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Synthesis and Biological Evaluation of 2,3′-Diindolylmethanes as Agonists of Aryl Hydrocarbon Receptor

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Abstract

Recent studies suggest that arylhydrocarbon receptor (AhR) may be a target for a number of diseases. Natural product malassezin is a AhR agonist with an interesting 2,3′-diindolylmethane skeleton. We have prepared a series of analogues of natural product malassezin using our recently developed method and tested the activity of these analogues against AhR in a cell-based assay. We found that a methyl substituent at 1′-N can significantly increase the activity and the 2-formyl group is not critical for some diindolylmethanes.

Keywords

AhR; diindolylmethane; agonist; indole; transcription factor

The arylhrydrocarbon receptor (AhR) is a basic helix-loop-helix transcription factor that is well conserved across many species.[1] AhR readily binds to various endogenous and xenobiotic polyaromatic heterocycles.[2] It is perhaps best known for its role in conferring the toxicity of environmental pollutant 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD or dioxin) **1** (Figure 1).[3] AhR binds to TCDD with remarkably high affinity and upon binding, the ligand-receptor complex travels to the nucleus where AhR then dimerizes with the aryl hydrocarbon receptor translocator (ARNT).[4] It is this active dimer that functions to promote or repress the transcription of a multitude of different genes, most notably CYP1A1.[5] AhR activation is highly regulated by a negative feedback mechanism to prevent continuous signaling.[6] It is believed that prolonged AhR signaling is very unfavorable.[7] Therefore, it has been proposed that the toxicity of dioxin is due to its exceptionally long metabolic stability (half-life of 7–10 years in human),[3] resulting in a continuously activated AhR.

The physiological role of AhR has been a long sought problem.[2–3] Recent data showed that AhR was involved in many important biological processes such as immune cell differentiation,[8] intestinal function,[9] and development of prostate.[10] It has been postulated that AhR may be a potential target for the treatment of benign prostatic hyperplasia,[11]

reduction of undesired immune responses during organ transplantation,[12] inflammation disorders,[13] and certain types of cancer.[14] Peterson and Safe reported that synthetic AhR agonist 6-methyl-1,3,8-trichlorodibenzofuran (MCDF) **2** blocked vascular endothelial growth factor in prostate and conferred protection against prostate cancer in vivo.[15] Safe also showed that a variety of substituted 3,3′-diindolylmethanes (DIMs) **3**, which are also AhR agonists, inhibit tumor growth in rat models.[16] Natural products indolo[3,2-b]carbazole (ICZ) **4**, 6-formylindolo[3,2-b]carbazole (FICZ) **5**, and malassezin **6** have been demonstrated to be agonists of AhR. Both ICZ and FICZ are categorized as indolocarbazoles,[17] while malassezin[18] is a formylated 2,3′-diindolylmethane.[19] Recent studies suggested that FICZ had anti-asthmatic effects by inhibiting Th2 cytokine production in a mouse model.[20] Herein we report our efforts towards the development of selective AhR modulators by preparing an assortment of 2,3′-diindolylmethanes.

We have recently reported a platinum-catalyzed indole annulation/arylation cascade reaction for the synthesis of diindolylmethane **9** from propargylic ether **7** (Scheme 1) and demonstrated its utility in the total synthesis of natural product malassezin.[21] Based on this method, we prepared a collection of malassezin analogues **10** and **11** following the sequence in Scheme 1. After the formation of **9** using the previously established indole annulation/arylation protocol, we cleaved the Boc-group under thermal conditions to produce diindolylmethanes **10**, which were then treated with $POCl_3$ and DMF to give the formylated malassezin analogues **11**.[18,21]

We next turned our attention to the investigation of the bioactivity of malassezin analogues. We employed the well-documented ethoxyresorufin-*O*-deethylase (EROD) assay to determine the EC_{50} values of our compounds towards AhR activation in HepG2 cells.[22] This assay measures the induction of cytochrome P450-1A1 (CYP1A1), which is a major outcome of AhR activation.[23] CYP1A1 selectively converts 7-ethoxyresorufin to a fluorescent product resorufin.

Malassezin was served as the positive control (Table 1). The EC_{50} values of our malassezin analogues varied quite dramatically depending on their respective substitution pattern. For the halogenated 2,3′-diindolylmethanes **11a–c,** 5′-chlorination resulted in a compound (**11a**) more potent than the positive control, while 7′-chlorination gave a slightly weaker agonist (**11b**). Compound **11c**, with a methoxy substitution on the 5′-position, behaved similarly to malassezin. Interestingly, compound **11d** with an *N*-methyl group on the 1′-position was surprisingly potent; its EC_{50} value was about five times lower than that of malassezin.

We then investigated the importance of the formyl group in malassezin analogues for AhR activation. In the case of compound pair **10a**/**11a**, the formyl group contributed significantly to the potency of **11a**. In contrast, other non-formylated compounds including **6′**, **10c**, and **10d** have similar potency as their formylated counterparts (**6**, **11c**, and **11d**).

Up to 12 fold increase of signal over DMSO treatment was observed for many of the malassezing analogues. Even at 10 nM concentration, compounds **11a** and **11d** could induce 5 and 6 fold increase of signals, respectively (Figure 2).

In summary, we have synthesized a collection of novel analogues of the natural product malassezin and investigated their bioactivity in an EROD assay. It was found that some of these compounds were more potent than the parent malassezin and may become lead compounds for further diseases-relevant studies.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References and notes

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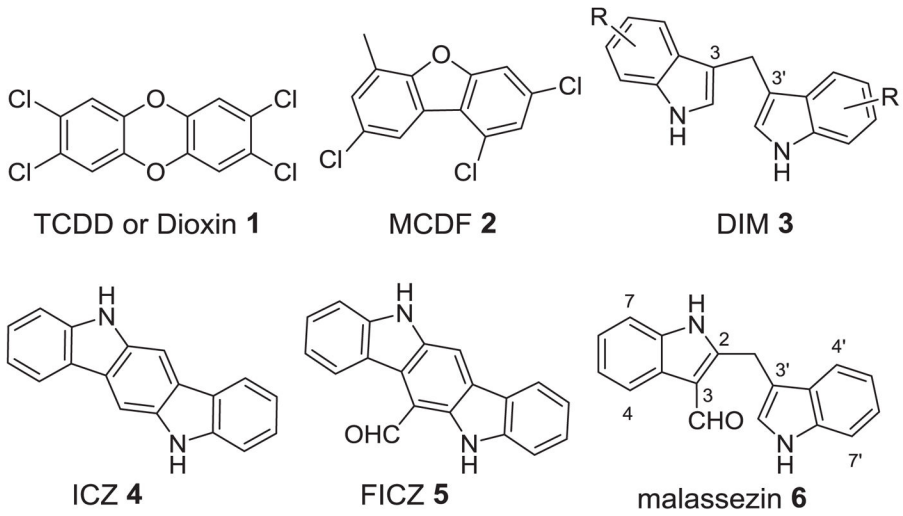


Figure 1.
Representative Synthetic and Natural AhR Agonists

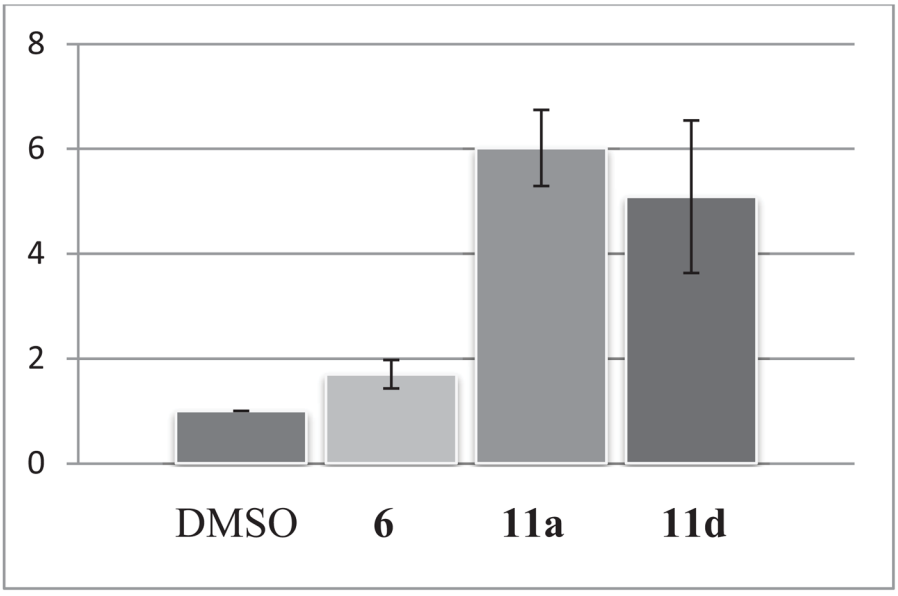
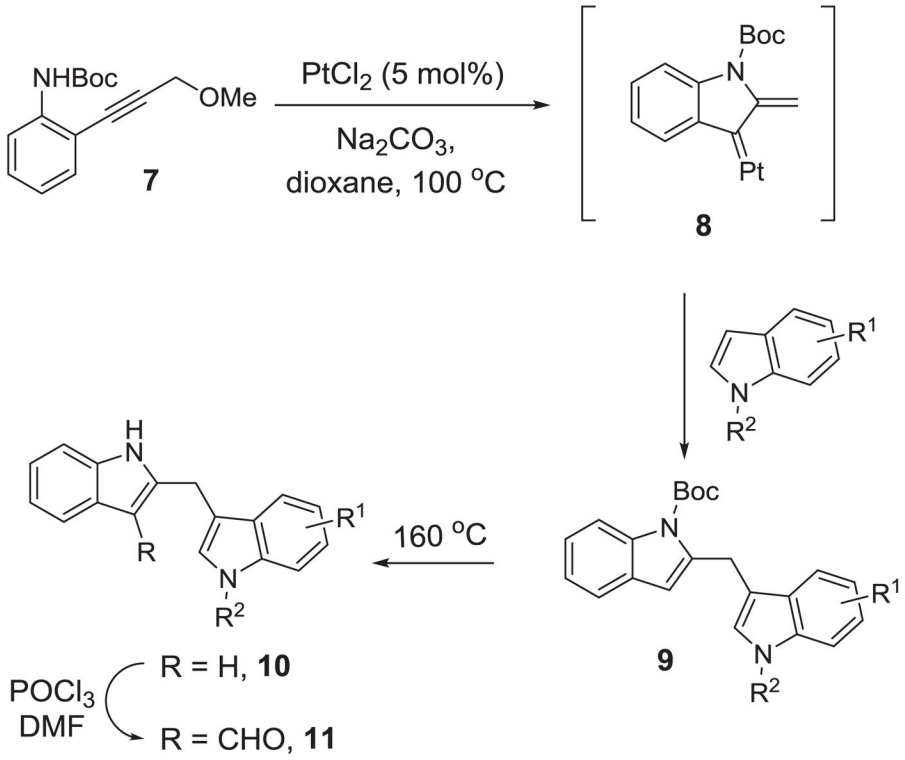


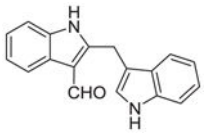
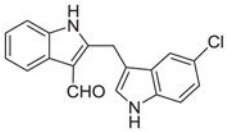
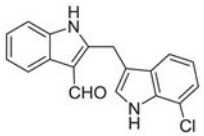
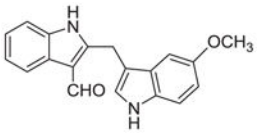
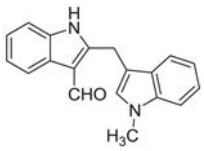
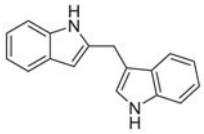
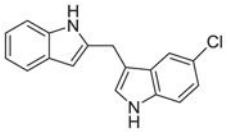
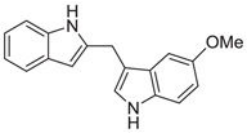
Figure 2.
Fold increase of signals (Y-axis) for compounds **6**, **11a**, **11d** over DMSO negative control at 10nM.

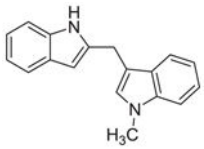


Scheme 1.
Synthesis of 2,3′-Diindolylmethanes

Table 1

AhR Activity of Diindolylmethanes

	Compounds	EC_{50} (μM)
6		0.27 ± 0.1
11a		0.093 ± 0.15
11b		0.32 ± 0.1
11c		0.23 ± 0.1
11d		0.055 ± 0.03
6′		0.215 ± 0.46
10a		> 6.7
10c		0.094 ± 0.27

	Compounds	EC_{50} (μM)
10d		0.086 ± 0.04