the members of the pepper family usually are herbs, shrubs or even small trees distributed mainly in tropical areas. Approximately a dozen of the *Piper* species have been used in traditional and folk medicines, and many secondary metabolites (including steroids, terpenes, alkaloids but also some biopolymers) have been isolated from the various species and investigated for their biological activity.

Amide alkaloids constitute the major *anti*-proliferative/cytotoxic constituents, and several of them have been found by bioactivity guided chromatographic separations. Among these cytotoxic compounds, piplartine (=piperlongumine (**1a**), Scheme 1) was among the most promising structures. Quite recently, **1a** came into the focus of scientific interest because of its interesting biological activities. This compound and analogs thereof have been shown to possess schistosomicidal and[1–4] *anti*-leishmanial[2,5] activity, to act as an anxiolytic[2,6–8] and *anti*-depressant,[2,6–8] to show *anti*-platelets effects,[2,6,8–15] and to act as an antitumor/cytotoxic agent.[16–24] Almost two decades after having isolated **1a**, another piperidine alkaloid was isolated from the aerial parts of *Piper rugosum* Lam., and its dimeric structure was elucidated by spectroscopic methods. This 'piplartine dimer A' contained a four membered ring system as a structural motif. Since then, several types of tetrasubstituted cyclobutanes have been isolated from the different kinds of plants



Scheme 1. Synthesis of cyclobutane derivatives **2a**, **3a**, **4a**–**e** and **5a**.

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of the genus *Piper*, for example, dipiperamides,[25–31] piperarborenines,[15,32–36] piperchabamides[20,25,37–43] and nigramides.[26,44,45] Because of the manifold biological activity of **1a**, we became interested in the cytotoxic properties of piplartine dimers, especially to reveal whether the alleged *anti*-proliferative properties are dependent of the stereochemical arrangement of the cyclobutane substituents.

2. Results and discussion

2.1. Synthesis

Among other routes, the photolytic homodimerization of piplartine (**1a**) seemed the most promising and straightforward route for the synthesis of piplartine dimers. Thus, irradiation (λ=366 nm) of piplartine (**1a**) on the silica surface of a TLC plate for 48 h (conversion of **1a**: 37%), led to a mixture of four piplartine dimers **2a**–**5a** (Scheme 1), as one could expect from the head-to-head and the head-to-tail photodimerization of *trans*-cinnamic acid derivatives. Prolonged irradiation time (5 days, leading to a complete conversion of **1a**) did not lead to improved yields of **2a**–**5a** but to increased amounts of decomposition products. The isomeric mixture was isolated in 73% yield (relative to conversion); its analysis by HPLC showed an isomeric ratio **2a**:**3a**:**4a**:**5a**=50:31.5:12:6.5%. Although separation of **2a**–**5a** was difficult, isolation of pure **2a**–**5a** was achieved (isolated yields: **2a** 21.6%, **3a** 16%, **4a** 4%, **5a** 3.4% rel to conversion). The pure major isomers **2a** and **3a** were easily isolated by flash chromatography (silica). The minor isomer **4a**, however, could be obtained by repeated chromatographic separation steps only. Several attempts to isolate the minor isomer **5a** by column chromatography (silica or reversed phase) using a variety of different solvent compositions failed, but **5a** was finally obtained by semi-preparative HPLC (reversed phase) starting from a mixed fraction **2a**/**5a**.

Variation of the photolytic conditions (irradiation of **1a** in *n*-hexane suspension, Hg-lamp, '*Rasotherm Glas*', 17 h), a method which was successfully applied for the dimerization of **6** to yield **7** (see below), led to a complete conversion of **1a**. However, lower yields of the photodimers **2a**–**5a** (**2a**: 4%, **3a**: 7.5%, **4a**: 1% and **5a**: 1.5%) and large amounts of polymeric material were obtained. Although the yields of the dimers **2a**–**5a** in these reactions were low, they were found still to be significantly higher compared with to photolysis of piplartine **1a** in solid state; this reaction gave **2a** in an isolated yield of 0.8%.

Dimer **2a** (a α-truxillic acid derivative) was identified as the known piplartine-dimer A, which has previously been isolated from *Piper tuberculatum* Jacq.,[7] *P. rugosum* Lam.[46] and *Piper arborescens* Roxb.,[35,47] while dimers **3a**–**5a** were hitherto unknown compounds. In contrast to **2a**, for dimer **3a** a β-truxinic structure was determined. Structurally related piperarborenines C, D and E were isolated from *P. arborescens*,[15] although a ε-truxillic structure was postulated at first by the isolators. Recently, an independent synthesis, however, led to revision of the structures,[33,34] and these piperarborenines are β-truxinic isomers. Isomer **4a** (possessing a δ-truxinic structure, racemic) is unprecedented, too, both in the series of piplartine-dimers, as well as in the series of piperarborenines. Independent synthesis of **4a** was achieved starting from the corresponding *trans*-cinnamic acid **6**, whose irradiation in *n*-hexane as a suspension (Hg-lamp, '*Rasotherm Glas*', 17 days) afforded the substituted δ–truxinic acid (racemic) **7** in an acceptable yield of 67%. Transformation of **7** to the corresponding bis-acid chloride using oxalyl chloride, followed by the reaction with dihydropyridone **8a** in the presence of molecular sieves[33,48,49] yielded the piplartine-dimer **4a** (23%), identical with the product of the photolysis of **1a**. Analogous reaction of **7** employing the saturated lactams **8b**–**d**, afforded compounds **4b**–**d** (yields: 22, 21 and 7%, respectively). The relatively low yields obtained in these reactions are in agreement with reported observations;[33] they are caused by side reactions (i.e., formation of anhydrides and decomposition) during the formation of the bis-acid chlorides from the corresponding truxinic acids and ring opening of the lactams (especially in the case of **4d**). Attempts to separate the enantiomers of **4a** and **7** are under investigation.

Photodimer **5a** was identified as a ε–truxillic isomer. Related naturally occurring piperarborenines are also unknown, but an analogous piperarborenine derivative has been synthesized by a Pd-mediated stereoselective arylation reaction of the cyclobutane ring.[33,34]

2.2. Characterization of dimers 2a–5a

Unfortunately, we were not able to grow crystals of compounds **2a**–**5a**, suitable especially for X-ray analysis. However, the new compounds were fully characterized by ESI mass spectrometry, IR, ^{1}H NMR and ^{13}C NMR spectroscopy (Table 1). For a complete assignment of the signals in the NMR spectra to the corresponding hydrogen and carbon atoms, 2D experiments were carried out (COSY, NOESY, gHSQC and gHMBC correlations).

Table 1
^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) data of the cyclobutane moiety in compounds **2a**, **3a**, **4a**–**e**, **5a** and **7**[a]

	Spin system	^{1}H NMR δ (ppm)[b]	Coupling constants (Hz)[c]	^{13}C NMR δ (ppm)[b]
2a	*AA′BB′*	4.91 (CHCO) 4.75 (CHAr)	$^3J_{AB}$=$^3J_{A'B'}$ 7.3 (*trans*) $^3J_{AB'}$=$^3J_{A'B}$ 11.6 (*cis*) $^4J_{AA'}$ −1.1 (*trans*) $^4J_{BB'}$ −0.5 (*trans*)	51.3 (CHCO) 42.3 (CHAr)
3a	*AA′XX′*	4.78 (CHCO) 4.16 (CHAr)	$^3J_{AA'}$ 9.9 (*cis*) $^3J_{XX'}$ 9.2 (*cis*) $^3J_{AX}$=$^3J_{A'X'}$ 6.9 (*trans*) $^4J_{AX'}$=$^4J_{A'X}$ −0.8 (*trans*)	48.1 (CHCO) 46.1 (CHAr)
4a	*AA′BB′*	4.20 (CHCO) 4.12 (CHAr)	$^3J_{AA'}$ 10.2 (*trans*) $^3J_{BB'}$ 9.3 (*trans*) $^3J_{AB}$=$^3J_{A'B'}$ 8.8 (*trans*) $^4J_{AB'}$=$^4J_{A'B}$−0.1 (*cis*)	50.6 (CHCO) 46.0 (CHAr)
4b	*AA′BB′*	4.03 (CHCO) 4.01 (CHAr)	$^3J_{AA'}$ 10.5 (*trans*) $^3J_{BB'}$ 9.2 (*trans*) $^3J_{AB}$=$^3J_{A'B'}$ 9.4 (*trans*) $^4J_{AB'}$=$^4J_{A'B}$−0.1 (*cis*)	51.0 (CHCO) 46.0 (CHAr)
4c	*AA′BB′*	4.25 (CHCO) 4.14 (CHAr)	$^3J_{AA'}$ 10.5 (*trans*) $^3J_{BB'}$ 9.3 (*trans*) $^3J_{AB}$=$^3J_{A'B'}$ 8.8 (*trans*) $^4J_{AB'}$=$^4J_{A'B}$−0.2 (*cis*)	47.6 (CHCO) 45.2 (CHAr)
4d	*AA′BB′*	4.07 (CHCO) 4.03 (CHAr)	$^3J_{AA'}$ 10.3 (*trans*) $^3J_{BB'}$ 9.3 (*trans*) $^3J_{AB}$=$^3J_{A'B'}$ 9.2 (*trans*) $^4J_{AB'}$=$^4J_{A'B}$−0.2 (*cis*)	50.8 (CHCO) 46.2 (CHAr)
4e	*AA′BB′*	3.69 (CHAr) 3.43 (CHCO)	$^3J_{AA'}$ 9.9 (*trans*) $^3J_{XX'}$ 9.7 (*trans*) $^3J_{AX}$=$^3J_{A'X'}$ 9.3 (*trans*) $^4J_{AX'}$=$^4J_{A'X}$ −0.1 (*cis*)	47.6 (CHCO) 47.2 (CHAr)
5a	A_2X_2	4.93 (CHCO) 3.96 (CHAr)	$^3J_{AX}$ 9.7 (*trans*)	49.4 (CHCO)[d] 46.5 (CHAr)
7	*AA′BB′*	3.52 (CHAr)[e] 3.20 (CHCO)	$^3J_{AA'}$ 9.7 (*trans*) $^3J_{XX'}$ 9.5 (*trans*) $^3J_{AX}$=$^3J_{A'X'}$ 9.6 (*trans*) $^4J_{AX'}$=$^4J_{A'X}$ −0.2 (*cis*)	47.3 (CHAr)[e] 44.3 (CHCO)

[a] Measured in $CDCl_3$, unless otherwise stated. For complete data, see experimental part.
[b] Cyclobutane protons *CH*Ar and carbons *C*HAr were assigned from correlations (HMBC) with C-2,6 and H-2,6 (^3J couplings) of the 3,4,5-trimethoxyphenyl substituents, respectively.
[c] Coupling constants were determined using the simulation program *PERCH*[54] (std±0.1 Hz).
[d] Measured at 201 MHz.
[e] DMSO-d_6 was used as the solvent.