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A biosynthetic microbial ability applied for the oxidative ring cleavage of non-natural heterocyclic quinones

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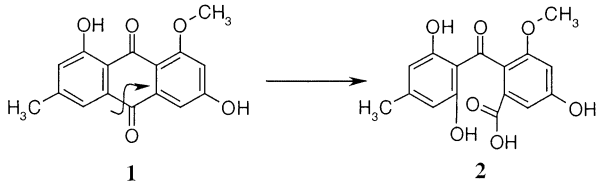
Abstract—The regiospecific oxidative ring cleavage of naphthothiazole and naphthooxazole quinonic derivatives was performed by a microbial methodology using a *Streptomyces* strain. The subsequent C–C bond cleaved products were new 2-substituted-5-(2-hydroxybenzoyl)thiazole or oxazole-4-carboxylic acid derivatives obtained in one step with 30–65% yields.

Biological Baeyer–Villiger oxygenation constitutes an important bioconversion type with interesting synthetic applications due to regioselective and/or asymmetric lactonization.[1] Baeyer–Villiger oxidations accept as substrates various aliphatic and alicyclic carbonyl derivatives. As far as we know no quinonic substrate has ever been depicted as a substrate for this reaction. By contrast, the biosynthesis of several natural products such as the so-called *seco*-anthraquinones, like xanthones,[2–4] ergochromes,[5] geodin,[6–9] and recently isolated metabolites such as balanol,[10] or coniochaetones,[11] was suggested to imply an unusual enzymatic Baeyer–Villiger process.[3,7] This conversion of anthraquinones to benzophenones and related metabolites was mainly demonstrated by feeding studies with $^{18}O_2$ and ^{13}C-acetate, and an oxidative ring cleavage of these anthraquinonic compounds is assumed to be the key step. There is indeed only one reported partial purification of the enzyme from *Aspergillus terreus*, presumed to be responsible for the oxidative ring cleavage of questin **1** to yield the corresponding benzophenone desmethylsulochrin **2**,[12] as shown in Scheme 1.



Scheme 1.

This biotransformation is remarkable because it generates in a simple way the introduction of new structural functionalities: a carboxyl group and a phenol group. Applied to non-natural substrates,[13] this biotransformation should be valuable for the easy access to a diversity of novel heterocyclic ketone structures. So, in an attempt to generate novel entities of putative pharmacological interest, we have explored such a microbial ring cleavage reaction leading to novel heteroaromatic ketone derivatives using as model substrate the heterocyclic naphthoquinone **3** (Table 1).

Among 50 fungal and bacterial strains tested, *Streptomyces platensis* NRRL 2364[13] was selected as giving in high yield (65% after purification) a single metabolite **4** with mass peak at m/z 316 (MH^+) corresponding to the expected molecular formula $C_{15}H_9NO_5S$. The ^1H, ^{13}C and 2D NMR spectra confirm that compound **4** is one of the four possible ring-cleavage products.[14] The structure of **4** was established unambiguously by X-ray crystallography.[15]

We have thus demonstrated the ability of *S. platensis* NRRL 2364[16] to convert the 2-furan-2-yl-naphtho[2,3-*d*]thiazole-4,9-dione **3** into the 5-(2-hydroxy-benzoyl)-2-(furan-2-yl)-thiazole-4-carboxylic acid **4**, as shown in

Keywords: biotransformation; oxidative ring cleavage; microbial reaction; *Streptomyces*; naphthoquinones.

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Table 1. Biotransformations by *S. platensis*[16]

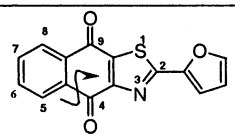
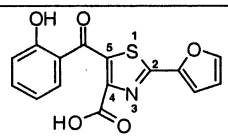
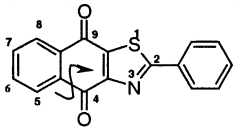
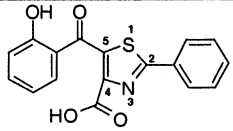
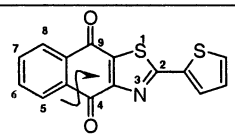
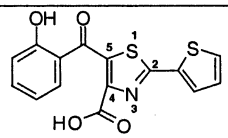
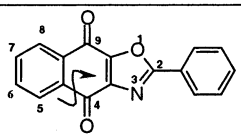
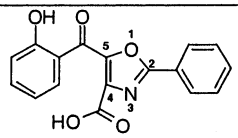
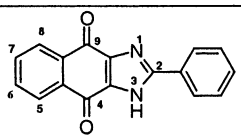
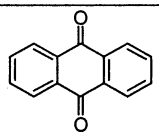
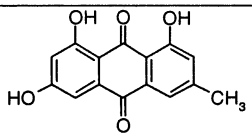
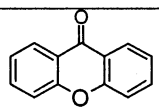
Substrate	Yield, Duration	Cleaved Product
3 	65%, 48H	 **4**
5 	50%, 40H	 **6**
7 	53%, 48H	 **8**
9 	30%, 48H	 **10**
11 		none
12 		none
13 		none
14 		none

Table 1. The oxidative ring cleavage is regioselective and occurs on the C_{4a}–C_4 bond as indicated in Fig. 1.

In order to explore the potential synthetic applications of such a biotransformation, we investigated other substrates as indicated in Table 1. Naphthothiazole-(**5**, **7**) and naphthooxazole-**9** derivatives are efficiently converted to open ketones, with a similar regioselectivity, whereas naphthoimidazole derivative **11** is not metabolized. Anthraquinonic derivatives like anthraquinone itself **12** or emodin **13** are not substrate for this reaction, and xanthone **14** is not cleaved either.

No lactone intermediate, as expected from a supposed Baeyer–Villiger like reaction, is detected among the microbial products. Nevertheless, such a lactone compound, corresponding to a phenol ester, may have been hydrolyzed in the incubation conditions. It is noteworthy that anthraquinones[5] as well as compound **3** are not cleaved by peracids under chemical Baeyer–Villiger oxidation conditions, emphasising the synthetic interest of the microbial reaction.

This reaction is the first reported example of a microbial oxidative ring cleavage applied to the transformation of non-natural heterocyclic quinones. Moreover the generated products are novel heteroaromatic ketones formed efficiently in a single step.

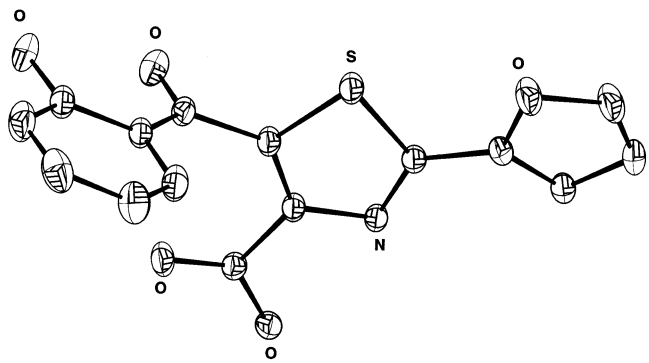


Figure 1. View of molecule **4** from X-ray coordinates.

Encouraged by these results, we want to conduct a substrate structure–ring cleavage relationship study and we are currently investigating this cleavage reaction in a biocombinatorial approach on a larger diversity of substituted heterocyclic quinones. Besides, in order to elucidate the biochemical cleavage mechanism, an enzymatic study is in progress.

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13. Patent WO 97/21684.
14. Data for compound **4**: mp 159°C; MS (APcI$^+$): m/z 316 (M+H)$^+$, MS (APcI$^-$): m/z 315 (M$^-$); ^{1}H NMR (250.13 MHz, DMSO-d_6): δ 7.97 (m, 1H), 7.50 (m, 2H), 7.30 (d, 1H, $J=3.48$ Hz), 6.92 (m, 2H), 6.77 (dd, 1H, $J=3.50$ Hz, $J=1.74$ Hz); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 189.2, 162.2, 159.0, 157.2, 147.4, 146.9, 146.2, 138.6, 135.9, 131.4, 123.1, 119.3, 117.4, 113.1, 111.6. Data for compound **6**: mp 173°C; MS (APcI$^+$): m/z 326 (M+H)$^+$, MS (APcI$^-$): m/z 325 (M)$^-$; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ 8.00 (m, 2H), 7.52 (m, 5H), 6.93 (m, 2H); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 189.6, 167.5, 162.3, 159.2, 146.9, 139.9, 136.0, 131.8, 131.5, 131.4, 129.5 (2C), 126.6 (2C), 122.9, 119.4, 117.4. Data for compound **8**: mp 172°C; MS (APcI$^+$): m/z 288 (MH–COOH+H)$^+$, MS (APcI$^-$): m/z 286 (M–COOH)$^-$, MS (ESI$^-$): m/z 330 (M–H)$^-$; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ 7.84 (m, 2H), 7.50 (m, 2H), 7.22 (m, 1H), 6.95 (m, 2H); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 189.0, 162.2, 161.5, 159.0, 146.6, 139.1, 135.9, 135.0, 131.4, 131.0, 129.5, 128.9, 123.1, 119.4, 117.4. Data for compound **10**: mp 181°C; MS (APcI$^+$): m/z 310 (M+H)$^+$, MS (APcI$^-$): m/z 309 (M)$^-$; ^{1}H NMR (250.13 MHz, DMSO-d_6): δ 8.02 (m, 2H), 7.57 (m, 5H), 6.96 (m, 2H); ^{13}C NMR (62.9 MHz, DMSO-d_6): δ 183.4, 161.8, 160.3, 158.6, 149.6, 135.7, 135.0, 132.0, 130.9, 129.4 (2C), 126.8 (2C), 125.5, 123.3, 119.4, 117.1.
15. The crystallographic data have been deposited with the Cambridge Crystallographic Data Centre under deposition numbers 161586 and 161587 for compounds **4** and **6**, respectively.
16. *General procedure*: *Streptomyces platensis* NRRL 2364 was maintained on nutrient agar slants (Difco, yeast malt extract agar 38 g/L) at 4°C. Erlenmeyer flasks (500 mL) containing 100 mL of medium composed of (g/L) yeast extract (5.0), soy bean peptone (5.0), NaCl (5.0), K_2HPO_4 (5.0) and glucose (20.0), adjusted to pH 7 with 1N HCl, were inoculated with a glycerol suspension of *S. platensis* NRRL 2364 and incubated on an orbital shaker (270 rpm) at 27°C. After 24 h, 150 mL of the shake flasks culture were used to inoculate a bioreactor (30 L fermentor, B. Braun Biostat C) containing 20 L of medium composed of (g/L)) yeast extract (5.0), soy bean peptone (5.0), NaCl (5.0), K_2HPO_4 (13.9), KH_2PO_4 (2.7) and glucose (20.0). The bioreactor was operated at 27°C with agitation at 250 rpm, air flow at 15 L/min. After 35 h of growth, the biomass was harvested by centrifugation and stored at −20°C. Cells (2.5 g wet weight) were transferred into 100 mL of 0.1 M phosphate buffer pH 6.1 in a shake flask (500 mL). The substrate (0.3 g/L) due to its low solubility was added to this cell suspension in a minimal volume of DMF, containing 10% Tween 80®. The biotransformation was conducted at 270 rpm in an orbital shaker at 27°C. When the biotransformation (monitored by HPLC) was completed, the incubation mixture was centrifuged, the supernatant was acidified, extracted with ethyl acetate and the resulting metabolite (30–65%) was purified by repeated solubilization and precipitation from alkaline extract.