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Bioactive Derivatives of Isopropylstilbene from Mutasynthesis and Chemical Synthesis

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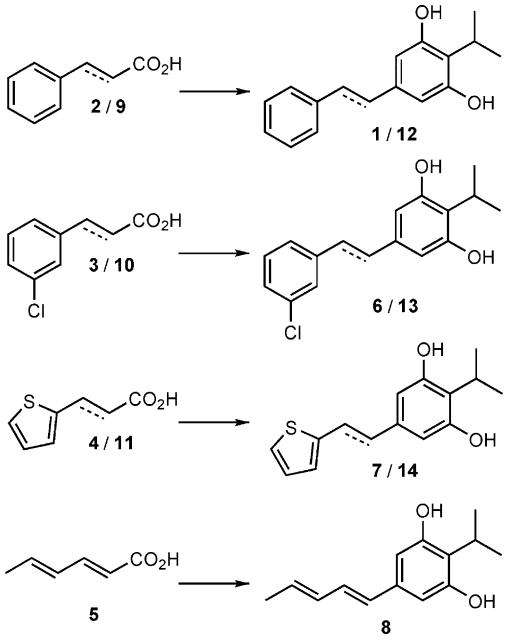
Isopropylstilbene is a natural product from *Photorhabdus luminescens* TT01, with multiple biological activities. A mutant deficient in the production of both anthraquinones and cinnamic acid was constructed, thus giving a clean background according to UV detection. This anthraquinone and stilbene deficient (ASD) mutant was used in mutasynthesis experiments to obtain new stilbene derivatives, which were detected by GC–MS. The structures of the new derivatives were confirmed by detailed MS analysis and then chemically synthesised; all of the natural and synthetic compounds were tested against protozoa that cause tropical diseases. Two compounds obtained by mutasynthesis showed the highest activity against *Trypanosoma cruzi*, the causative agent of Chagas disease, and *Leishmania donovani*, which causes leishmaniasis.

Isopropylstilbene (**1**) is a natural product produced by all bacteria of the genus *Photorhabdus*; these are entomopathogenic and live in symbiosis with nematodes of the genus *Heterorhabditis*.[1] Isopropylstilbene shows insecticidal and antibiotic activity,[2] and is required for the development of the nematode.[3] Its biosynthesis differs from that of the well-known plant stilbene biosynthesis[4] in that the resorcinol ring is derived from the condensation of two acyl moieties,[3] as has recently been elucidated.[5] Briefly, the biosynthesis starts from cinnamic acid (**2,** Scheme 1), which is produced from phenylalanine by the phenylalanine ammonium lyase (PAL) StlA and is subsequently elongated by a ketosynthase. The elongated product is cyclised by an unusual ketosynthase with a leucine derived 3-oxo-isoheptanoyl thioester to form a cyclohexanedione (CHD), which is aromatised in a final step to a dialkylresorcinol (DAR) as in **1**.

Natural products play an important role in drug development[6] because of their evolutionary adaption to biological targets. Nevertheless, natural products are usually only lead structures and require optimisation for stability or efficacy. Besides chemical synthesis or semisynthesis, precursor-directed biosynthesis or mutasynthesis can be applied to obtain natural-product derivatives with hopefully improved properties, as has been well reviewed.[7–9] In medicinal chemistry, halogen atoms



Scheme 1. Derivatives of cinnamic acid (**2**) and phenyl propionic acid (**9**) by mutasynthesis with the mutant ASD strain. The dashed line shows the position of the double bond, where both the unsaturated and saturated acid were used; first and second compound numbers refer to unsaturated and saturated molecules, respectively.

or hetero aromatic rings are often introduced, thereby probably altering the degradation or metabolism of the compounds in the target organism. An already multifunctional compound,[3] **1** was recently the starting point for new compounds showing both an inhibitory effect on soluble epoxide hydrolase (sEH) and antiproliferative activity against particular cancer cell lines (e.g., HepG2 (hepatocarcinoma) and U937 (histiocytic lymphoma)).[10]

To produce new derivatives of **1** by mutasynthesis, we used a *Photorhabdus luminescens* mutant (BMM901) that lacked the ability to produce **2** and therefore also stilbenes, as a result of an insertion in the PAL gene[3] (Figure 1). Additionally, an insertion was introduced into the *antB* gene (encoding the phosphopantheteinyl transferase (PPTase) involved in anthraquinone (AQ) biosynthesis) to prevent the production of this usually highly abundant class of compound.[11] The resulting double mutant was named ASD (anthraquinone and stilbene deficient).

Upon addition of **2** to this strain, stilbene production was restored, as expected, and no AQ was detected, thus facilitating compound detection and isolation (Figure 1C). The mutasynthesis experiments were performed by growing ASD in a

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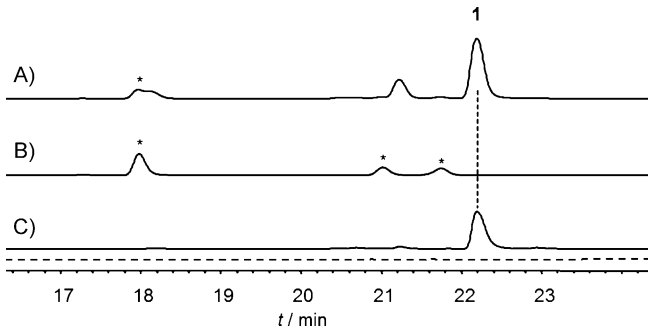


Figure 1. HPLC/UV chromatograms (250–550 nm) of *P. luminescens* strains: A) wild-type, B) PAL mutant, and C) ASD mutant without (-----) and with **2**. Anthraquinones are indicated with an asterisk (*); chromatograms are shown with the same scale.

medium containing different derivatives of **2**. Compounds **2**–**5** resulted in the production of new DAR derivatives (**6**–**8**; Scheme 1), which were analysed by GC–MS (chromatogram for **1** in Figure 2A). No new compound was detected upon addition of further acids (Figure S1 in the Supporting Information; GC–MS data not shown).

The production of a chlorinated derivative upon addition of *meta*-chloro cinnamic acid (**3**; Figure 2B) was sufficient to allow purification of the respective stilbene derivative by flash chromatography. 1D and 2D NMR experiments and HRMS data of the isolated compound proved the expected chloro-

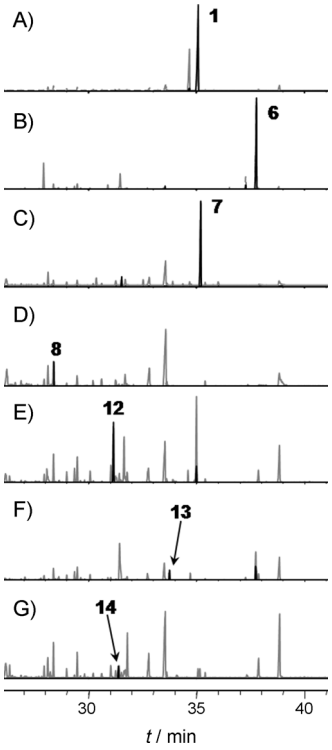
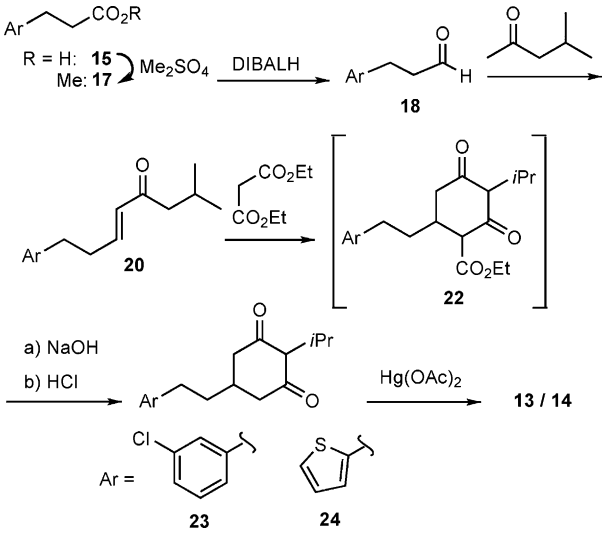


Figure 2. GC–MS chromatograms of mutasynthesis experiments with mutant ASD in medium containing A) **2** (*m/z* ratio, 398), B) **3** (432), C) **4** (404), D) **5** (352), E) **9** (400), F) **10** (434), and G) **11** (406). Total ion chromatogram (grey) and extracted ion chromatogram of the corresponding DAR derivative (indicated by bold compound number).

isopropylstilbene structure (**6**, Table S1, Figure S2). Interestingly, addition of the related *para*-chloro cinnamic acid (Figure S1) did not lead to the formation of the related stilbene derivative. Similarly, the addition of **4** and **5** resulted in the production of the DAR derivatives **7** and **8**, respectively (Figure 2C, D; no isolation of these derivatives was performed).

Further experiments were conducted with reduced propionic acid derivatives rather than the acrylic acids (Scheme 1), as such natural products have also been identified in some *Photorhabdus* strains.[5] Addition of 3-phenylpropionic acid (**9**), 3-(3-chlorophenyl) propionic acid (**10**) and 3-(2-thienyl) propionic acid (**11**) resulted in production of new natural products with masses matching the corresponding products (**12**–**14**, respectively; Figure 2E–G). However, because of low production, no purification was attempted. In order to prove the structures of these compounds, the recently described synthesis route to both **1** and **12** was adapted (Scheme 2).[12] The reaction was started with acid **15** (for Ar see **23** and **24**) because of its com-



Scheme 2. Synthetic route to **13** and **14**. Intermediate **22** was not isolated (for Ar see **23** and **24**). DIBALH: diisobutylaluminium hydride.

mercial availability. Treatment with dimethyl sulphate formed the methyl ester **17**, which was reduced with DIBALH to give the desired aryl propionaldehyde **18**. Instead of the original Wittig reaction (requiring an elaborate purification), aldol condensation with 4-methyl-2-pentanone (**19**) was employed, and similar yields of α,β-unsaturated ketone **20** were achieved, while the purification effort was dramatically reduced. The subsequent steps were as described above. Briefly, a Michael addition with diethyl malonate followed by intramolecular Claisen condensation produced a six-membered ring with an attached ester function (**22**) in a one-pot reaction. Subsequent saponification and decarboxylation of the resulting β-keto acid led to a CHD (**23** and **24**), which were aromatised by mercury(II) acetate to the final products **13** and **14**, respectively.

With **12**–**14** in hand, a comparison with the new mutasynthesis products proved their identity by GC–MS retention time and MS fragmentation pattern (Figure S3). Furthermore, bioac-

tivity tests against protozoa causing tropical diseases[13] and cytotoxicity tests against rat myoblast L6 cells were performed with both isolated **6** and synthesised CHDs (**23**, **24**) and DAR derivatives (**13**, **14**). Consistent with previously published results,[12] DARs showed higher activity than CHDs. When comparing unsaturated with saturated stilbene derivatives, the saturated compounds (**12**–**14**) were more active against *Trypanosoma cruzi* (the causative agent of Chagas disease) than **1** and **6**. In contrast, **1** and **6** were more active than **12**–**14** against malaria-causing *Plasmodium falciparum*. Chemically synthesised **13** and **14** were the most active compounds against *Leishmania donovani* and *T. cruzi* (IC_{50} values of 3.71 and 8.80 μM, respectively); these two mutasynthesis compounds were more active than the parent natural products, although the production titres were too low to be isolated (Table 1).

In this work, new bioactive derivatives of isopropylstilbene (**1**) were produced by a mutant of *P. luminescens* optimised for mutasynthesis and purification of new products. However, some derivatives were only produced in small amounts, thus indicating low flexibility of the downstream enzymes in stilbene biosynthesis. Additionally, it was shown that these compounds are available by chemical synthesis and that derivatives can be more active than the original natural product. Thus, mutasynthesis and chemical synthesis provide the possibility to produce and evaluate even more derivatives of this simple but potent class of natural products.

Table 1. Bioactivity against protozoa causing neglected tropical diseases

	IC_{50} [μM]				
	T.b. rhod.	*T. cruzi*	*P. falc.*	*L. don.*	Cytotoxicity[a]
1	0.90	18.5	7.67	3.77	15.2
6	14.7	17.0	8.00	6.75	26.0
12	0.35	9.40	12.0	5.73	19.0
13	1.58	16.3	9.70	3.71	19.2
14	1.26	8.80	16.4	7.47	7.47
23	33.7	92.9	38.6	14.9	165
24	23.6	134	49.2	7.94	202
ref.[b]	0.005	1.73	0.006	0.21	0.02

[a] Evaluated for L6 cells. [b] Reference compounds: *Trypanosoma brucei rhodesiense*, melarsoprol; *T. cruzi*, benznidazole; *Plasmodium falciparum*, chloroquine; *Leishmania donovani*, miltefosine; L6 cells, podophyllotoxin.

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Keywords: biological activity · biomimetic synthesis · mutasynthesis · natural products · *Photorhabdus*

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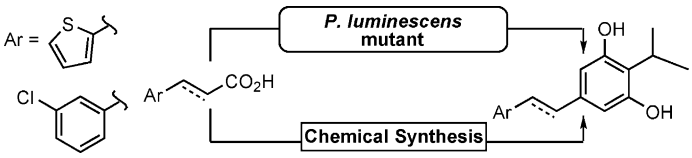

It's just not natural! The production of new isopropylstilbene derivatives was achieved by mutasynthesis with a *Photorhabdus* ASD (anthraquinone and stilbene deficient) mutant, blocked in anthraquinone and cinnamic acid biosynthesis. Chemical synthesis of selected compounds allowed for their biological evaluation, which led to compounds with better activity than the natural products.