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Condensed Aromatic Peptide Family of Microbial Metabolites, Inhibitors of CD28–CD80 Interactions

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Abstract—Three condensed aromatic peptides SCH79235 (**1**), SCH79236 (**2**), and SCH204698 (**3**) were isolated from the fermentation broth of a *Streptomycete* microorganism. The structure of SCH204698 (**3**) was established by extensive NMR spectral data. All these compounds exhibited good activity against CD28–CD80 binding with an IC_{50} of 0.42, 0.38 and 0.22 μM, respectively.

Antigen-specific activation of T lymphocytes involves two major signaling pathways: one triggered by cross-linking of T cell receptor to antigen–MHC complex, and the other triggered by the encounter of CD28 and its ligands CD80 and/or CD86 on antigen presenting cells.[1] CD28 costimulation serves to enhance production of lymphokines required for T cell proliferation and to prevent anergy induction through a cyclosporin A resistant signal transduction pathway.[2–4] Therefore, the CD28 costimulation provides a specific target of pharmacological intervention for transplant rejection and autoimmune diseases.[5,6] To facilitate the discovery of inhibitors of CD28 costimulation, we have developed a scintillation proximity assay (SPA)-based screen, using genetically engineered and derivatized proteins of CD28, CD80, and CD86. As part of our continuing investigation of natural products as leads for treating autoimmune diseases, we screened ethyl acetate extracts of microbial fermentation broths. Fermentation broth of a microorganism belonging to a *Streptomycete* sp. was identified that displayed distinct activity in the CD28–CD80 binding assay.[7] Bioassay guided fractionation of this extract led to the isolation of compounds **1**–**3**.

A 100-L fermentation broth was extracted with ethyl acetate, the organic extract was dried over anhydrous sodium sulfate and the solvent removed to yield 15.2 g of crude extract. This extract was then loaded on a Sephadex LH-20 (10 × 75 cm) column packed in methanol and eluted with methanol and collected 15 mL fractions. The elution of the active compounds were monitored by HPLC [PLRP-S 0.39 × 15 cm, mobile phase acetonitrile: 0.05% trifluoroacetic acid (45:55)] and by CD28–CD80 binding assay. The active fractions were pooled based on purity and CD28–CD80 binding inhibition to produce two active cuts. Removal of solvents from these combined fractions yielded 0.086 and 2.52 g fraction I and II respectively. Fraction I contained compounds **2** and **3** and the fraction II contained compounds **1** and **2**. About 500 mg of fraction II was fractionated on a PLRPS column (2.5 × 30 cm) and eluted with acetonitrile and 0.05% trifluoroacetic acid (45:55). Acetonitrile was removed from the pure peak eluates and freeze-dried to yield 67 and 34 mg of **1** and **2**, respectively. The fraction I contained compound **2** and a related minor peak with similar UV and CD28–CD80 binding inhibitory activity. This fraction was further purified on an YMC ODS column (2.5 × 300 cm) eluting with a mixture of acetonitrile and 0.05 N TFA 45:55, to yield 15.7 and 5.9 mg pure **2** and **3**, respectively.

Both SCH 79235 (**1**) and SCH 79236 (**2**) showed identical molecular ion at m/z 1328 in FABMS. The nature of the molecular ion as a cluster suggested the presence of several halogen atoms in the molecule. Analysis of this cluster with various numbers of halogen atoms

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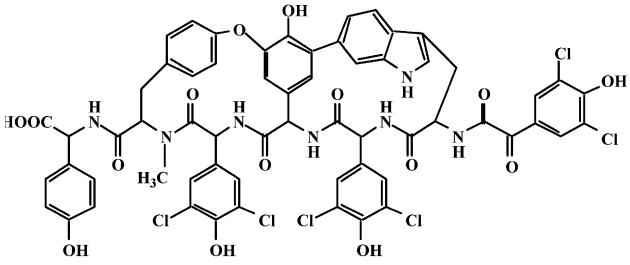
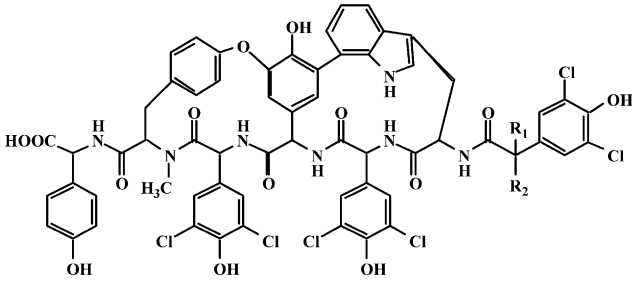


1. Complestatin (Chloropeptin II)



2. R_1 & R_2 = O (Chloropeptin I)
3. R_1 = -OH, R_2 = CH_2COCH_3

suggested SCH 79235 and SCH 79236 contains six chlorine atoms. The molecular formula of **1** was established as $C_{61}H_{45}N_7O_{15}Cl_6Na$ by HRMS (obsd 1348.0960, calcd 1348.1003). The ^{13}C NMR showed 61 carbon signals in agreement with the established molecular formula. The ^1H NMR indicated the presence of one methyl, two methylenes, seven α-amino carbon type methines, and many aromatic carbons due to several aromatic rings. APT ^{13}C NMR revealed them as eight >C=O, 20 aromatic =CH–, 24 aromatic =C<, six >CH–N, two $>CH_2$ and one $–CH_3$. The methyl signal appeared to be *N*-methyl group. These results suggested this compound must be an aromatic condensed peptide similar to those of vancomycin family. Further investigation confirmed **1** and **2** are identical to Complestatin (Chloropeptin II) and Chloropeptin I, respectively.

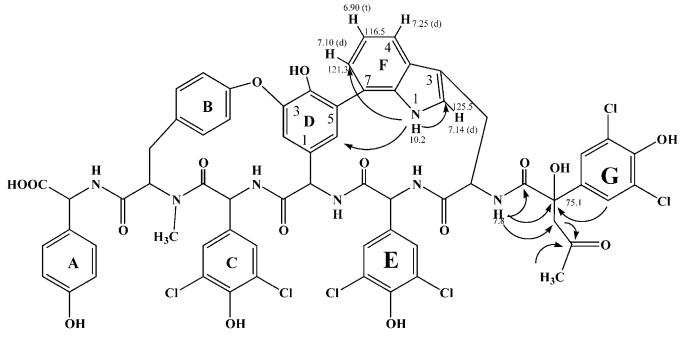
Complestatin was initially isolated in 1980 as an inhibitor of the human complement system.[8] Chloropeptin I was reported in 1994 along with complestatin (Chloropeptin II) as inhibitors of *gp*120-CD4 binding.[9]

Compound **3** showed *m*/*z* 1376 in FABMS. The cluster molecular ion in FAB and UV maxima revealed the **3** to be an analogue of **1** and **2**. The molecular formula was established as $C_{64}H_{51}N_7O_{16}Cl_6$ an additional C_3H_6O units than either compounds **1** and **2**. The ^1H and ^{13}C NMR of **3** was also similar to those of **1** and **2**. The ^1H NMR showed five additional protons, three-proton singlet at δ 2.10 and an additional two proton doublet at δ 2.5 due to a methyl and methylene group, respectively. The ^{13}C NMR showed four additional carbon signals at δ 28.9, 30.9, 75.1, and 205.1 compared to compound **2** and the peak at δ 185.9 due to the keto-amide carbon in chloropeptin I was absent. Further analysis revealed the changes were at keto group of keto-amide functionality.

The presence peak at δ 75.1 suggested that **3** must be an alkylated product. The presence of a methylene, methyl and carbonyl groups suggested that probably $CH_3–CO–CH_2–$ group must have been added to the keto function of keto-amide functionality.

The UV spectrum of **1** (MeOH) displayed maxima at 215, 245, and 290 nm and **2** and **3** displayed maxima at 215, 245, and 305 nm. The UV maxima of **1**, **2**, and **3** suggested the UV chromophores of **3** is identical to that of **2** suggesting the attachment of **D** and **F** rings in **3** is similar to that in **2** or C-5 of ring **D** is attached to C-7 of ring **F**. Further extensive analysis of NMR data revealed the structure of this compound as **3**. These results were further confirmed by HMBC correlation studies.

All three condensed aromatic peptides **1**, **2**, and **3** showed binding inhibition in the CD28–CD80 assay[7] with an IC_{50} of 0.42, 0.38 and 0.22 μM, in presence of Fetal Bovine Serum (FBS) and 0.01, 0.01, and 0.008 μM in the absence of FBS, respectively. Compound **3** was the most potent inhibitor CD28–CD80 binding, isolated from the nature so far. These compounds are also inhibitors of CD4–*gp*120 binding with IC_{50} ranges from 0.13 to 0.5 μM in the absence of FBS.



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