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Synthesis and evaluation of macrocyclic diarylether heptanoid natural products and their analogs

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ABSTRACT

The macrocyclic diarylether heptanoid (MDEH) natural products have been used in folk medicine for centuries. MDEHs are reported to exert anti-tumor properties by inhibiting the activation of NF-κB. Here we report the synthesis of a small MDEH library (first reported synthesis of racemic platycarynol) using a Grubbs cross metathesis/Ullmann cyclization strategy. Evaluation of the library led to the identification of MDEH **9b** which sensitizes pancreatic cancer cells to gemcitabine mediated growth inhibition and apoptosis.

Natural products are highly desired as lead candidates in the drug discovery process. This can be attributed to not only their historical role in treating and preventing human disease, but also their structural diversity.[1,2] Naturally occurring macrocyclic diarylether heptanoids (MDEHs) are found in plant materials that have been used in folk medicine for centuries (Chart 1). They are a subset of the larger class of diarylheptanoids and are characterized by two aromatic rings linked via a seven carbon chain and an ether bond to form the inherent macrocycle.[3] The diarylheptanoids have been reported to exert anti-inflammatory and anti-tumor activities.[4] Macrocyclic diarylheptanoids isolated from natural sources have been reported to inhibit tumor necrosis factor-α (TNF-α) and NO production. These natural products have also been shown to inhibit nuclear factor-κB (NF-κB) transcription activity in a gene reporter assay.[5]

NF-κB is a pleiotropic family of transcription factors implicated in pancreatic tumor progression.[6] A protein–protein interaction between NF-κB and IκBα retains NF-κB in the cytoplasm. Upon activation of the pathway by pro-inflammatory cytokines such as TNF-α, IκBα is rapidly phosphorylated and ubiquitinated. This process leads to the degradation of IκBα by the proteasome and subsequent translocation of NF-κB into the nucleus. In the nucleus, NF-κB activates transcription of a variety of genes including those involved in cell proliferation, apoptosis, and chemoresistance.[7]

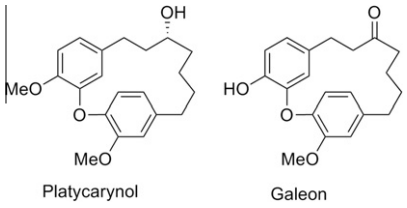

Chart 1. MDEH natural products.

Many chemotherapeutic agents including gemcitabine have been reported to activate the NF-κB pathway and this event is thought to provide tumor cells with a mechanism of resistance to such agents.[8] We aimed to increase the diversity of NF-κB inhibitors by seeking out scaffolds found in natural sources and evaluating them as NF-κB inhibitors and potential anti-cancer agents.

A 15-member macrocycle with a *para*-substituted phenyl ring is a common structural feature found in acerogenin MDEH natural products. These were isolated from the stem bark of *Acer nikoense* Maxim (Aceraceae) used in folk medicine to treat hepatic disorder. The first total synthesis of the acerogenin natural product used an intramolecular S_NAr ring closure to generate the macrocycle. The macrocyclization step required the insertion of a nitro group followed by its removal post cyclization.[3] A more direct route used an Ullmann reaction for macrocyclization to access acerogenins C and A.[9,10] We recently reported the synthesis of a related natural product ovalifoliolatin B which has a *trans* double bond in the

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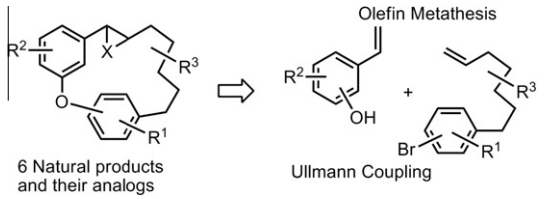


Chart 2. Retrosynthetic analysis of MDEH natural products and their analogs.

macrocycle using a Grubbs cross metathesis/Ullmann cyclization strategy.[11] Here we extend this strategy to generate an MDEH library.

In this report we describe the first total synthesis of the MDEH natural product platycarynol. We used a Grubbs cross metathesis/Ullmann cyclization strategy to generate the natural product and a small library of MDEH analogs. The library was evaluated in NF-κB assays and the best MDEH analog **9b** was found to sensitize pancreatic cancer cells to gemcitabine mediated growth inhibition and apoptosis.

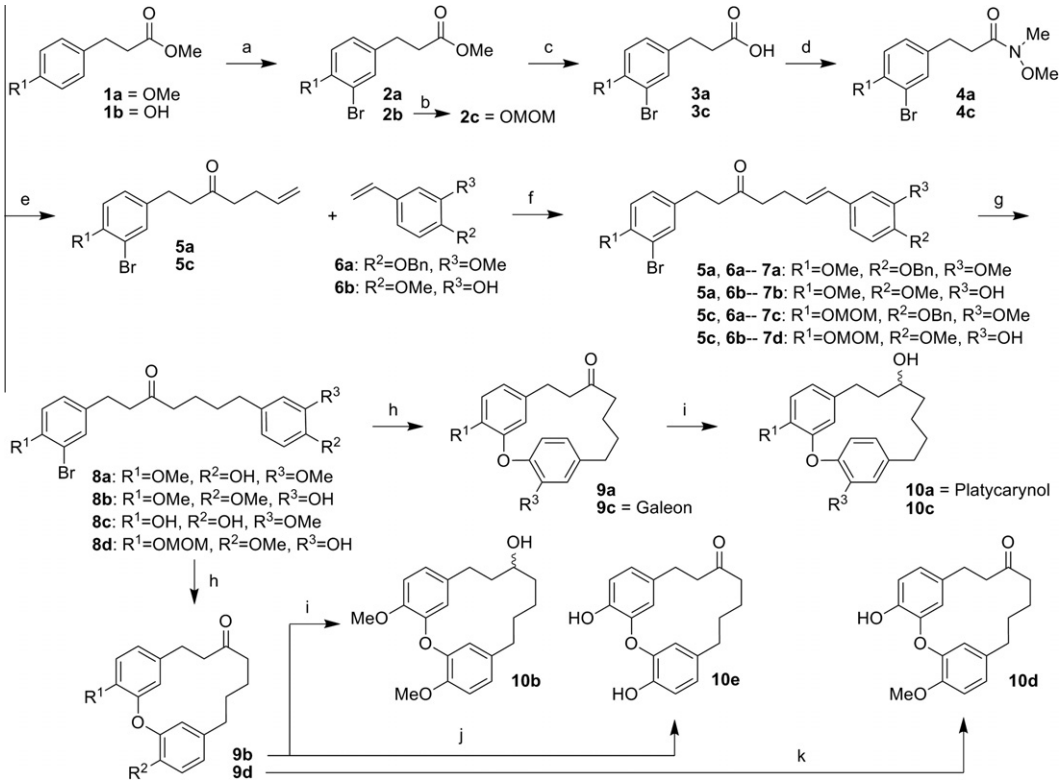
Synthesis of MDEH analogs with variations in the substitution patterns on the aryl rings, the heptanoid chain, and the diarylether linkage were envisioned as summarized in the retrosynthetic analysis described in Chart 2. Ullmann cross coupling followed by ring closing metathesis would result in the structural framework that can be manipulated to generate the desired library.

We were able to successfully generate the linear compounds using Ullmann cross coupling. However, despite our best efforts with varying solvents, dilution, temperature and catalysts, we failed to generate the ring closed products.[11] A reason for this failure could be attributed to the reduced probability of end-to-end contact of the alkenes templated on the diarylether backbone. Therefore, we reversed the sequence with olefin cross-metathesis between the alkene analogs (**5**) and styrene analogs (**6**) before the Ullmann reaction mediated macrocyclization. This approach yielded the desired MDEH core that was elaborated to the desired natural products and their analogs.

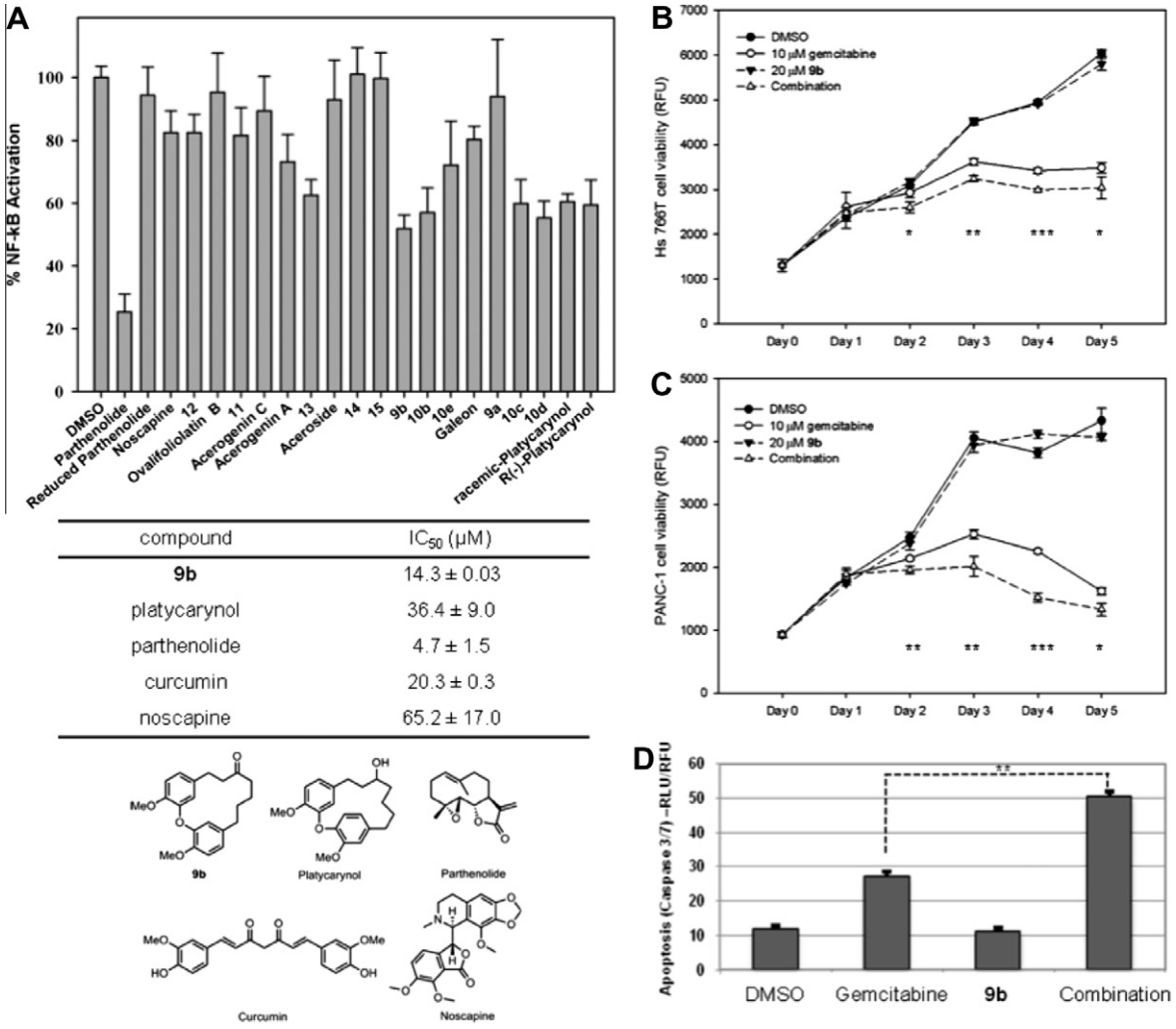
Briefly, methyl-3-(4-methoxyphenyl)propanoate **1a** and methyl-3-(4-hydroxyphenyl)propanoate **1b** were brominated to generate **2a** and **2b**, respectively, (Scheme 1). The ester groups on the brominated compounds were hydrolyzed to corresponding acids (**3**) under basic conditions. In a two-step process, the acids (**3**) were converted to the Weinreb amides (**4**) and subsequently reacted with the Grignard reagent 3-butenylmagnesium bromide to generate the desired alkene fragments (**5**).

The linear diarylheptanoid fragment (**8**) required for Ullmann cyclization was generated in moderate yields in a two-step sequence, that is, Grubbs II catalyzed cross metathesis of **5** with **6** to form **7**, followed by reduction of the resulting double bond via catalytic hydrogenation. As expected, in addition to the desired cross-coupled products, we isolated homodimers of both the alkene fragments and the styrene fragments.

Macrocyclization of fragments **8a** and **8c** using copper oxide in a sealed tube resulted in *meta–para* linked MDEH analog **9a** and the natural product galeon **9c** (Scheme 1). Reduction of the ketone in **9a** and **9c** resulted in racemic platycarynol **10a** and MDEH analog **10c**. On the other hand Ullmann cyclization of fragments **8b** and **8d** resulted in *meta–meta* linked diarylether MDEH analogs **9b** and **9d**. Reduction of the ketone in **9b** yielded analog **10b**. Removal of the MOM protecting group in **9d** yielded a monomethylated analog **10d** while treating **9b** with aluminium chloride resulted in another analog **10e**. This structure was originally reported as



Scheme 1. Synthesis of MDEHs. Reagents and conditions: (a) **2a**: Br_2, $AlCl_3$, CH_2Cl_2, 0 °C, 97%; **2b**: Br_2, CH_2Cl_2, 0 °C, 98%; (b) MOMCl, K_2CO_3, acetone, 0 °C, 98%; (c) **3a**: 2 N NaOH, MeOH, room temp, 98%; **3c**: LiOH, THF/H_2O, 95%; (d) (i) CDMT, NMM, THF; (ii) MeNH–OMe·HCl, room temp.; (e) 3-butenylmagnesium bromide, THF, 0 °C, 65–70% over two steps; (f) **7a**, **7c**: Grubbs II, CH_2Cl_2, reflux, 4 h, 26–30%; **7b**, **7d**: Grubbs II, toluene, reflux, 2 h, 30–53%; (g) **8a**, **8c**: Pd/C, CH_2Cl_2/MeOH, 175 psi; **8b**, **8d**: Pd/C, CH_2Cl_2, room temp, 55–82%; (h) CuO, K_2CO_3, pyridine, sealed tube, Δ, 12–52%; (i) $NaBH_4$, MeOH, 84–94%; (j) $AlCl_3$, CH_2Cl_2, reflux, 41%; (k) 6 N HCl, MeOH, 80%.



compound	IC_{50} (μM)
9b	14.3 ± 0.03
platycarynol	36.4 ± 9.0
parthenolide	4.7 ± 1.5
curcumin	20.3 ± 0.3
noscapine	65.2 ± 17.0

Figure 1. Engelhardione analog **9b** inhibits NF-κB transcription activity and sensitizes pancreatic tumor cells to gemcitabine mediated growth inhibition and apoptosis. (A) **9b** Inhibits NF-κB transcription activity in stable κB-luciferase expressing A549 cells at 20 μM. The table summarizes the results from dose response experiments. (B) and (C) **9b** Sensitizes Hs 766T and PANC-1 cells to gemcitabine mediated growth inhibition (data representative of three independent experiments). (D) **9b** Sensitizes PANC-1 cells to gemcitabine mediated caspase activation (data representative of two independent experiments). All data are expressed as the mean ± SD and $^{*}P < 0.5$, $^{**}P < 0.1$, $^{***}P < 0.01$.

the natural product engelhardione and was recently revised based on elegant work by the Sun group.[13] This is the first report describing the synthesis of racemic platycarynol.

Analysis of the ^{1}H NMR spectra of the alcohols **10a** and **10c** showed doubling of the methoxy peaks on the para-disubstituted phenyl ring in the macrocycle. This suggests the generation of atropisomeric mixtures of platycarynol (**10a**) and MDEH analog **10c**. Careful chromatography allowed us to separate a small amount of one of the two atropisomers in platycarynol. The spectral data of the separated material matched reported natural product R(−)-platycarynol.[12]

To add to the diversity we generated analogs of the intermediates from the recently reported ovalifoliolatin B synthesis (Fig. S1). These include the reduction of the keto group on the linker in methyl acerogenin C (**12**) to generate analog **13**. We also elaborated acerogenin C to the known compound aceroside following literature procedure.[3] To explore the size effects on the heptanoid linker, we functionalized the double bond in ovalifoliolatin B to generate epoxide and aziridine containing compounds **14** and **15** as racemic mixtures. In summary, we assembled a small library of 18 MDEH compounds (see Fig. S1 in Supplementary data) including seven natural products for evaluation as inhibitors of NF-κB activity.

A cell line specifically designed to monitor the transcription activity of NF-κB in response to TNF-α was used to screen the MDEH library. Parthenolide, a known NF-κB inhibitor and a recently reported NF-κB inhibitor noscapine, an isoquinoline alkaloid natural product with anti-tussive activity were used as positive controls.[14–16]

Results from the κB-luciferase reporter assay screen are summarized in Figure 1A (and Fig. S2). We selected **9b** and platycarynol, the most active *meta–meta* and *meta–para* diarylether compounds, respectively, for dose response studies (Figs. 1A and S3). The IC_{50} data show that analog **9b** was (i) more active than platycarynol, (ii) ∼four fold more active than noscapine, (iii) comparable or marginally better than the linear diarylheptanoid curcumin, another well characterized NF-κB inhibitor, and (iv) ∼three fold less active than parthenolide. To further demonstrate the ability of **9b** to inhibit NF-κB activation, we evaluated the expression levels of IκBα after TNF-α stimulation of HEK 293 cells by Western blotting (Fig. S4). Cells treated with **9b** show a decrease in IκBα protein expression at 60 and 120 min, suggesting that **9b** inhibits NF-κB mediated transcription of the IκBα gene.

Pancreatic cancer (PC) is one of the most lethal human cancers, with a 5-year survival rate of <4% and a median survival of less than six months. The clinically silent onset of PC has been attrib-

uted to the up-regulation of inflammatory signals and subsequent activation of NF-κB.[17] Constitutive NF-κB activity has been implicated in PC progression.[18,19] It is also well known that many chemotherapeutic agents induce the NF-κB pathway and this activation is implicated as a possible mechanism of chemoresistance by tumor cells. Therefore we decided to examine whether inhibition of NF-κB by **9b** could lead to PC cell growth inhibition and/or sensitize cells to chemotherapeutics. To test this, we selected commonly used PC cell lines, Hs766T and PANC-1, and examined the ability of **9b** to inhibit cell growth over a five-day period, alone and in combination with the chemotherapeutic agent gemcitabine (Fig. 1B and C).

Our results show that **9b** alone did not exhibit growth inhibitory effects (dark triangles vs dark circles). However, **9b** did sensitize Hs766T and PANC-1 tumor cells to gemcitabine mediated growth inhibition (Fig. 1B and C). Increased caspase activity in cells is indicative of apoptosis. To determine whether the growth inhibitory effect seen with the combination of **9b** and gemcitabine involved apoptosis, PANC-1 cells were treated with **9b** alone or in combination with gemcitabine and caspase 3/7 activity was measured after 48 h (Fig. 1D). Consistent with the growth inhibitory effects, we found increased levels of caspase activation with the combination of **9b** and gemcitabine, when compared to gemcitabine or **9b** alone. Together these data suggest that **9b** sensitizes PC cells to gemcitabine mediated growth inhibition and apoptosis.

In conclusion, we report the first total synthesis of the natural product platycarynol. We evaluated a small library of MDEH analogs as NF-κB inhibitors. This led to the identification of a *meta–meta* linked analog **9b** as a low micromolar inhibitor. Growth inhibition and apoptosis studies showed that **9b** sensitizes pancreatic cancer cells to gemcitabine, a commonly used chemotherapeutic agent for pancreatic cancer. We are currently exploring **9b** as an adjuvant in combination therapy with other chemotherapeutic agents in a panel of cancer cell lines.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.11.025.

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