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Biotransformation of natural compounds. Oxido-reduction of Sch-642305 by *Aspergillus ochraceus ATCC 1009*

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ABSTRACT

Sch-642305 is the major compound produced by the endophytic fungi *Phomopsis* sp. *CMU-LMA*. Incubation of Sch-642305 with *Aspergillus ochraceus ATCC 1009* resting cells leads to three new derivatives through an oxido-reduction of the six-membered ring of the molecule. Reduction of the double bound leads to compound (**1**), which subsequently undergoes carbonyl reduction to (**2**) and ring hydroxylation to (**3**). According to the previously solved crystal structure of Sch-642305 coupled with ^{1}H NMR NOE correlation and the crystal structure of compound **1**, the absolute configurations of the new derivatives were established. In contrast to the parent compound Sch-642305, compound (**1**) exhibits antimicrobial activity against Gram-negative bacteria. Furthermore, while all derivatives exhibit cytotoxic activity against various cancer cell lines, compound (**2**) achieved an IC_{50} of 4 nM against human myelogenous leukemia K 562, compared to 20 nM for the parent Sch-642305.

Scientists use various tools to improve the properties of target compounds to enhance their bioavailability, bioactivity, stability or targeting.[1] In the field of bioactive natural compounds, these techniques involve a variety of disciplines, from classical and combinatorial organic chemistry[2] to in silico screening[3] and gene cluster reorganization.[4,5] To introduce soft modifications that are compatible with biological conditions and involve naturally occurring reactions, biotransformation represents an ideal compromise.[6–9] In addition to chemical diversification, biotransformation using enzymes or whole cells offers the opportunity to help elucidate biosynthetic pathways and anticipate mammalian metabolism and toxicity. We have shown recently that biocatalysis can simulate the mammalian metabolisms of natural targets[10] and form unexpected analogs from abundant unfunctionalized natural compounds.[11]

In this Letter, we report the biotransformation of Sch-642305, a bicyclic, 10-membered macrolide that was first isolated from *Penicillium verrucosum* as a bacterial DNA primase inhibitor.[12] We have produced large quantities of this compound from the endophytic fungus *Phomopsis* sp. *CMU-LMA* and submited it to biocatalysis by microbial resting cells. Among nine screened microorganisms chosen for their exceptional oxido-reduction potential, we selected *Aspergillus ochraceus ATCC 1009,* which gives rise to three Sch-642305 analogs. Their antibiotic and cytotoxic activities were compared to the parent compound. The compound Sch-642305 is produced by the fungal strain *Phomopsis* sp. *CMU-LMA*,[13] isolated from healthy wild galanga (*Alpinia malaccensis*) as previously described.[14] The structure of Sch-642305 was deduced from spectroscopic data[15] (Tables 1 and 2) and confirmed by X-ray crystals structure analysis[16] (Fig. 1). We engaged in the generation of new derivatives through microbial biocatalysis with the aim of enhancing chemodiversity and modulating bioactivity. Based on our knowledge of oxido-reductive microorganisms,[17–19] we

Table 1
^{13}C NMR data (δ) for compounds **1**, **2**, **3** and the parent compound Sch-642305 (δ values in ppm). Spectra were recorded in $CDCl_3$, 500 MHz

Position	Sch-642305	**1**	**2**	**3**
1	200.3	211.0	67.6	210.1
2	131.1	35.8	27.7	44.6
3	146.6	32.5	26.9	72.2
4	67.1	70.9	72.8	74.3
5	37.1	41.7	36.2	36.6
6	46.3	47.8	35.1	46.9
7	22.9	21.2	22.5	22.6
8	22.3	22.2	22.9	22.1
9	22.9	22,7	25.9	25.4
10	30.3	33.0	26.5	33.7
11	73.9	74.1	74.3	74.5
12	172.2	173.2	174.9	173.8
13	38.9	39.8	36.4	38.8
14	18.6	19.7	20.6	19.9

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Table 2
^{1}H NMR data (δ) for compounds **1**, **2**, **3** and the parent compound Sch-642305 (δ values in ppm). Spectra were recorded in $CDCl_3$, 500 MHz

Position	Sch-642305	**1**	**2**	3
1	–	–	3.93 m	–
2	6.02 d (9.9)	2.20 m 2.66 m	1.53 m	2.34 dd (3.4, 11.3) 2.96 dd(3.4, 11.3)
3	6.99 dd (4.3, 5.5)	1.78, 1.94 m	1.91 m	4.28 q (3.0, 3.0, 11.3)
4	4.25 dd (3.4, 5.6)	4.10 q (7.3)	4.03 m	3.94 t (1.8)
5	2.80 m	2.36 m	2.24 m	2.77 m
6	2.66 m	2.66 m	1.73 m	2.56 m
7	2.11 m	1.35 m	1.30, 1.73 m	1.73 m
8	1.29, 1.58 m	1.35 m	1.30, 1.73 m	1.53 m
9	1.13, 1.78 m	1.35 m	1.30 m	1.33 m
10	1.29,2.01 m	1.35 m	1.30 m	1.33, 1.94 m
11	5.06 m	5.07 m	5.95 m	5.09 m
13	2.62 dd (2.4, 16.8)	2.66 m	2.84 dd (5.1, 7.9) 2.42 dd (2.3, 11.4)	2.67 m
14	1.27 d (6.6)	1.23 d (6.4)	1.24 d (6.2)	1.23 d (6.3)

selected nine fungi, all of which were used as fresh resting cells in the presence or absence of 10% added glucose. These included *Rhizopus arrhizus ATCC 1145, Aspergillus terreus MMP 2296, Aspergillus ochraceus ATCC 1009, Aspergillus niger ATCC 16404, Beauveria bassiana ATCC 7159, Mucor plumbeus CBS 11016, Cylindrocarpon radicicola ATCC11011, Geotrichum candidum CBS 23376* and *Cunninghamella echinulata NRRL 3655*. A series of analytical incubations were dedicated to identify the optimal strains and conditions.[20] Finally, we selected *A. ochraceus ATCC 1009,* which led, in the presence of glucose, to the highest conversion yield and compound diversity, for further investigations with the presence of 10% glucose.[21] Figure 2 represents the HPLC chromatogram obtained after 12 days of incubation of Sch-642305 with *A. ochraceus ATCC 1009*. The *m*/*z* values were deduced from HPLC–MS analysis on positive and negative electrospray modes. Besides the residual starting compound Sch-642305, three major peaks, **1**, **2** and **3**, were observed that corresponded to *m*/*z* 254, 256 and 270, respectively. Three minor peaks were also detected corresponding to *m*/*z* 254, 284 and 286. Kinetic monitoring of the three major compounds and the substrate Sch-642305 by HPLC (Fig. 3) showed a

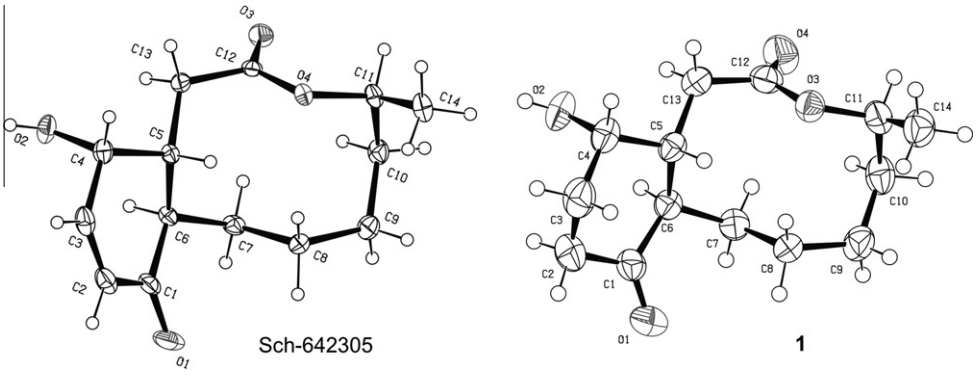

Figure 1. ORTEP diagrams showing the solid-state structures of Sch-642305 and compound **1** (for details see Ref. 16).

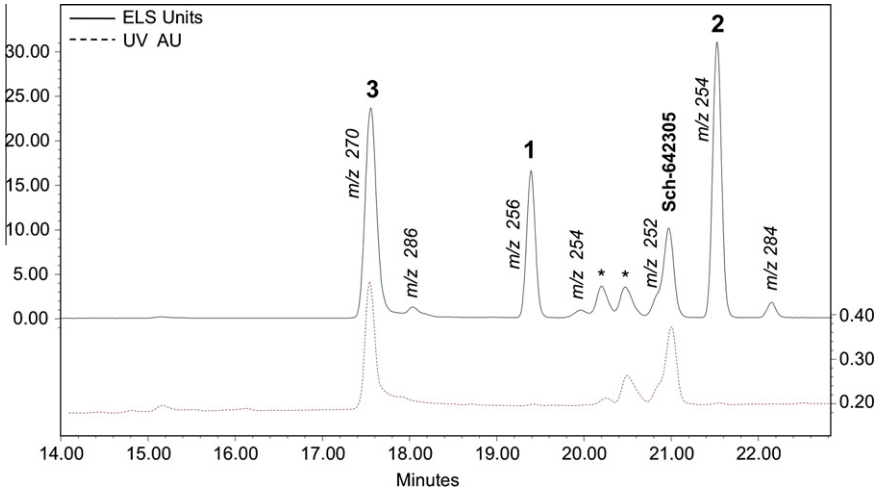

Figure 2. HPLC chromatogram obtained after 12 days of incubation of Sch-642305 with *Aspergillus ochraceus* in the presence of 10% glucose.

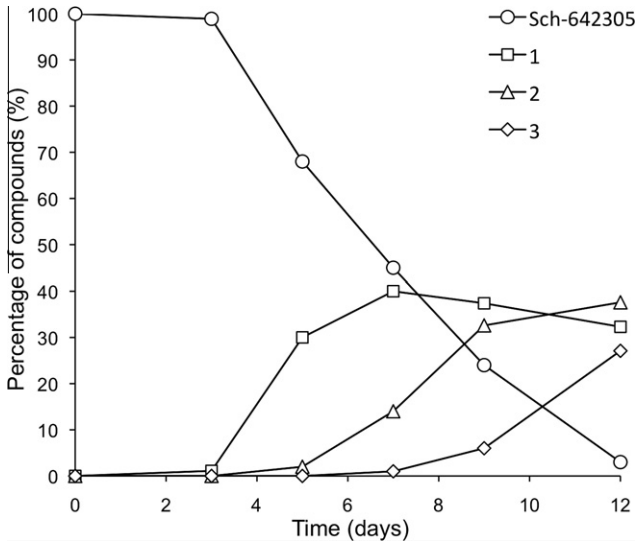

Figure 3. Kinetic monitoring of the biotransformation of Sch-642305 by *Aspergillus ochraceus ATCC 1009.*

lag period of three days, followed by a constant decrease in the substrate, which completely disappeared in about 12 days. Compound **1** is formed first; it reached a maximum of 40% at day seven and then decreased slowly. The second derivative, compound **2**, began to form at day five, reached 33% at day nine and then increased slowly. Compound **3** started to appear at day seven in parallel with an equivalent decrease in Sch-642305 and reached a maximum of about 30% in 12 days. Careful observation of the period from day 9 to day 12 suggests that compound **3** was a direct product from Sch-642305, while products **1** and **2** remained stable. Biotransformation of 200 mg of Sch-642305 yielded approximately 30 mg each of pure **1**, **2** and **3**, which allowed us to achieve the structural investigations and cell based bioassays.[22,23] Tables 1 and 2 regroup the ^{1}H and ^{13}C NMR data of the three metabolites compared to the substrate Sch-642305. At approximately 170 ppm (172–175 ppm), the lactone carbonyl C-12 is conserved in compounds **1**, **2** and **3**, while the methine carbons C-2 and C-3 at 131 and 146 ppm shift to lower ppm values in compounds **1**, **2** and **3** (26.9–72.2 ppm), supporting the reduction of the double bound. The C-4 bearing the hydroxyl group (67.1–74.3 ppm) is also conserved in all compounds. The carbonyl C-1 at 200.3 ppm is conserved in compounds **1** and **3** (211 and 210.1 ppm, respectively) and replaced by a hydroxyl group in compound **2** (67.6 ppm). The C-3 in compound **3** is at 72.7 ppm, supporting the presence of a hydroxyl at this position. By coupling NMR data with HR/ESI-MS and IR data (see experimental section), the structures of compounds **1**, **2** and **3** are presented in Figure 4. Absolute configurations of compounds Sch-642305 and **1** were deduced from the X-ray crystal structures (Fig. 1) and those of compounds **2** and **3** were assigned according to the NMR–NOE correlations (Fig. 4). Thus for compound **2**, the NMR–NOE spectrum showed an interaction between protons H1 and H6 confirming their cis configuration. Interactions between protons H3 and H4 in compound **3** also drive to the same conclusion.

The formation of **1** and **2** is the consequence of two consecutive reductions affecting the double bound and then the carbonyl (cf. kinetic). It is plausible that **3** could derive from **2** by direct hydroxylation of the nonactivated carbon C-3. This type of oxidation is well documented and generally catalyzed by Cyt-P450 type enzymes.[24] This hypothesis is not fully consistent with the kinetic monitoring that suggests the formation of **3** directly from **1**. The compound at retention time 22.2 (Fig. 2) with *m*/*z* 286 may correspond to a derivative bearing two hydroxyl groups at carbons 2 and 3, directly resulting from the epoxidation of **1** followed by epoxide hydrolysis. Unfortunately, we did not obtain any evidence for the formation of the epoxide, nor did we have sufficient quantities of minor compounds to go further in structure achievements.

The antibiotic and cytotoxic activities of **1**, **2** and **3** were compared to the parent Sch-642305.[25] Only the biotransformation derivative **1** would exhibit significant antibiotic activity against the Gram-negative bacteria *E. coli* (Table 3). Furthermore, while all derivatives exhibited cytotoxic activity against various cancer cell lines, compound (**2**) reached an IC_{50} of 4 nM against human myelogenous leukemia K 562, as compared to 20 nM for the parent Sch-642305 (Table 4).

In conclusion, three new compounds were obtained in good yields through the microbial oxido-reduction of Sch-642305 by *A. ochraceus ATCC 1009.* These new derivatives could also be considered as natural compounds because they were obtained through further enzymatic conversion of an abundant natural precursor. Mild and safe reaction conditions are consistent with green chemistry guidelines and exhibit high efficiency and total enantioselectivity.

Furthermore, bioassays confirm that access to Sch-642305 analogs through biocatalysis opens a quick and efficient access to more active compounds. As Sch-642305 was reported to mildly inhibit DNA primase,[12] we are currently undertaking a large screening of bioconversion derivatives on this key target.

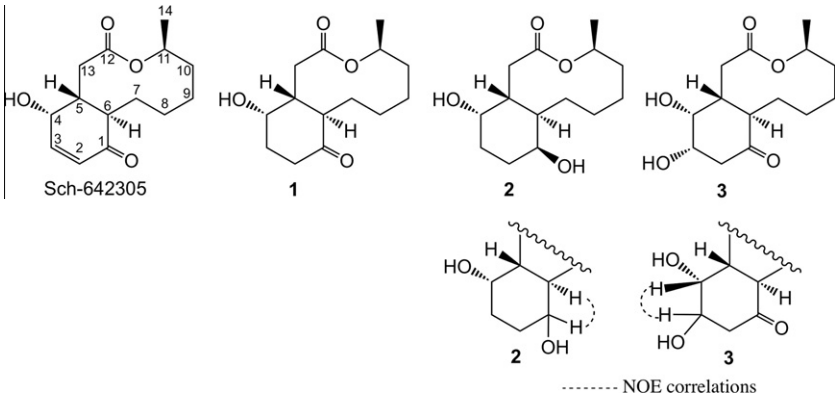

Figure 4. Structure of Sch-642305 and its three major derivatives (**1**, **2** and **3**) issued from biotransformation by *Aspergillus ochraceus ATCC 1009.* ^{1}H NMR NOE correlation corresponds to hashed lines.

Table 3
Antimicrobial activity of isolated compounds compared to the parent compound Sch-642305 and antibiotic controls

Compound	*E. Coli*[a]	*B. Subtilis*[b]	*M. Luteus*[a]
Sch-642305	0	100	38
1	50	0	0
2	0	0	0
3	0	62.5	0

[a] Compared to 30 μg chloramphenicol.
[b] Compared to 10 μg gentamycin

Table 4
In vitro cytotoxic activity was screened on two cell lines. IC_{50} was determined in triplicate (for details see Ref. 15).

Compound	MDA 231	K 562
Sch-642305	2.0×10^{-8}	2.0×10^{-8}
1	4.0×10^{-7}	2.0×10^{-7}
2	4.0×10^{-8}	4.0×10^{-9}
3	1.2×10^{-8}	2.0×10^{-8}

References and notes

1. Harvey, A. L. *Drug Discovery Today* **2008**, *13*, 894.
2. Lazo, J. S.; Wipf, P. *J. Pharmacol. Exp. Ther.* **2000**, *293*, 705.
3. Stockwell, B. R. *Nature* **2004**, *432*, 846.
4. Van Lanen, S. G.; Shen, B. *Curr. Opin. Microbiol.* **2006**, *9*, 252.
5. Bol, D.; Ebner, R. *Pharmacogenomics* **2006**, *7*, 227.
6. Liu, J. H.; Yu, B. Y. *Curr. Org. Chem.* **2010**, *14*, 1400.
7. Asakawa, Y.; Noma, Y. *Comprehensive Nat. Prod. II Chem. Biol.* **2010**, *3*, 803.
8. Asakawa, Y.; Noma, Y. *Comprehensive Nat. Prod. II Chem. Biol.* **2010**, *3*, 893.
9. Noma, Y.; Asakawa, Y. *Comprehensive Nat. Prod. II Chem. Biol.* **2010**, *3*, 669.
10. Decor, A.; Bellocq, D.; Thoison, O.; Lekieffre, N.; Chiaroni, A.; Ouazzani, J.; Cresteil, T.; Gueritte, F.; Baudoin, O. *Bioorg. Med. Chem.* **2006**, *14*, 1558.
11. Leverrier, A.; Martin, M. T.; Servy, C.; Ouazzani, J.; Retailleau, P.; Awang, K.; Mukhtar, M. R.; Gueritte, F.; Litaudon, M. *J. Nat. Prod.* **2010**, *73*, 1121.
12. Chu, M.; Mierzwa, R.; Xu, L.; He, L.; Terracciano, J.; Patel, M.; Gullo, V.; Black, T.; Zhao, W.; Chan, T. M.; McPhail, A. T. *J. Nat. Prod.* **2003**, *66*, 1527.
13. Five liter of Potato Dextrose Broth PDB (Difco) were inoculated with *Phomopsis* sp. CMU–LMA spores and cultivated at 27 °C and 200 rpm for five days. The medium was extracted with 3 × 1 L of ethyl acetate. The organic layers were evaporated under reduced pressure affording 1.9 g of whole extract. Compound was purified by silica gel chromatography using a Combiflash Companion Chromatograph and a ready-to-use RediSep column (80 g, 35–70 μm mesh). The solvent consisted of a mixture of ethyl acetate/heptane, 30–70% for 45 min, then 50–50% for 45 min. This procedure afforded 325 mg of pure Sch-642305.
14. Bussaban, B.; Lumyong, S.; Lumyong, P.; Seelanan, T.; Park, D. C.; McKenzie, E. H.; Hyde, K. D. *Mycologia* **2005**, *97*, 1002.
15. *Sch-642305:* white powder; $[\alpha]_D^{25}$ + 75.8 (c 1.0, MeOH); IR ν_{max} 2919, 2859, 1690, 1276, 758 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table (Tables 1 and 2); HRESIMS *m/z* 253.1448 $[M+H]^+$ (calculated for $C_{14}H_{21}O_4$ 253.1440). *Compound* ***1****:* amorphous solid; $[\alpha]_D^{25}$ – 10.6 (c 1.0, MeOH); IR ν_{max} 3400, 2925, 1697, 1278, 1251, 1064, 958, 730 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table (Tables 1 and 2); HRESIMS *m/z* 277.1482 $[M+Na]^+$ (calculated for $C_{14}H_{22}O_4Na$ 277.1416). *Compound* ***2****:* amorphous solid; $[\alpha]_D^{25}$ + 59.0 (c 1.0, MeOH); IR ν_{max} 3498, 2958, 1698, 1279, 1250, 1064, 957, 860 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table (Tables 1 and 2) HRESIMS *m/z* 279.1571 $[M+Na]^+$ (calculated for $C_{14}H_{24}O_4Na$ 279.1572). *Compound* ***3****:* white powder; $[\alpha]_D^{25}$ – 30.6 (c 1.0, MeOH); IR ν_{max} 2928, 1698, 1276, 1250, 1064, 957, 860 cm^{-1}; ^1H NMR and ^{13}C NMR data, Table (Tables 1 and 2); HRESIMS *m/z* 293.1324 $[M+Na]^+$ (calculated for $C_{14}H_{22}O_5Na$ 293.1365).
16. *Crystal data for Sch-642305:* $C_{14}H_{20}O_4$ (molecular mass 252.30), thin elongated rod, 0.58 × 0.25 × 0.19 mm, orthorhombic, space group P $2_12_12_1$, a = 5.6506(2) Å, b = 12.5744(4) Å, c = 18.4526(13) Å, V = 1311.11(11) $Å^3$, Z = 4, ρ_{calcd} = 1.278 g cm^{-3}, $F(0\,0\,0)$ = 544, λ (CuK_α) = 1.54187 Å, $2\theta_{max}$ = 72.09°, $-6 \leqslant h \leqslant 5$, $-15 \leqslant k \leqslant 11$, $-22 \leqslant l \leqslant 22$, 7411 measured reflections, 2400 independent, R(int) = 0.0415, μ = 0.759 mm^{-1}, T_{min} = 0.728 and T_{max} = 1.000, 166 parameters were refined against all reflections, R1 = 0.0417, wR2 = 0.1108 (using all data) based on observed F values, R1 = 0.0351, wR2 = 0.0828 (3288 reflections with $I > 2\sigma(I)$), extinction coefficient = 0.0119(8), $\Delta\rho_{min}$ and $\Delta\rho_{max}$ = −0.204 and 0.188 e $Å^{-3}$, GOF = 1.159 based on F^2. After extensive refinements, the compound was assumed 100% enantiopure as (4*S*,5*R*,6*R*,11*S*)-4-hydroxy-11-methyl-4,5,6,7,8,9,10,11-octahydro-1*H*-benzo[*d*]oxecine-1,12-dione.
Crystal data of compound ***1****:* $C_{14}H_{22}O_4$, Mr = 254.32, parallelepiped, 0.58 × 0.23 × 0.19 mm, orthorhombic, space group P $2_12_12_1$, a = 6.2185(2) Å, b = 11.4026(4) Å, c = 18.9071(13) Å, V = 1340.65(11) $Å^3$, Z = 4, ρ_{calcd} = 1.260 g cm^{-3}, $F(0\,0\,0)$ = 552, λ(CuK_α) = 1.54187 Å, $2\theta_{max}$ = 68.13°, $-7 \leqslant h \leqslant 4$, $-13 \leqslant k \leqslant 10$, $-22 \leqslant l \leqslant 14$, 7497 measured reflections, 2429 independent, R(int) = 0.0313, μ = 0.743 mm^{-1}, T_{min} = 0.728 and T_{max} = 1.000, 166 parameters were refined against all reflections, R1 = 0.0409, wR2 = 0.0780 (using all data) based on observed F values, R1 = 0.0287, wR2 = 0.0641 (2049 reflections with $I > 2\sigma(I)$), extinction coefficient = 0.0041(4), $\Delta\rho_{min}$ and $\Delta\rho_{max}$ = −0.117 and 0.143 e $Å^{-3}$, GOF = 1.104 based on F^2. After extensive software and calculation refinements the compound assumed 100% enantiopure as (4*S*,8a*R*,12*S*,12a*R*)-12-hydroxy-4-methyldecahydro-1*H*-benzo[*d*]oxecine-2,9-dione.
Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre under the respective following deposit number CCDC-786076 for Sch-642305 and CCDC- 805294 for compound 1. Copies of these data can be obtained, free of charge on application to the Director, CCDC 12 Union Road, Cambridge CB2 1EZ, UK (fax: (+44) 1223 336033; or e-mail: deposit@ccdc.cam.uk).
17. Arseniyadis, S.; Rodriguez, R.; Dorado, M. M.; Alves, R. B.; Ouazzani, J.; Ourisson, G. *Tetrahedron* **1994**, *50*, 8399.
18. Ouazzani, J.; Arséniyadis, S.; Alvarez-Manzaneda, R.; Rumbero, A.; Ourisson, G. *Tetrahedron Lett.* **1991**, *32*, 1983.
19. Herlem, D.; Ouazzani, J.; Khuong-Huu, F. *Tetrahedron Lett.* **1996**, *37*, 1241.
20. The strains were cultivated in a liquid medium consisting of (per liter of water) 10 g of corn steep, 0.5 g of $MgSO_4$, 2 g of $NaNO_3$, 0.5 g of KCl and 0.02 g of $FeSO_4$. The microorganisms were allowed to grow at 27 °C in rotary shaker mixtures (200 rpm). After three days, the biomass was recovered by filtration, washed twice with water, compacted and then added directly to water containing a suspension of the substrate Sch-642305. In a typical analytical incubation, 5 g of wet biomass was introduced into a 30 mL aqueous suspension of 10 mg of Sch-642305 in a 200 mL Erlenmeyer flask with or without 10% glucose.
21. 200 mg of Sch-642305 were incubated with 100 g of wet *Aspergillus ochraceus ATCC 1009* biomass in 0.5 L of water. The aqueous phase was recovered by filtration and extracted three times with ethyl acetate (3 × 500 mL). The organic layers were evaporated, giving 279 mg of extract. The extract was separated by flash chromatography on a CombiFlash Companion Chromatogram using a Redisep 24 g column eluted with a 30:70 mixture of Heptane/Ethyl acetate for 160 min with a flow rate of 30 mL min^{-1}. Three pure fractions were recovered consisting of 29.6 mg of **1**, 33.5 mg of **2** and 35.3 mg of **3** and 46 mg of a mixed fraction.
22. Tempete, C.; Werner, G. H.; Favre, F.; Rojas, A.; Langlois, N. *Eur. J. Med. Chem.* **1995**, *30*, 647.
23. Perri, V.; Rochais, C.; Santos, J. S. D.; Legay, R.; Cresteil, T.; Dallemagne, P.; Rault, S. *Eur. J. Med. Chem.* **2010**, *45*, 1146.
24. Holland, H. L.; Weber, H. K. *Curr. Opin. Biotechnol.* **2000**, *11*, 547.
25. Antibacterial activity was measured by the disk inhibition zone method against *Bacillus subtilis ATCC 6633*, *micrococcus luteus ATCC 10240* and *Echerichia coli ATCC 25922*. Discs were soaked with 100 μg of compound. Inhibition was compared to disks containing 10 μg gentamycin and 30 μg chloramphenicol. Cytotoxicity assays were conducted on two cell lines: human breast adenocarcinoma cells MDA-MB-231 (ATCC HTB-26) and human myelogenous leukemia cells K-562 (ATCC CCL-243). Experiments were conducted according to published procedures.[18,19] Cells were grown in D-MEM medium supplemented with 10% fetal calf serum (Invitrogen) and plated in 96-well microplates. After 24 h of growth, cells were treated with target compounds dissolved in DMSO from 10^{-5} to 10^{-10} M. After 72 h, MTS reagent (Promega) was added, and the absorbance was monitored (490 nm) to measure the inhibition of cell proliferation compared to untreated cells. IC_{50} determination experiments were performed in duplicate.