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# Design, Synthesis, and Biological Evaluation of Angiogenesis Inhibitors: Aromatic Enone and Dienone Analogues of Curcumin

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**Abstract**—The quest to find new antitumor compounds is an ongoing research endeavor in many laboratories around the world. The use of small-molecule angiogenesis inhibitors promises to be a potentially effective method for cancer treatment and possible prevention. Many antiangiogenic compounds are in various stages of laboratory evaluations and clinical trials. Curcumin is a natural product that has exhibited potent antiangiogenic properties. Based on a simple pharmacophore model, using standard drug design concepts, aromatic enone and aromatic dienone analogues of curcumin were prepared and/or obtained commercially. These compounds were screened for antiangiogenic properties via an in vitro SVR assay and were found to inhibit cell proliferation.


Cancer is a general term used to describe many disease states, each of which are characterized by abnormal cell proliferation. The causes that bring about this abnormal cellular behavior are specific to each type of cancer. Success of tumor-targeted therapy is limited by this diversity. One common denominator for all types of cancer is the requirement of a suitable blood supply.[1] Therefore, tumor vasculature has emerged as a potential target for therapeutic intervention.

New blood vessel growth from preexisting vasculature stimulated by biochemical signals is termed *angiogenesis* or *neovascularization*. Tumor masses require a constant supply of oxygen and nutrients, as well as efficient waste removal for development. Diffusion from nearby capillaries supplies adequate nutrition for tumors less than 2 mm,[2] but for continued growth the tumors must develop their own blood supply. Alteration of the delicate balance of angiogenic stimulating factors and angiogenic inhibitors results in the phenotypic change from quiescence to active endothelial proliferation. This *angiogenic switch* is not completely understood. Angiogenic tumors produce positive regulators of angiogenesis, mobilize angiogenic promoters from the extracellular matrix, and/or stimulate normal host cells to inappropriately produce angiogenic substances. The goal of antiangiogenic therapy is to interfere with these mechanisms and prevent tumor cells from developing a viable blood supply. Success here has the potential to induce a persistent dormant state.

Curcumin is a natural product isolated from the spice turmeric. The compound has been shown to have antiangiogenic properties in vitro and in vivo.[3] Although the specific mechanism of action for its activity is not fully understood, this small molecule carotenoid pigment is a promising lead compound for structure modification. Our efforts toward the design of novel curcumin analogues have focused on the truncation of the general structure to either a single enone or dienone system (Fig. 1).

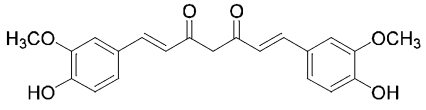


**Figure 1.** Structure of curcumin.

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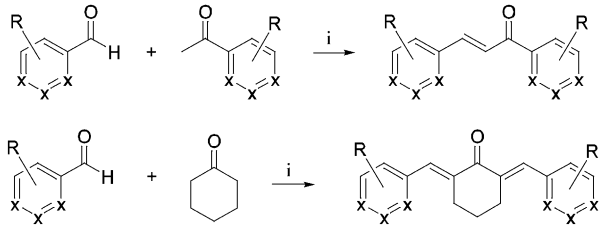


**Scheme 1.** Generalized synthetic scheme for the preparation of enone and dienone analogues of curcumin: (i) 40% KOH, EtOH, 5 °C. Let stir 10 h (rt). X = C, N. R = OH, OMe, Cl, F.

All of the compounds presented in Tables 1 and 2 were prepared according to the same general synthetic procedure.[4] The utilization of classic Claisen–Schmidt reaction conditions allows for the condensation of an aromatic aldehyde with a ketone to afford an α,β unsaturated ketone product (Scheme 1).

In the case of our enone analogues the following procedure was used: A mixture of the aldehyde (10 mmol, 1 equiv) and the appropriately substituted aromatic ketone (10 mmol, 1 equiv) is dissolved in 15 mL of ethanol and allowed to stir for several min at 5 °C (ice bath). 10 mL of a 40% KOH solution in water is added drop wise to the flask over several min. The mixture is then allowed to stir at room temperature for approximately 10 h. The reaction is next neutralized with a dilute solution of hydrochloric acid. A precipitate forms and is collected by suction filtration. Recrystallization from ethanol affords the pure product. In the event that a solid was not formed, the solution was extracted with anhydrous ether (3×, ~10 mL). The organic layers are collected, consolidated, and concentrated under reduced pressure to afford the crude product. Recrystallization and or column chromatography was then utilized in order to isolate the pure product. The experimental procedure for isolating the dienone analogues is identical save for the use of a 2:1 ratio of aldehyde/ketone in the reaction flask.

The endothelial cell proliferation assays used in this study were developed by Arbiser et al.[5] Primary murine endothelial cells, garnered from adult C57BL/6 mice, were infected with an ecotropic retrovirus encoding the simian virus 40 (SV40) large T 58-3 allele coupled with a resistance to neomycin. The resulting cells were then selected in a solution of Dulbecco's modified Eagle's medium (DMEM), 5% fetal calf serum and 0.6 mg/mL geneticin. The clone cells were then stained with 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocyanine perchlorate coupled to acetylated low density lipoprotein (diI-Ac-LDL). Fluorescence microscopy was used to identify the positive clones. The clone MS1 was expanded and infected with an additional retrovirus which encoded for activated H-ras (mutant allele at glycine-12 and threonine-59) as well as hygromycin resistance. The resulting, immortalized endothelial cell line was designated SVR.

For use in the in vitro assays, SVR cells were plated 10,000 cells/well on a multi-well gelatinized dish. The cells were then cultured for 24 h at 37 °C in 1 mL/well of 5% DMEM under an atmosphere of 10% $CO_2$. After 24 h of incubation, the medium was aspirated and replaced with 1 mL of a fresh 5% DMEM solution containing either 3 or 6 μg of our individual test compounds. In order to prepare these test solutions each compound was dissolved in a minimal amount of dimethylsulfoxide (DMSO). A measured amount of these stock (1 mg/mL) solutions was added to the fresh incubation medium in order to achieve our desired concentrations for testing. For this reason, each plate utilized one column as a DMSO control group for each concentration. After the addition of the test solutions, the dishes were allowed to culture under the same conditions for an additional 72 h. At the end of the three day incubation period, the wells were aspirated. Each well was then rinsed with 1 mL of Dulbecco's Phosphate Buffered Saline (DPBS) and aspirated again in order to remove any dead cells or lingering DMEM. The remaining, live cells were then removed from the bottom of each well using 0.5 mL of a 1× trypsin-EDTA solu-

**Table 1.** Percent inhibition of in vitro endothelial cell proliferation for the enone analogues of curcumin

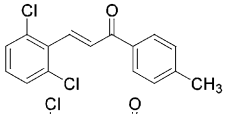
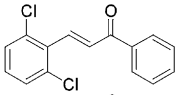
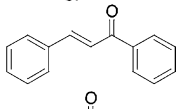
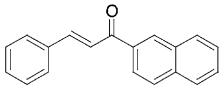
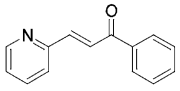
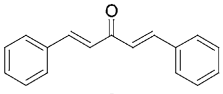
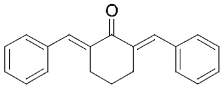
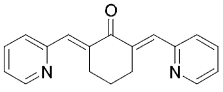
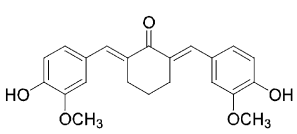
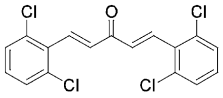
| | Structure | 3 μg/mL | 6 μg/mL |
|---|---|---|---|
| **2** |  | 98.2 | 98.1 |
| **3** |  | 92.9 | 97.5 |
| **4** |  | 92.8 | 94.4 |
| **5** |  | 92.2 | 94.7 |
| **6** |  | 89.1 | 96.9 |

**Table 2.** Percent inhibition of in vitro endothelial cell proliferation for the dienone analogues of curcumin

| | Compd structure | 3 μg/mL | 6 μg/mL |
|---|---|---|---|
| **7** |  | 96.6 | 97.7 |
| **8** |  | 94.4 | 97.7 |
| **9** |  | 92.9 | 96.7 |
| **10** |  | 90.1 | 96.0 |
| **11** |  | 87.1 | 90.4 |

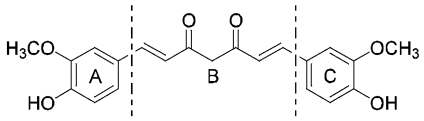



**Figure 2.**

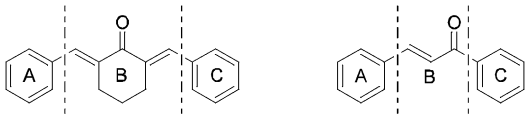



**Figure 3.**

tion (0.5% trypsin, 0.53 mM EDTA-4Na in HBSS without Ca, Mg, or $NaCO_3$). The enzymatic activity of the trypsin was neutralized by the addition of 0.5 mL of fresh 5% DMEM after approximately 8 min. After each well was thoroughly mixed to insure homogeny, 0.5 mL of the media from each well was mixed with 9.5 mL of Isotonin Coulter Electrolyte Solution and the number of cells/well were counted using a Coulter Cell Counter. The total cell count of each compound test well was then compared to the DMSO control well for each concentration. The percent inhibition of cell growth was calculated from these values.

Small-molecule antiangiogenic analogues of curcumin are attractive targets. Examination of curcumin suggests that the two aromatic regions might be critical for potential ligand–receptor binding. A reasonable approach following standard medicinal chemistry design concepts was to explore compounds with systematic differences in the carbon chain connecting the two aromatic regions (Fig. 2). A preliminary pharmacophore model has curcumin divided into three regions. Region A requires an aromatic ring, Region B is composed of a symmetrical dienedione linker, and Region C also requires an aromatic ring. Based on this simple model, various linkers in Region B were prepared or obtained commercially. By modifying the linker in Region B to an enone or a dieneone, two new series possessing antiangiogenic properties were discovered. In Figure 3, the aromatic enone and dienone lead compounds may also be divided into three regions.

Table 1 displays five compounds, **2**–**6** with excellent inhibition. The parent aromatic enone, **4**, is chalcone, a well-known natural product that has previously been reported as having antitumor properties.[6] In an effort to explore stereoelectronic effects, aromatic substituents were explored, represented by compounds **2** and **3**. Initially, assuming potential nucleophilic addition with the enone, it was hypothesized that substituents in positions 2 and 6 of the aromatic ring in Region A might affect inhibition via stereoelectronic effects resulting from a conformational change. Adjusting the aromatic rings in Regions A and C led to **5**. Substitution of the phenyl ring in Region A with a pyridyl ring, **6**, yielded a compound with excellent inhibition.

Table 2 displays another five compounds, **7**–**11**, with excellent inhibition. The parent aromatic dienone, **8**, is a cyclohexanone derivative, and **7** is an acetone derivative. As before with the enone series, the importance of stereoelectronic effects and the affect of conformational changes were explored by altering the aromatic substituents in Regions A and C. This approach led to **11**. Substitution of both phenyl rings, Regions A and C, with pyridyl rings, **8**, yielded a compound with excellent inhibition. Since curcumin has a 3-methoxy 4-hydroxy aromatic substitution pattern, it was decided to examine compounds with similar functional group patterns, represented by **10**.

Aromatic enone and aromatic dienone analogues of curcumin are excellent antiangiogenic compounds, having inhibition patterns equivalent or better than the parent natural product. The compounds can easily be prepared using classic Claisen–Schmidt reaction conditions, which is important for future animal model studies. Molecular modeling coupled with structure–activity studies are currently underway to help refine the preliminary pharmacophore model.

## References and Notes

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