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Aspercyclide A–C, three novel fungal metabolites from *Aspergillus* sp. as inhibitors of high-affinity IgE receptor

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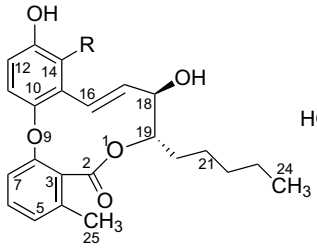
Abstract—Immunoglobin E (IgE) binds to the high-affinity IgE receptor on mast cells and basofils and causes release of inflammatory compounds that leads to allergic diseases. Inhibition of the ligand binding could lead to blockade of the release of inflammation causing compounds and thus alleviate asthma and other allergic diseases. Natural product screening and bioassay-guided isolation of an extract of *Aspergillus* sp. led to the identification of three novel 11-membered macrocyclic biphenyl ether lactones, aspercyclides A–C. Aspercyclide A inhibited the IgE binding with an IC_{50} of 200 μM. The isolation, structure elucidation, absolute stereochemistry, and the binding activities of these compounds are described.

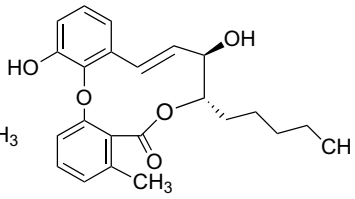
Allergic disorders are caused by mediators from mast cells and basofils.[1] Allergen specific immunoglobin E (IgE) binds to the high-affinity IgE receptor on these cells. Cross linking of these complexes by multivalent allergens activates a signal transduction pathway resulting in the release of mediators containing granulocytes. The IgE receptor comprises of four chains (α, β, and γ2). The α-chain (the extracellular soluble domain) binds to the constant region of IgE whereas the β and γ chains are required for membrane insertion and signal transduction. Molecules that bind to the high-affinity IgE receptor prevent IgE binding and release of inflammatory compounds, thus preventing symptoms associated with allergic diseases. Therefore such molecules that target the high-affinity receptor and block IgE binding may be efficacious in treating asthma, allergic rhinitis, and other forms of atopy.[2–4]

Screening of natural product extracts of microbial origin using a Myeloma α1/α2-IgE receptor binding ELISA assay led to the identification of a fungal extract with the requisite activity. Bioassay-guided fractionation of the extract led to the isolation of three novel compounds named aspercyclide A (**1a**), B (**1b**), and C (**2**). Aspercyclide A inhibited the IgE receptor binding activity with an IC_{50} value of 200 μM. The isolation, structure elucidation, absolute stereochemistry, and biological activity of these compounds are described.

The producing organism (MF6215) was isolated from a soil sample collected in Olduvai Gorge, Upper Strata in Tanzania and was identified as *Aspergillus* sp. It was grown on a seed medium containing in (g/L) yeast extract (4), malt extract (8), glucose (4), and Junlon (1.5) in distilled water at pH 7.0 at 25 °C for 5 days. It was then transferred to AD4 liquid production medium consisting of (in g/L) glucose (100), corn meal (10), soybean meal (4), oat flour (5), peptone (5), yeast extract (5), $CaCO_3$ (5), V8 juice (80 mL), and trace elements (1 mL) in distilled water at pH 7.0. Fifty milliliter portions of the production media were transferred into



1a: R = CHO
1b: R = CH_2OH



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Keywords: Aspercyclides; Fungal metabolites; IgE receptor antagonist.
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250 mL nonbaffled-Erlenmeyer flasks and grown for 19 days at 22 °C.

Fermentation broth was extracted with 1.2 volumes of methyl ethyl ketone (MEK) by shaking on a shaker for 30 min. A 30 mL MEK extract was chromatographed on Sephadex LH20 in MeOH. A 2/3rd portion of the active fraction was chromatographed on silica gel preparative TLC using 3% MeOH–CH_2Cl_2 leading to the isolation of **1a** (4.2 mg, 210 mg/L), **1b** (2.0 mg, 100 mg/L), **2** (2.0 mg, 100 mg/L) as colorless amorphous powders.[5]

Aspercyclide A (**1a**). HREIMS analysis of **1a** produced a molecular formula of $C_{24}H_{26}O_6$ (found *m/z* 410.1780, calcd *m/z* 410.1729) suggesting the presence of 12 degrees of unsaturations. The UV spectrum of **1a** exhibited absorption bands at λ_{max} 212, 266, and 348 nm indicating the presence of multiple independent highly conjugated systems. The infrared spectrum showed absorption bands for hydroxy (ν_{max} 3442 cm^{-1}), ester (1719), aldehyde, and aromatic groups. The ^{13}C NMR spectrum (Table 1) of aspercyclide A showed the presence of 24 carbon signals that were assigned to two methyls, four methylenes, seven olefinic methines, two oxymethines, and one aldehyde. Of the remaining carbon signals, one was assigned to an ester carbonyl and the rest to olefinic quaternary carbons.

One of the methyl groups appeared as a triplet in the ^1H NMR spectrum (Table 1) of **1a** at δ 0.95 (J = 6.8 Hz) indicative of a terminal ethyl group. The second methyl appeared as a singlet at δ 2.38 indicative of an aromatic methyl group. The ^1H NMR spectrum showed the presence of a pair of doublets for *ortho*-coupled aromatic protons at δ 7.53 and 6.95, and two doublets (δ 6.90 and δ 6.69) both coupled to a triplet (δ 7.15) typical of a 1,2,3-aromatic system. The ^1H–^1H connectivity was verified by the COSY spectrum. This was suggestive of the presence of 1,2,3-tri- and 1,2,3,4-tetra-substituted aromatic rings. The remaining pair of olefinic protons appeared as a doublet (δ 6.45, J = 16.0 Hz) and a doublet of doublets (δ 6.07, J = 16.0, 9.2 Hz) indicating a *trans*-olefin. The two oxymethines appeared as a triplet (δ 4.17, J = 9.2 Hz) and a doublet of a triplet (δ 5.30, J = 2.0, 9.2 Hz). The COSY spectrum of **1a** indicated that one side of the olefin (H-17) was coupled to the oxymethine triplet (H-18), which showed cross peaks to the other oxymethine H-19, which in turn showed cross peaks to the methylene (H_2-20). Extended COSY correlations of H_2-20 to the respective side chain protons established C16–C24 spin system (Fig. 1). This spin system was supported by requisite HMBC correlations. The three contiguous spin systems were connected to each other by HMBC experiments acquired with $^nJ_{CH}$ = 7, 5, and, 3 Hz (Fig. 1). The olefinic methyl group displayed the HMBC correlations to three carbons C-3 (δ 127.7), C-4 (δ 136.7), and C-5 (δ 125.7). The aromatic triplet at δ 7.15 showed three bond HMBC correlations to C-4 and to an oxygen-bearing aromatic carbon (δ 155.4) assigned to C-8. Lastly, a four-bond HMBC correlation (only at $^nJ_{CH}$ = 3 Hz) of H-7 to the only ester

Table 1. ^1H and ^{13}C NMR assignments of aspercyclide A (**1a**), B (**1b**), and C (**2**) in $CDCl_3$

Position	**1a**		**1b**		**2**	
	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	169.3		168.1		169.1	
3	127.7		127.2		128.1	
4	136.7		135.2		136.8	
5	125.7	6.90, d, 8.0	125.6	6.85, d, 8.0	126.4	6.93, d, 8.0
6	131.2	7.15, t, 8.0	129.7	7.18, t, 8.0	131.7	7.16, t, 8.0
7	114.2	6.69, d, 8.0	112.8	6.75, d, 8.0	116.1	6.67, d, 8.0
8	155.4		153.5*		155.0	
10	146.3		145.6		143.3	
11	134.8	7.53, d, 9.0	124.9	7.19, d, 8.8	151.4	
12	118.9	6.95, d, 9.0	116.0	6.84, d, 8.8	116.8	6.99, dd, 8.0, 1.2
13	161.2		154.4*		127.4	7.09, t, 8.0
14	119.8		130.3		122.8	6.70, br d, 8.0
15	136.5		136.5		133.8	
16	124.3	6.45, d, 16.0	123.8	6.13, d, 16.0	129.5	6.30, d, 16.0
17	142.2	6.07, dd, 16.0, 9.2	138.4	5.81, dd, 16.0, 9.2	138.8	5.99, dd, 16.0, 9.2
18	78.1	4.17, t, 9.2	78.0	4.08, t, 9.2	78.0	4.07, dt, 9.2, 4.5
19	78.5	5.30, dt, 2.0, 9.2	77.3	5.20, dt, 2.0, 9.2	78.5	5.25, dt, 2.0, 9.2
20	33.1	2.15, m		2.08, m		2.09, m
		1.73, m	31.6	1.71, m	32.9	1.72, m
21	26.6	1.53	25.2	1.65, m	26.7	1.58, m
22	23.9	1.50, m	22.5	1.55, m	23.9	1.42, m
23	33.0	1.40, m	31.7	1.44, m	32.9	1.42, m
24	15.3	0.95, t, 6.8	14.0	0.94, t, 6.8	15.3	0.95, t, 7.2
25	20.6	2.38, s	19.3	2.37, s	20.5	2.4, s
26	196.8	10.12, s	61.4	4.82, ABq, 12.0		
OH-13		11.57, s		7.76, br s		
OH-18		Not observed		Not observed		1.77, d, 4.5
OH-11						Not observed

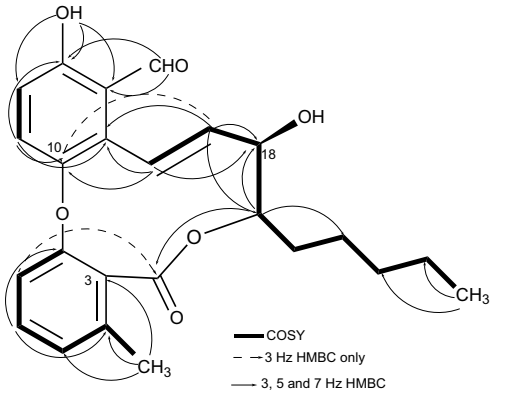

Figure 1. Selected HMBC correlations of **1a**.

carbonyl (δ 169.3) indicated the ester substitution at C-3.

The HMBC correlations of the olefinic proton H-16 to the aromatic carbons C-10 (δ 146.3) and C-15 (δ 136.5) helped to connect one end of the olefin (C-16) to the second aromatic ring at C-15. The remaining three substitutions of the second aromatic ring were established by HMBC correlations of H-11 to C-10, C-13 (δ 161.2), and C-15; OH-13 to C-12 (δ 118.9), C-13 and C-14 (δ 119.8); and the aldehyde proton to C-13 and C-14. The downfield shifted methine proton (H-19) displayed key HMBC correlation to the ester carbonyl (C-2) thus establishing the connection of C-19 to C-2 via O-1 and confirming the 11-membered lactone ring. The HMBC experiments were not useful for the establishment of the diphenyl ether linkage, which was established by the observation of reciprocating NOE's between aromatic protons H-7 and H-11 (Fig. 2) and was supported by the chemical shifts of the oxygen-bearing carbons C-8 and C-10.

The relative stereochemistry of aspercyclide A was elucidated by the ChemDraw 3D modeling, coupling constants and NOE measurements. H-18 exhibited 9.2 Hz couplings each with H-17 and H-19. ChemDraw 3D minimized structure revealed dihedral angles of 160° and 170° between H17–H18 and H18–H19 that supported the observed 9.2 Hz J values. H-19 displayed 2 and 9.2 Hz couplings with the methylene protons at

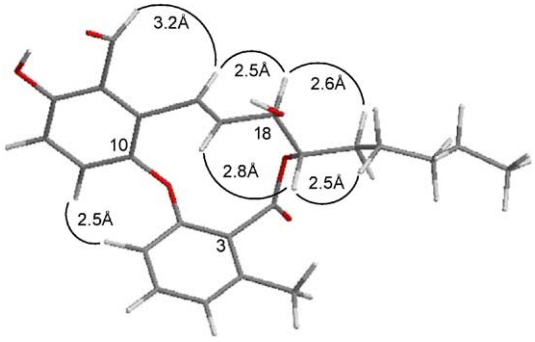

Figure 2. ChemDraw 3D model of **1a** showing inter-atomic distances and selected NOE's.

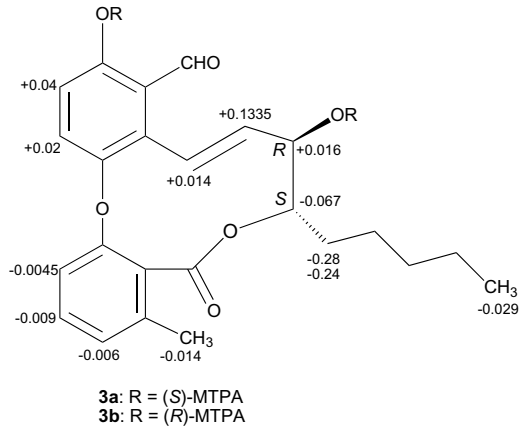

Figure 3. $\Delta\delta$ ($\delta S - \delta R$)-MTPA esters of **1a**.

C-20 supported by dihedral angles of 64° (H-20β) and 178° (H-20α), respectively. Irradiation of the olefinic proton H-17 exhibited strong NOE to H-19 indicating their *syn*-disposition. The other olefin proton H-16 afforded NOE enhancements to the aldehyde proton H-26 and H-18 suggesting the *syn*-relationship of H-16 and H-18. These two sets of NOE's suggested the opposite facial transpositions of the H17–H19 and H16–H18 and confirmed *anti*-relationships of H-18 and H-19.

The absolute stereochemistry of aspercyclide A was elucidated by Mosher ester method.[6] The reaction of **1a** with (*R*)- and (*S*)-MTPA chlorides afforded bis-(*S*)- and (*R*)-MTPA esters **3a** and **3b**. The chemical shift differential $\Delta\delta$ ($\delta S - \delta R$, Fig. 3) suggested *R* and *S* stereochemistries at C-18 and C-19, respectively.

Aspercyclide B (**1b**). HREIMS of **1b** afforded a molecular formula of $C_{24}H_{28}O_6$ (found *m/z* 412.1886, *m/z* calcd 412.1886) indicating that it possessed two extra hydrogens compared to **1a**. The UV spectrum exhibited absorption bands at λ_{max} 214 and 280 nm and indicated the absence of the absorption maxima of λ_{max} 348 nm due to the loss of extended conjugation. The comparison of the ^{1}H and ^{13}C NMR spectra of **1b** and **1a** indicated the absence of the aldehyde group and the presence of an ABq for an oxy-methylene (δ_H 4.82; δ_C 61.4). The ^{1}H and ^{13}C NMR shifts of **1b** were fully assigned by 2D NMR spectra including HMBC and are summarized in Table 1. Thus, structure **1b** was assigned for aspercyclide B.

Aspercyclide C (**2**). A molecular formula of $C_{23}H_{26}O_5$ (found *m/z* 382.1746, calcd *m/z* 382.1780) was obtained for **2** by mass spectral analysis. The comparison of the formula of **2** with **1a** suggested the loss of a CO group. The UV spectrum of **2** was similar to the UV spectrum of **1b**. The ^{1}H and ^{13}C NMR spectra of **2** indicated the absence of the aldehyde group compared to **1a** and the presence of an additional aromatic proton suggesting the substitution of the aldehyde group with the proton. However, analysis of the ^{1}H NMR spectrum suggested that the substitution pattern of the aromatic ring connected to olefin must be different from **1a** and **1b**. The remaining part of the spectrum of **2** was similar to the

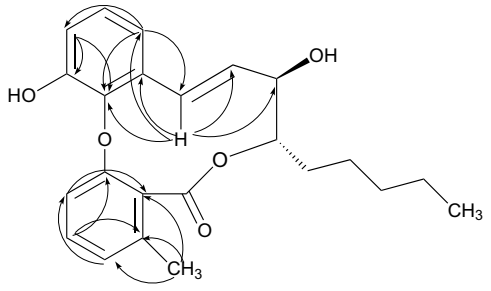

Figure 4. Selected HMBC correlations of **2**.

corresponding spectrum of **1a** and **1b**. The ^{1}H NMR spectrum (Table 1) showed the presence of a pair of aromatic doublets and a triplet all with *ortho*-couplings suggesting a 1,2,3-substitution leading to two potential substitution positions for the free phenolic group either at C-11 or at C-14. The structure was unambiguously assigned by the HMBC correlations of H-16 (Fig. 4), which showed strong HMBC correlations to three aromatic carbons (C-10, C-14, and C-15). Of these, only one of the carbons bears an oxygen substitution thus establishing the phenolic group at C-11. This assignment was supported by the oxygen induced chemical shifts of C-10 and C-11. Based on these data structure **2** was assigned to aspercyclide C.

IgE receptor binding activity. Aspercyclides A–C were evaluated in a receptor binding ELISA assay using Myeloma IgE, the truncated soluble α-chain of the IgE receptor[7] in the supernatant from Baculo virus infected Sf9 cells, biotin labeled goat anti-IgE, Eu labeled streptavidin, and monoclonal anti-KT3 antibody[8] in 96-well plates.[9] cDNA coding for the truncated α-chain of IgE fused to the KT3 peptide (Lys-Pro-Pro-Thr-Pro-Pro-Pro-Glu-Pro-Glu-Thr) was cloned into Baculo virus. Under standard assay conditions IgE bound to the receptor with EC_{50} values in the low nanomolar range. Aspercyclide A inhibited the IgE binding to its receptor by an IC_{50} value of 200 μM. The other two compounds were much less active ($IC_{50} > 200$ μM) suggesting that the aldehyde group plays a prominent role in the binding activity.

In summary, we have described the isolation and structure of three novel 11-membered diphenyl ether macrolide[10] fungal metabolites. One of these macrolides showed modest inhibition of IgE binding to high-affinity IgE receptor. While anti-IgE antibodies have been developed as potent IgE antagonists, the search for small molecule antagonists has not been successful except for the discovery of β-hairpin[11] and zeta ($IC_{50} = 0.032$ μM)[4] peptides. To the best of our knowledge aspercyclide A is the first small molecule inhibitor of this protein–protein interaction.

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5. Physical and spectral properties: (**1a**) $[\alpha]_D^{23}$ +191 (*c*, 1, CH_3OH), UV (CH_3OH) λ_{max} 212 (log ε 4.30), 266 (3.84), 348 (3.41); IR (neat) ν_{max} 3442, 1719, 1651, 1603, 1455, 1251 cm^{-1}; (**1b**) $[\alpha]_D^{23}$ +210 (*c*, 1.06, CH_3OH), UV (CH_3OH) λ_{max} 214 (log ε 4.28), 280 (3.43); IR (neat) ν_{max} 3325, 1714, 1585, 1454, 1251, 1233 cm^{-1}; (**2**) $[\alpha]_D^{23}$ +122.5 (*c*, 0.4, CH_3OH), UV (CH_3OH) λ_{max} 204 (log ε 4.21), 278 (3.23); IR (neat) ν_{max} 3378, 1710, 1580, 1250, 1217 cm^{-1}.
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9. Typically, receptor was incubated for 6–8 h at 37 °C with IgE in 125 μL incubation buffer (1% BSA, 0.05% Tween 20, 0.02% azide in phosphate buffer saline (PBS)). ELISA plates were coated with anti-KT3 (10 μg/mL) in PBS plus 0.02% azide at 4 °C overnight, blocked for 1 h with incubation buffer and washed three times with a wash buffer (0.05% Tween 20 and 0.02% azide in PBS). An aliquot of 80 μL of the reaction mixture per well was added and incubated overnight at room temperature, washed three times, and 100 μL/well biotin labeled anti-IgE was added. Plates were incubated for 1.5 h at 37 °C, washed three times and 100 μL/well Eu-streptavidin was added. After incubation for 1 h at room temperature 100 μL of the enhance reagent was added and plates were read after 20 min incubation at room temperature.
10. The STN substructure search of diphenyl ether with 11-membered ring without any substitutions provided no hit.
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