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Malassezin—A Novel Agonist of the Arylhydrocarbon Receptor from the Yeast *Malassezia furfur*

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Abstract—The yeast *Malassezia furfur* converts tryptophan into several indole compounds. One of these, malassezin, was identified as 2-(1*H*-indol-3-ylmethyl)-1*H*-indole-3-carbaldehyde (**1**). It was synthesized from *N*-Boc-indole-3-carbaldehyde in five steps with 12% overall yield. The compound easily cyclizes to indolo[3,2-*b*]carbazole (**7**) which is known to interact with the arylhydrocarbon receptor (AHR). Similarly, malassezin was found to induce cytochrome P450 as an agonist of AHR ($EC_{50} = 1.57\,\mu M$) in rat hepatocytes.

Introduction

Lipophilic yeasts of the genus *Malassezia* are part of the residential flora of human skin and many warm blooded animals.[1,2] They are also responsible for skin diseases such as pityriasis versicolor in human beings, but its pathogenesis is not fully understood.[3,4] In pityriasis versicolor the affected skin area exhibits fluorescence under UV light (366 nm) and in the final stage depigmentation appears due to interrupted melanin synthesis.[5] The latter effect may be ascribed to an inhibition of human tyrosinase[6–8] by a secondary metabolite produced by the fungus.

Recently, we reported a new minimal medium for the cultivation of *Malassezia furfur* (CBS 7019) which simulates phenomena characteristic for the clinical variance of pityriasis versicolor.[9,10] With tryptophan as the single nitrogen source, the formation of a series of alkaloids and simple degradation products exhibiting a variety of colour and fluorescence phenomena is induced.[11] In this publication we describe the structure of malassezin (**1**), a novel arylhydrocarbon receptor (ACR) agonist from cultures of *M. furfur*.

Results and Discussion

Isolation and structural elucidation

Malassezin (**1**) (Fig. 1) was originally obtained by a tyrosinase assay guided fractionation of the EtOAc extract from cultures of *M. furfur*. The crude extract was subjected to gel permeation chromatography on Sephadex LH-20, followed by preparative TLC and HPLC on reverse-phase C-8 silica gel to yield < 1 mg of malassezin as a colourless solid ($t_R = 21.2$ min on analytical HPLC using CH_3CN:H_2O as the eluent with a gradient of 15:85 to 60:40 in 25 min).

Malassezin (**1**) exhibits a molecular ion at *m*/*z* 274.1113 in the HRMS (EI) corresponding to the molecular formula $C_{18}H_{14}N_2O$. The NMR signals of **1** summarized in Table 1 were best resolved in DMSO-d_6:CCl_4 (4:1). The aromatic protons 4-H to 7-H (and their counterparts 4′-H to 7′-H) form a AA′BB′-pattern in the ^{1}H NMR spectrum that is supported by COSY experiments. Five singlets can be assigned to a formyl group (δ_H 10.20), an indole 2-H (δ_H 7.25), two indole NH groups and a

Abbreviations: CYP, cytochrome P450; DMEM, Dulbecco's modified Eagle's medium; EGTA, ethylene glycol-bis(β-aminoethyl ether) *N*,*N*,*N*′,*N*′-tetraacetic acid; EROD, 7-ethoxyresorufin *O*-deethylase; FCS, fetal calf serum; Tris, tris(hydroxymethyl)aminomethane.

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†X-ray crystal structure analysis.

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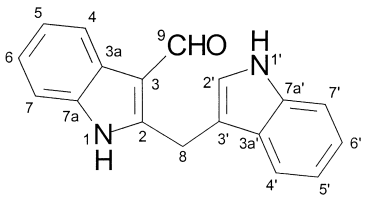


Figure 1. Malassezin (**1**).

methylene group (δ_H 4.55) that appears in the ^{13}C NMR spectrum at unusually high field (δ_C 21.92). These facts and an IR (KBr) absorption at 1630 cm^{-1} suggest the presence of both an indole and an indole–carbaldehyde moiety connected by a methylene group. In order to elucidate the position of the formyl group we compared the UV/Vis spectrum of **1** with those of 1:1 mixtures of indole and indole-2- and 3-carbaldehyde, respectively. Since correspondence was found only in the latter case, the formyl group can be located at C-3 of the indole nucleus. This suggests structure **1** for malassezin, in accord with the occurrence of NOE's between the methylene protons and H-2′ and H-4′ of the indole nucleus. Structure **1** was proven by a total synthesis that provided the compound in quantities sufficient for biological evaluation.

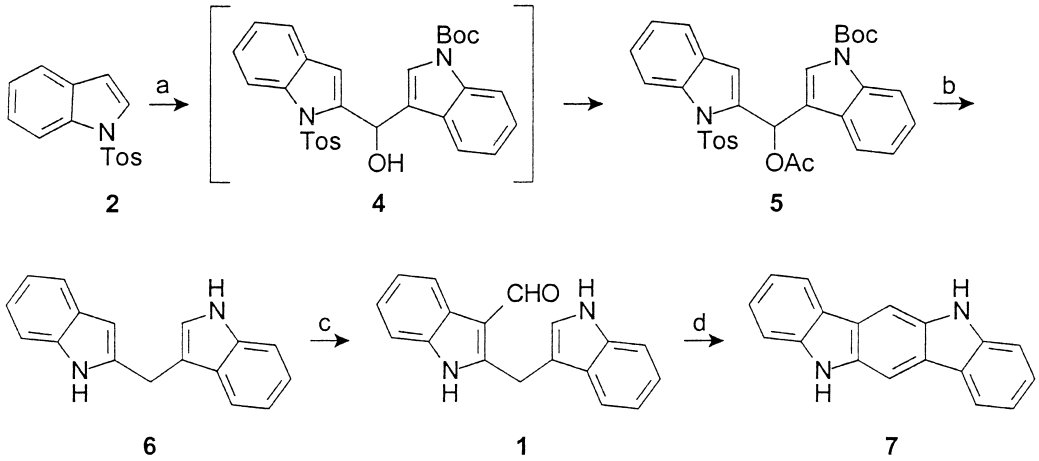
Table 1. ^1H and ^{13}C NMR data (600 MHz) of malassezin (**1**) in CCl_4:DMSO-d_6 (1:4)

Pos.	^1H NMR[a]	COSY	^{13}C NMR[b]
1	11.98[c] (s)		
2			151.3
3			113.0
3a			126.5
4	8.01 (d, 6.7)	5	120.1
5	7.1 (m)	4,6	121.8
6	7.1 (m)	5,7	122.6
7	7.35 (d, 7.0)	6	111.6
7a			136.2
1′	10.98[c] (s)		
2′	7.25 (s)		123.7
3′			110.8
3a′			125.5
4′	7.48 (d, 8.1)	5′	118.2
5′	6.91 (t, 7.5)	4′,6′	118.5
6′	7.02 (t, 7.6)	5′,7′	121.1
7′	7.31 (d, 7.9)	6′	111.5
7a′			135.4
8	4.55 (s)		21.9
9	10.20 (s)		184.4

[a]δ (Multiplicity, *J* in Hz) in ppm.
[b]δ in ppm.
[c]Exchanges with D_2O.

Total synthesis

Recently, we have shown that indole and indole-3-carbaldehyde occur in cultures of *M. furfur*.[9] Commencing with these compounds, we synthesized **1** in five steps (Scheme 1). Coupling of the anion obtained by *ortho*-metalation[12,13] of *N*-tosyl-indole (**2**) with *N*-Boc-indole-3-carbaldehyde (**3**) afforded the alcohol **4** in 74% yield. Experiments to remove the hydroxy group in **4** by hydrogenolysis with H_2/Pd-C were unsatisfactory. However, treatment of the crude alcohol **4** with acetyl chloride followed by reaction of the resulting acetate **5** with sodium in liquid ammonia led to reductive removal of the acetoxy group with simultaneous cleavage of all protective groups.[14] Pure 2,3′-methylenebisindole[15] (**6**) was thereby obtained in varying yields ranging from 40–52%. Best results were obtained by using only a small excess of sodium. In the final step, **1** was synthesized by a Vilsmeier formylation.[16] At room temperature the reaction proceeds sluggishly, however, by careful heating to 40 °C, the desired product **1** was obtained in 74% yield. At higher temperatures, only the condensation product **7** was isolated as a poorly soluble solid. Malassezin shows a high tendency for crystallization, and suitable crystals for an X-ray crystallographic analysis (Fig. 2) could be grown from the mother liquor at −20 °C. Synthetic malassezin (**1**) agreed in all its spectroscopic data with the natural product, however, it was devoid of any in vitro tyrosinase activity. This may be explained by the presence of small amounts of an unknown tyrosinase inhibitor in the natural malassezin fractions. Efforts to identify this elusive compound are under way.



Scheme 1. Reagents and conditions: (a) 1. *tert*-BuLi, THF, −20 °C, 2. *N*-Boc-indole-3-carbaldehyde (**3**) in THF, 3. CH_3COCl, −20 °C to rt, 57%; (b) Na, liquid NH_3, THF, −78 °C, 40–52%; (c) $POCl_3$, DMF, 40 °C, 74%; (d) cat. HCl, THF, reflux, 78%.

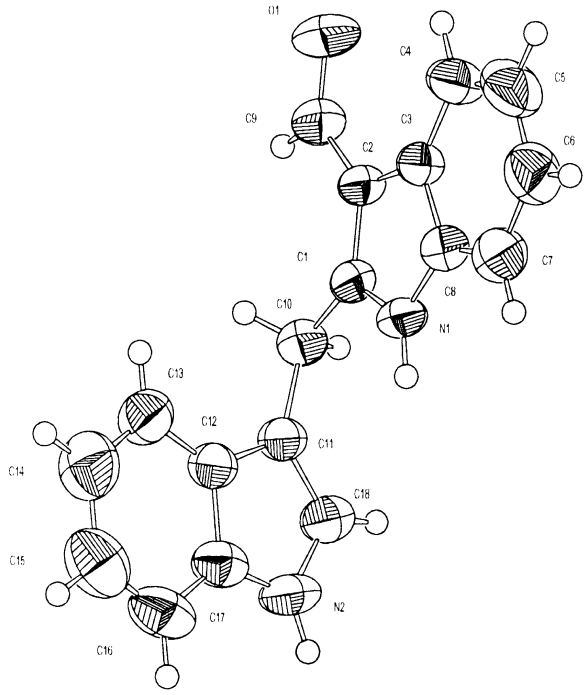
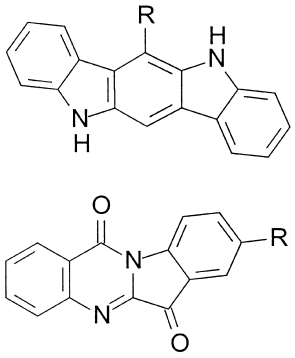


Figure 2. X-ray crystallographic structure of malassezin (**1**) (cyrstallographic numeration of atoms was used).[17,18]



	R	EC_{50} (M)
7	H	2.6×10^{-7}
8	CHO	7.0×10^{-11}
9	H	6.1×10^{-6}
10	CH_3	1.7×10^{-6}
11	Br	1.7×10^{-7}

Figure 3. Natural AHR agonists: indolo[3,2-*b*]carbazole (**7**), 6-formylindolo[3,2-*b*]carbazole (**8**) and tryptanthrines (**9**–**11**).

As expected,[19,20] heating of **1** in THF with a catalytic amount of concd HCl afforded the indolocarbazole **7** in 78% yield. The NMR data and MS fragmentation pattern of **7** were in agreement with those of the literature.[21]

Biological activity

The arylhydrocarbon receptor (AHR) is involved in cell growth, differentiation and regulation of gene expression.[22] It was shown that the AHR plays a role in the transcriptional regulation of certain genes encoding drug-metabolizing enzymes. Likewise, agonists of the AHR induce an increased transcription of cytochrome P450 (CYP) 1A1. The agonistic potency can be described by the EC_{50} values for the induction of CYP 1A1-dependent 7-ethoxyresorufin *O*-dealkylase (EROD) activity.[23]

Indolo[3,2-*b*]carbazole[19,22,24] (**7**) is a strong agonist of the AHR and an in vivo dimerization product of (1*H*-indol-3-yl)-methanol, a breakdown product from vegetables of the *Brassica* genus.[25,26] Noteworthy, **7** was also detected in faeces, urine, the gastrointestinal tract and livers of rats, and also in human faeces. 6-Formylindolo[3,2-*b*]carbazole (**8**) was found to be the strongest AHR agonist known so far (Fig. 3).[20,27] The first AHR agonists with a tryptanthrine skeleton (**9**–**11**) were isolated from cultures of *Candida* yeasts containing anthranilic acid and 5-substituted tryptophans (Fig. 3).[28]

In our experiments with rat hepatocytes malassezin (**1**) was submitted to EROD bioassays because of its structural similarity to the known AHR-agonists (Fig. 3). **1** was found to act as an AHR agonist inducing CYP 1A-catalysed EROD activity ($EC_{50} = 1.57\,\mu M$) in a concentration range similar to methyltryptanthrine (**10**).[28] Malassezin is a non-planar molecule (see X-ray crystal structure, Fig. 2) and does not fit into a 7×14 Å rectangle. Thus, it does not fulfill major structural requirements for potent AHR agonists.[22] Further investigations are needed to elucidate if malassezin represents a novel prototype of AHR agonists. Alternatively, it may be easily converted into a potent agonist such as indolo[3,2-*b*]carbazole (**7**). In supernatants of cell cultures no **7** could be detected by fluorescence/HPLC analysis. However, the formation of minor amounts of **7** sufficient for AHR activation, e.g. by intracellular metabolism, cannot be excluded. It remains to be elucidated if the yeast benefits from the biosynthesis of an AHR agonist and/or if AHR activation by malassezin contributes to the pathogenesis of pityriasis versicolor.

Conclusion

Malassezin (**1**) is the first alkaloid described from cultures of a lipophilic *Malassezia* yeast. Compared to the non-active 2,3′-methylenebisindole (**6**) the activity of **1** as AHR agonist indicates the crucial influence of the formyl group as previously described for **7** and **8**.[20] Alternatively, **1** may be converted intracellularly into the potent AHR agonist **7**.

Experimental

Material and methods

All common reagents and components of the yeast medium were obtained from Sigma-Aldrich-Fluka (Deisenhofen, Germany) and used without further purification unless otherwise indicated. Yeast strains where supplied from the Centraalbureau voor Schimmelcultures, The Netherlands. Merck silica gel 60 plates (0.5 mm) were used for preparative TLC. Analytic HPLC was performed on a Merck-Hitachi L-6200A instrument, preparative HPLC on a Gilson Model 302. For separation LiChrospher reversed phase material (RP-8, anal: 4×250, prep: 10×250 mm) was used with

isocratic acetonitrile/water mixtures. Flow rates were 1 and 6.25 mL/min, respectively. Compounds were monitored at 220 nm and dried on a Lyovac GT2. The extinction of the dopaquinone was measured with a Beckman DU-68 spectrophotometer ($\lambda_{max} = 475$ nm).

All chemical reactions were carried out under an argon atmosphere in flame-dried vessels with dry, freshly distilled solvents under anhydrous conditions unless otherwise indicated. Dry and air sensitive liquids were handled via syringes. Dry THF was distilled from sodium/benzophenone, pyridine and DMF were purchased as dry solvents. Solvents for work-up procedures were distilled before use. Reactions were monitored by TLC on 0.25 mm silica gel plates (Merck, 60 F_{254}) unless otherwise indicated, using UV light for visualizing and ethanolic *p*-anisaldehyde (2.5%) or acidic $KMnO_4$ solutions as developing agents. Silica gel (Merck 60, particle size 0.043–0.063 mm) was used for flash column chromatography.

NMR spectra were recorded on Bruker AMX-600 (^{1}H: 600.19 MHz, ^{13}C: 150.92 MHz) and ARX-300 (^{1}H: 300.13 MHz, ^{13}C: 75.47 MHz) instruments and calibrated with the residual undeuterated solvent as the internal reference. The following abbreviations were used to denote the multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br broad; app apparent. IR spectra: Perkin-Elmer 1420 or Bruker IFS 45 FT-IR. High-resolution mass spectra (HRMS): Finnigan MAT 90 or MAT 95 Q, electron ionization (EI). X-ray crystal structure analysis: Enraf-Nonius CAD4. UV/VIS: Perkin-Elmer Lambda 16. Melting points (mp) are uncorrected and were recorded on a Reichert Thermovar.

DMEM, penicillin and streptomycin were purchased from Biochrom (Berlin, Germany), FCS and collagenase from Gibco (Karlsruhe, Germany), and isocitrate dehydrogenase from Roche (Mannheim, Germany). Solutions for treatment of cell cultures were obtained by dissolving malassezin (**1**) in DMSO and preparing successive dilutions resulting in final DMSO concentrations of 0.5% in the culture medium.

Cultures of *Malassezia furfur* and isolation of malassezin (1); Medium

The medium for the induction of alkaloid synthesis consisted of Tween 80 ultra® (30 mL), cycloheximide (0.5 g), chloramphenicol (0.05 g) and agar (20 g). The volume of the medium was adjusted to 1000 mL with distilled water. After sterilization, sterile filtered L-tryptophan at a concentration of 0.3 g% was added at 50 °C and 10 mL portions of the medium were poured into sterile Petri dishes (10 cm in diameter). The pH was adjusted to 5.5 using HCl (0.1 M).

Cultivation and isolation

A suspension of CBS 1878 *M. furfur* was smeared on the minimal medium by a swab. After incubation for 14 days at 30 °C, the content of the Petri dishes was pureed and extracted with EtOAc for 12 h. The extract was filtrated over glass wool, evaporated to dryness and dissolved in methanol. The crude extract was fractionated by chromatography on Sephadex LH-20 with methanol as eluent and further separated by preparative TLC with toluene:ethyl formate:formic acid (10:5:3). The main zones were partitioned between H_2O and EtOAc and the organic phases subjected to the tyrosinase bioassay. The active fractions still contained several components which were separated by HPLC. Malassezin (**1**) was isolated from a tyrosinase-active peak with $t_R = 21.2$ min on analytical HPLC using CH_3CN: H_2O as the eluent with a gradient of 15:85 to 60:40 in 25 min.

Synthesis

3-Formylindole-1-carboxylic acid *tert*-butyl ester (3). To a solution of indole-3-carbaldehyde (7.26 g, 50 mmol) in dry THF (100 mL) were added Boc_2O (12.00 g, 55 mmol) and a catalytic amount of DMAP at 0 °C under argon. The TLC showed complete reaction after 30 min, and the solvent was removed in vacuo (bath temperature < 35 °C). Crystallization from MeOH afforded **3** as yellowish needles (11.02 g, 90%), mp 110 °C. TLC: R_f 0.55 (hexanes:EtOAc 5:1); ^{1}H NMR ($CDCl_3$) δ 1.70 (s, 9H), 7.34 (dt, 1H, $J = 1$ Hz, $J = 7.0$ Hz), 7.39 (dt, 1H, $J = 1$ Hz, $J = 7.0$ Hz), 8.12 (dd, 1H, $J = 1$ Hz, $J = 7.5$ Hz), 8.18 (s, 1H, H-2), 8.25 (dd, 1H, $J = 1$ Hz, $J = 7.5$ Hz); ^{13}C NMR ($CDCl_3$) δ 27.3, 85.0, 114.6, 120.9, 121.5, 123.9, 125.4, 125.5, 135.4, 135.9, 148.2, 185.1; IR (KBr) 1744, 1679, 1558 cm^{-1}; MS (EI) *m*/*z* 245.1 (M^+). Anal. ($C_{14}H_{15}NO_3$); calcd: C, 68.56; H, 6.21; N, 5.71. Found: C, 68.80; H, 6.18; N, 5.72.

3-{Hydroxy-[1-(4-methylphenylsulfonyl)-1*H*-indol-2-yl]-methyl}-indole-1-carboxylic acid *tert*-butyl ester (4). 3.24 mL (5.5 mmol) of *tert*-BuLi (1.7 M in pentane) were added at −20 °C to a vigorously stirred solution of 1-[(4-methylphenyl)sulfonyl]-1*H*-indole (**2**)[29] (1.36 g, 5.01 mmol) in dry THF (40 mL). The mixture was brought to −5 °C within 40 min and allowed to warm to 20 °C for 5 min. The solution was treated at −20 °C with **3** (1.23 g, 5.0 mmol) dissolved in dry THF (20 mL), stirred for 1 h and then warmed to 20 °C. The reaction mixture was carefully quenched with water (40 mL), and the mixture was extracted with ether (3×50 mL). The combined organic layers were washed with brine and dried over $MgSO_4$. Chromatography with hexanes:EtOAc (20:1 to 5:1 gradient) afforded **4** as a yellowish solid (1.91 g, 74%), mp 151 °C. TLC: R_f 0.31 (hexanes:EtOAc 5:1); ^{1}H NMR ($CDCl_3$) δ 1.67 (s, 9H), 2.34 (s, 3H), 3.78 (d, 1H, $J = 5$ Hz), 6.36 (s, 1H), 6.58 (d, 1H, $J = 5$ Hz), 6.93 (d, 1H, $J = 8$ Hz), 7.03 (dt, 1H, $J = 8.5$ Hz, $J = 1$ Hz), 7.17 (d, 2H, $J = 8.5$ Hz), 7.18 (s, 1H), 7.21–7.36 (m, 4H), 7.69 (d, 2H, $J = 8.5$ Hz), 8.16 (d, 1H, $J = 8.5$ Hz), 8.18 (d, 1H, $J = 8.5$ Hz); ^{13}C NMR ($CDCl_3$) δ 21.5, 28.1, 63.4, 83.8, 111.9, 114.7, 115.6, 119.8, 120.7, 121.4, 122.5, 123.7, 124.1, 124.3, 125.2, 126.3, 128.5, 128.8, 129.9, 136.7, 135.8, 137.5, 142.0, 145.1, 149.6; IR (KBr) 3443, 1734, 1372, 1173 cm^{-1}; HRMS (EI) *m*/*z* 516.1923 [M^+], calculated for $C_{29}H_{28}NO_5S$, 516.1717.

3-{Acetoxy-[1-(4-methylphenylsulfonyl)-1*H*-indol-2-yl]-methyl}-indole-1-carboxylic acid *tert*-butyl ester (5). 6.47 mL (11 mmol) of *tert*-BuLi (1.7 M in pentane) were added at −20 °C to a vigorously stirred solution of **2** (2.71 g, 10 mmol) in dry THF (80 mL). The mixture was heated to −5 °C within 40 min and allowed to warm up to rt for 5 min. The solution was treated at −20 °C with **3** (2.45 g, 10 mmol) dissolved in dry THF (35 mL), stirred for 2 h, and then treated with acetyl chloride (1.36 mL, 20 mmol) and warmed to rt overnight. The reaction mixture was carefully quenched with water (50 mL), and the mixture was extracted with ether (3×80 mL). The combined organic layers were washed with $NaHCO_3$ (5%, 3×70 mL), brine and dried over $MgSO_4$. Chromatography with hexanes:EtOAc (10:1) afforded **5** as a yellowish solid (3.18 g, 57%), mp 172 °C, besides unreacted alcohol **4** (852 mg, 1.65 mmol). TLC: R_f 0.42 (hexanes:EtOAc 5:1); ^{1}H NMR (acetone-d_6) δ 1.65 (s, 9H), 2.15 (s, 3H), 2.32 (s, 3H), 6.95 (t, 1H, J=1 Hz), 7.23 (t, 1H, J=1 Hz), 7.26 (d, 2H, J=8.5 Hz), 7.27 (t, 2H, J=7 Hz), 7.35 (2 ddd, 2 H, J=1.5, J=6 Hz, J=6 Hz and J=1.5 Hz, J=6 Hz, J=10 Hz), 7.54 (d, 1H, J=6 Hz), 7.57 (d, 1H, J=6 Hz), 7.74 (d, 2H, J=8 Hz), 7.90 (t, 1H, J=1 Hz), 8.13 (d, 1H, J=8 Hz), 8.16 (d, 1H, J=8 Hz); ^{13}C NMR (acetone-d_6): δ=21.1, 21.6, 28.3, 65.6, 85.1, 112.5, 115.7, 116.2, 119.7, 120.8, 122.4, 123.8, 124.9, 125.7, 126.1, 126.6, 127.6, 129.5, 130.1, 130.8, 136.5, 136.5, 138.3, 139.8, 146.4, 150.2, 170.3; IR (KBr) 1736, 1371, 1175 cm^{-1}; UV/VIS (CH_3OH) λ_{max} (lgε) 220 (4.63), 254 (4.41), 284 (3.97), 292 (3.96); HRMS (EI) m/z 558.1825 [M$^+$], calcd for $C_{31}H_{30}N_2O_6S$, 558.1805.

2,3′-Methylenebisindole (6). 20–25 mL of ammonia were condensed into a solution of **5** (370 mg, 0.66 mmol) in dry THF (3 mL) at −78 °C under mechanical stirring. The yellow muddy liquid was treated portion-wise with sodium (50–100 mg) until the blue colour remained permanent for 30 min. The reaction was quenched with NH_4OAc (2 g, 26 mmol) and warmed up to 20 °C. The mixture was diluted with saturated aqueous NH_4OAc (30 mL) and extracted with ether (3×50 mL). The combined organic layers were washed with brine and dried over $MgSO_4$. Chromatography with hexanes:EtOAc (5:1) afforded **6** as a colourless solid that turned pink on exposure to air[15] (85 mg, 52%), mp 130–134 °C. TLC: R_f 0.43 (hexanes:EtOAc 3:1); ^{1}H NMR (acetone-d_6) δ 4.25 (s, 2H), 6.28 (q, 1H, J=1 Hz), 6.94 (dt, 1H, J=7.5 Hz, J=1.5 Hz), 6.96 (dt, 1H, J=7.5 Hz, J=1.5 Hz), 6.99 (dt, 1H, J=7.5 Hz, J=1.5 Hz), 7.09 (dd, 1H, J=8 Hz, J=1 Hz), 7.22 (dt, 1H, J=1 Hz), 7.27 (d, 1H, J=8.5 Hz), 7.39 (d, 1H, J=8 Hz), 7.43 (d, 1H, J=8 Hz), 7.52 (d, 1H, J=8 Hz), 9.89 (br. s, 1H), 10.01 (br. s, 1H); ^{13}C NMR (acetone-d_6) δ 25.0, 100.2, 111.4, 112.1, 113.3, 119.4, 119.5, 119.6, 120.2, 121.1, 122.1, 124.0, 128.4, 129.9, 137.5, 137.8, 140.2; IR (KBr) 3396 cm^{-1}; UV/VIS (CH_3CN) λ_{max} (lgε) 223 (4.76), 272 (4.13), 280 (4.13), 289 (4.04); MS (EI) m/z 246.1 (M$^+$). The data are in agreement with those given in ref 15.

2-(1*H*-Indol-3-ylmethyl)-1*H*-indol-3-carbaldehyde, malassezin (1). A solution of $POCl_3$ (0.072 mL, 0.8 mmol) in dry DMF (2 mL) was stirred at 0 °C for 20 min under argon and then warmed to 20 °C for 15 min. The liquid was treated at 0 °C with **6** (180 mg, 0.73 mmol) dissolved in dry DMF (3 mL), and the ice bath was removed after 20 min. Careful warming to 40 °C completed the formylation reaction as indicated by TLC. The dark brown solution was poured into saturated aqueous $NaHCO_3$ (15 mL) at 20 °C and extracted with ether (3×20 mL). The combined organic layers were washed with NaOH (1 M, 320 mL) and brine, dried over $MgSO_4$ and concentrated in vacuo (5 mL). A first batch of **1** (102 mg) crystallized from ether at −20 °C in form of pinkish crystals. Chromatography of the mother liquor with hexanes:EtOAc (5:1–3:1 gradient) afforded a second batch (46 mg) of **1** (total yield: 148 mg, 74%), mp 240 °C. TLC: R_f 0.52 (hexanes:EtOAc 1:1); ^{1}H NMR (DMSO-d_6) δ 4.57 (s, 2H), 6.94 (t, 1H, J=7 Hz), 7.06 (t, 1H, J=8 Hz), 7.13 (t, 2H, J=3 Hz, J=4.5 Hz), 7.26 (d, 1H, J=2.5 Hz), 7.34 (d, 1H, J=8 Hz), 7.38 (d, 1H, J=4 Hz), 7.49 (d, 1H, J=8 Hz), 8.05 (t, 1H, J=4 Hz), 10.28 (s, 1H), 10.98 (s, 1H), 11.90 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 22.1, 110.9, 111.6, 111.8, 113.2, 118.3, 118.7, 120.3, 121.3, 122.0, 122.7, 123.8, 125.7, 126.7, 135.5, 136.3, 151.4, 184.6; IR (KBr) 3402, 3379, 1618 cm^{-1}; UV/Vis (CH_3CN) λ_{max} (lgε) 219 (4.73), 243 (4.23), 268 (4.20), 282 (4.15), 290 (4.17); HRMS (EI) m/z 274.1106 [M$^+$], calculated for $C_{18}H_{14}N_2O$, 274.1116.

Indolo[3,2-*b*]carbazole (7). A solution of **1** (13 mg, 0.045 mmol) in THF (15 mL) was treated with one drop of concd HCl in an open flask. The mixture was refluxed overnight, cooled to room temperature and filtered to yield **7** as a yellowish precipitate (9 mg, 78%), mp >350 °C. TLC (RP-18): R_f 0.60 (MeOH 100%); ^{1}H NMR (DMSO-d_6) δ 7.10 (t, 2H, J=7.5 Hz), 7.31 (t, 2H, J=7.5 Hz), 7.42 (d, 2H, J=7 Hz), 8.09 (s, 2H), 8.17 (d, 2H, J=7 Hz), 10.98 (s, 2H); ^{13}C NMR (DMSO-d_6) δ 101.3, 111.3, 118.5, 121.1, 123.4, 123.5, 126.3, 135.9, 142.0; IR (KBr) 3403 cm^{-1}; UV/Vis (MeOH) λ_{max} (qual.) 213, 249, 270, 320, 334 nm; MS (EI) m/z 256 (M$^+$). The data are in agreement those given in ref 21.

EROD Bioassay

Cell culture and treatment. Hepatocytes were prepared from male Wistar rats (Charles River, Kisslegg, Germany) as described earlier.[30] In brief, after anesthesia with sodium pentobarbital (100 mg/kg b.w., ip), livers were perfused in a two-step procedure with a calcium-free, EGTA-supplemented buffer and with a calcium-containing buffer supplemented with collagenase (500 mg/L), respectively, each step taking 10 min. Hepatocytes were dispersed in a BSA-containing washing buffer, filtered through silk gauze, and washed with washing buffer. Viability was measured with a trypan blue assay, and preparations showing viability >90% were seeded on collagenated Petri dishes (6 cm diameter) at a density of 70,000 cells/cm^2 in DMEM containing 20% FCS, dexamethasone (0.1 μM), penicillin (100 U/mL), and streptomycin (100 μg/mL). After 3 h, medium was replaced by fresh medium, malassezin (**1**) was added in DMSO (0.5% final concentration), and the cells were incubated for additional 48 h. DMSO served as a control.

Analysis of CYP 1A activity

Cells were washed with ice-cold saline and scraped of with a tris-buffered glucose solution (50 mM Tris, 200 mM glucose, pH 7.4). The cells were then centrifuged at 1000 g for 5 min and homogenized by sonication (50 W) on ice. Activity of the CYP 1A isozymes in the homogenates were measured by determination of their EROD activity according to the method of Burke and Mayer.[31] Protein contents were analyzed according to Lowry.[32]

Statistical analysis

Treatments of primary hepatocytes were carried out three times in duplicate. Dose–response curves, EC_{50}-values, and 95%-confidence intervals were calculated using a computerized log-probit procedure (Origin 5.0, Microcal, Northampton, USA).

Tyrosinase bioassay

335 μL of a L-DOPA stock solution (6.4 mM in 0.05 M phosphate buffer at pH 7.0) and 805 μL of the buffer were mixed. After addition of 107 μL tyrosinase (EC 1.14.18.1; 500 U/mL in phosphate buffer) the extinction was measured over 1 min indicating the formation of dopaquinone. Fractions of the crude extract obtained by chromatography on Sephadex LH 20 were dissolved in DMSO (100 μL) and added to the reaction with pure DMSO as control. Inhibitory activity of the single fractions was measured as reduced increase in extinction in comparison to the control.

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