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Discovery of Potent Cell Migration Inhibitors through Total Synthesis: Lessons from Structure−Activity Studies of (+)-Migrastatin

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The possibilities of enhancing the process of drug discovery through the use of nonpeptidal small-molecule natural products (secondary metabolites) are well established.[1] In some instances, the natural products themselves (cf. steroids, β-lactams, erythronolides, paclitaxel) are developed and ultimately registered as drugs. Perhaps more commonly, the natural product serves as a template for medicinal chemistry investigations, en route to eventual drugs. In other cases, natural products highlight substructural motifs which can be inserted as chemical cassettes in other molecular settings to produce valuable drugs (cf. Lipitor).[2] By extension it could well be argued that tamoxifen,[3] which does not per se carry a substructure found in a naturally occurring product, was clearly inspired by lessons learned from steroids and their binding to steroid receptors.

It is not uncommon that one would seek to improve the performance level of a natural product-inspired drug by alterations of its structure. While modified structures may have superior properties to the natural products themselves, the modified analog may not be accessible from inducible biosynthetic pathways.[4] The feasibility of introducing desired changes by chemical means, starting with the natural product, may well be circumscribed by limitations of conducting chemistry in the context of the native functional groups.

We have become attracted to the notion that the power of modern chemical synthesis can free the natural product-inspired discovery process from the often serious limitations of operational biosynthetic pathways, and from the limitations of post facto partial synthesis in the face of competing functional groups already in place. The idea is to divert a total synthesis program, so as to incorporate de novo, improved structural characteristics, not otherwise accessible due to the limitations discussed above (Figure 1). Through diverted total synthesis, the enormous teaching and stimulating potential of natural products can be leveraged still further. Two recent examples from the field of cytotoxic drugs that have produced serious structural candidates for accelerated clinical development served to encourage us to persist pursuing diverted total synthesis in drug discovery.[5,6]

Below we describe an even more striking application of this concept in the context of the discovery of a novel series of prospective anti-angiogenesis agents.[7] The potential relevance of angiogenesis modulation to cancer and inflammation is well appreciated.[8] The program we describe had its origins elsewhere, with the isolation and characterization of migrastatin by Imoto[9] and subsequently by researchers at Kosan Biosciences.[10] The name, migrastatin, is in keeping with the most notable biological property of the agent, i.e., its ability to inhibit tumor cell migration. Although

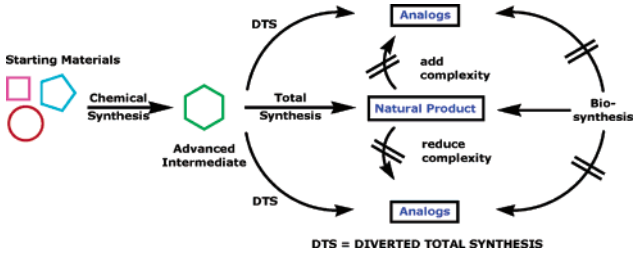

Figure 1. Diverted total synthesis.

the IC_{50} of migrastatin in the chamber cell migration assay (vide infra) was rather modest (29 μM), we envisioned the natural product as a plausible lead compound in a search directed to more potent anti-angiogenic prospects. As is our custom, our participation in this research began with a total synthesis of migrastatin.[11] The synthesis served, in the first instance, to provide solid chemical backing for the published structural and configurational assignments of migrastatin. Moreover, the lessons learned through the total synthesis enabled a more venturesome structure−activity relationship (SAR)-mapping of the migrastatin family than would have been possible from migrastatin itself.

Before describing the synthesis and exciting drug discovery exercises, we summarize the critical assays used in charting our progress. The effects of the migrastatins on the migration of 4T1 mouse breast tumor cells were investigated. 4T1 cells, which are highly aggressive and invasive, are routinely used as models for studying human breast cancer, because the progressive spread of 4T1 cells to lymph nodes, lungs, and other organs can be seen to mimic the metastasis of human mammary cancer. Two different assays were employed to monitor the effects of our compounds on 4T1 cell migration: (i) the wound-healing assay and (ii) the more quantitative chamber cell migration assay.[12]

Standardized scratches (cf. wounds) were made through a confluent 4T1 cell layer. Those cells did not migrate across the empty space thus created in the absence of serum. Addition of serum containing migration-enabling factors induced the transport of 4T1 cells across the hitherto empty space after overnight incubation. In this way we could explore the effects of fully synthetic migrastatin and its derivatives on the serum-induced 4T1 cell migration at various concentrations.

These findings were further confirmed and quantified by the chamber assay. In this assay, cells were seeded on the upper chamber of a transwell insert. Growth factor-containing serum was added to the lower chamber. After incubation for 6−8 h, cells which migrated from the upper chamber through the membrane to the lower compartment were counted.

In addition to the effect of migrastatin and its analogs on tumor cell migration, we investigated their impact on the migration of

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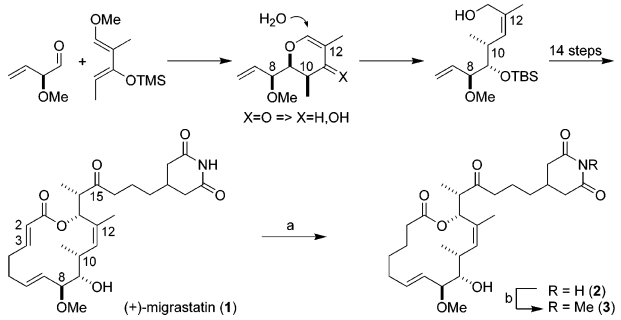
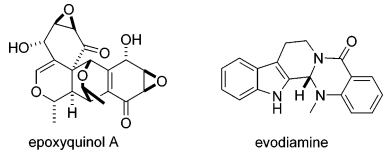
Scheme 1. Total Synthesis of Migrastatin and Migrastatin Analogs[a]



[a] Reagents and conditions: a) $[(PPh_3)CuH]_6$ (50%), b) MeI, Cs_2CO_3 (90%).

Table 1. Chamber Cell Migration Assay

compound	IC_{50} (4T1 tumor cells)
migrastatin (**1**)	29 μM
2,3-dihydro-migrastatin (**2**)	10 μM
2,3-dihydro-*N*-methyl-migrastatin (**3**)	7 μM
migrastatin core (**7**)	**22 nM**
2,3-dihydro-migrastatin core (**8**)	**24 nM**
migrastatin lactam (**13**)	**255 nM**
migrastatin ketone (**14**)	**100 nM**
(*R*)-isopropyl-migrastatin (**17**)	146 μM
(*S*)-isopropyl-migrastatin (**18**)	227 μM
epoxyquinol A	26 nM
evodiamine	315 nM

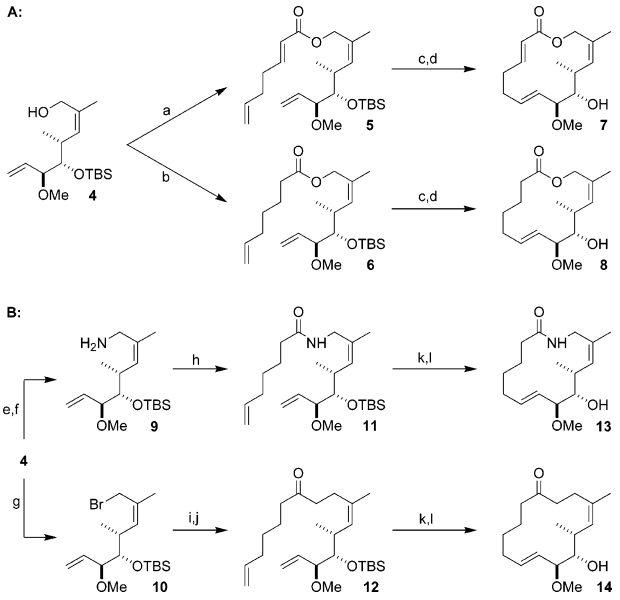


normal primary cells using endothelial cells (HUVECs). Endothelial cell migration is assumed to be critical for the angiogenesis process.

With migrastatin now available, SAR-directed studies commenced. We were concerned with the potential biovulnerability of the conjugated 2,3-double bond of migrastatin **1** (Scheme 1). Fortunately, the product of regiospecific reduction of the 2,3-double bond with the Stryker reagent was slightly more potent than migrastatin in the chamber cell migration assay (see compound **2** in Scheme 1 and Table 1). Also well tolerated was methylation of the nitrogen function (see compound **3**).

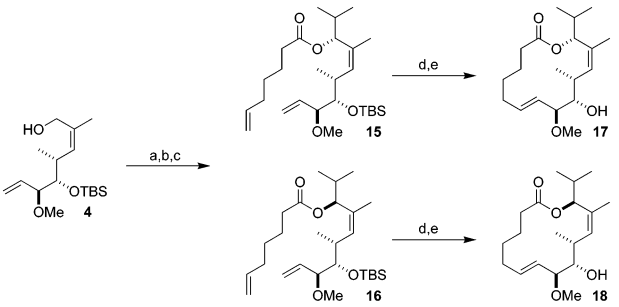
However, it was upon examination of structures not available from migrastatin itself that major breakthroughs in potency in the context of our process were realized. Thus, through diverted total syntheses (vide supra) starting with advanced intermediate **4** we reached the migrastatin core **7** and its 2,3-dihydro derivative **8** (Scheme 2A). Remarkably, their IC_{50} in the migration inhibition assay were reduced by ca. 3 orders of magnitude (22 and 24 nM, respectively, Table 1) relative to our starting point migrastatin. With a view to reaching compounds of potentially enhanced in vivo stability (for example against esterases in mouse plasma), the total synthesis program directed to **1** and then to **7** and **8** was further diverted, as shown, to provide migrastatin lactam **13** and migrastatin ketone **14** (Scheme 2B). While some loss of potency relative to the migrastatin core structures **7** and **8** was sustained, the compounds were still considerably more active than the natural product in the chamber assay (255 and 100 nM, respectively, Table 1), although the erosion of activity in the HUVEC determination was greater.[13,14]

Scheme 2. Total Synthesis of Primary Macrocycles[a]



[a] Reagents and conditions: a) Yamaguchi acylation (48%), b) Et_3N, DMAP, 6-heptenoyl chloride (89%), c) Grubbs catalyst,[15] toluene, reflux (47 and 73%), d) HF·pyridine, THF (78 and 90%), e) diphenylphosphoryl azide (87%), f) PPh_3, H_2O (90%), g) CBr_4, PPh_3 (95%), h) EDCI, 6-heptenoic acid (70%), i) 1-benzenesulfonyl-oct-7-en-2-one, DBU (75%), j) Na/Hg (79%), k) Grubbs catalyst,[15] toluene, reflux (70 and 75%), l) HF·pyridine, THF (90 and 95%).

Scheme 3. Total Synthesis of Macrocycles with a Side-Chain Mimic[a]



[a] Reagents and conditions: a) Dess−Martin oxidation (95%), b) *i*-PrMgCl (90%, 3:2-mixture of **16** and **15**), c) Yamaguchi acylation (70 and 75%), d) Grubbs catalyst,[15] toluene, reflux, e) HF·pyridine, THF (65 and 65% over two steps).

Interestingly, attempts to protect the lactone functionality of **7** and **8** from esterases by incorporation of a large branched group at C13 failed in the context of compounds **17** and **18**, synthesized by nonstereoselective addition of *i*-PrMgCl to the aldehyde derived from **4** (Scheme 3). These compounds were found to be even less potent than the natural product in the chamber cell migration assay (Table 1).

Comparison of our data with those of two recently discovered natural products with potent anti-angiogenic properties, epoxyquinol A[16] and evodiamine,[17] served to validate the chamber assay (Table 1).

Our set of new analogs was also tested in the wound-healing assay (Figure 2). The results further substantiated the activity trends observed in the chamber cell migration assay (Table 1). As an example, the effects of 2,3-dihydro-migrastatin core **8** and migrastatin **1** at 200 nM concentration on serum-induced 4T1 cell migration are shown in Figure 2C and D. While macrolactone **8** almost completely inhibited cell migration (Figure 2C), many cells invaded the space left by the wound in the presence of migrastatin

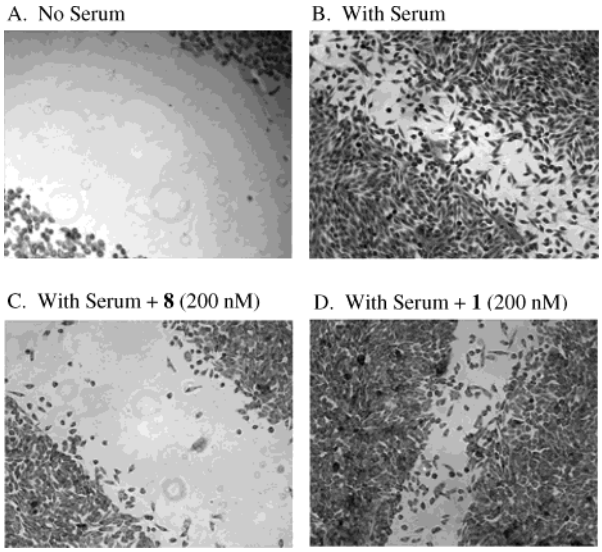


Figure 2. Wound-healing assay.

itself (Figure 2D). In this context, we have found that our most promising cell migration inhibitors do not have any cytotoxic or anti-proliferative effects up to 20 μM.[12]

In summary, by drawing from the teachings of nature and tapping diverted total syntheses to build focused structural diversity, we have uncovered a powerful and novel line of accessible potential angiogenesis inhibitors. It will be appreciated that the diverted total syntheses used in reaching the most promising compounds **7**−**14** are much less complicated than those required to get to migrastatin itself. The core compounds have now been prepared in significant quantity, and the study of their in vivo efficacy is underway. We hope to build upon these findings, even as our totally novel core compounds are being evaluated in a progression of pre-clinical models.

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Supporting Information Available: Physical data for compounds **7**, **8**, **13**, **14**, **17** and **18** and experimental details for the assays employed (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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