Point of VIEW

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Chemistry and Biology in Search of Antimetastatic Agents

Lucy Pérez[†] and Samuel J. Danishefsky[†,‡,*]

[†]Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021 and [‡]Department of Chemistry, Columbia University, Havemeyer Hall, 3000 Broadway, New York, New York 10027

ABSTRACT A recent publication is a great example of the inherent potential of exploring the chemical space inspired by natural products. Expanding access to chemical space through organic synthesis allows the discovery of valuable new small-molecule mediators of biological activity.

Metastasis remains a major hurdle in cancer treatment and is considered the most life-threatening aspect of the disease. Anticancer drug development strategies are generally aimed at direct inhibition of cancer cell growth. Most current chemotherapy treatments are antiproliferatives, which seek to retard growth of the primary tumor or even reduce the existing tumor burden. Therapeutic agents that can inhibit metastasis could well be effective means of preventing colonization, thereby enabling the containment of primary tumors in a chemically manageable form (*1*). A recent report in the *Journal of the American Chemical Society* highlights how organic chemistry, through the synthesis of small molecules based on natural products, can support the discovery of agents of potential value in oncology (*2*). The penetration of cancer cells into surrounding tissues and blood vessels is the opening phase of the metastatic cascade. Indeed, it has been shown to be rate-limiting in an experimental model (*3*). A judicious choice of synthesis-enabled probe structures could lead to a better understanding of the process of cell migration. It is not beyond imagination that with an improved understanding of the phenomenon, opportunities could emerge for new agents that are effective at preclinical and clinical levels. Thus, this field offers potential synergies between organic chemistry and medicine.

Rho-family GTPases are involved in the control of cell morphology and motility in untransformed cells (*4*). Rho-family GTPases are one of the main branches of the Ras superfamily of small GTPases. Similar to Ras, these proteins function as bimolecular switches by switching conformations in response to the presence or absence of GDP and GTP (*5*). Rho GTPases (Rho, ras-related C3 botulinum toxin substrate (Rac), and cell division cycle 42 (Cdc42)) play crucial roles in cell migration in a large number of cell types by mediating different cytoskeletal changes (*6*). Cell motility is a complex and highly regulated process; the initial response of a cell to a migration-promoting agent is to polarize and extend protrusions in the direction of the migration. Whereas activation of RhoA has been linked to the formation of stress fibers and focal adhesions, Rac and Cdc42 have been connected to the formation of lamellipodia and filopodia, respectively. However, the relative contribution of each of the Rho GTPases to cytoskeletal rearrangements depends on the specific condition and cell type (*7*).

It could therefore be of great benefit to have specific modulators for each member of the Rho GTPase family. In this way, their individual contributions to the overall process could be dissected. To this end, Zheng and coworkers (*8*) used computer-based virtual screening to design and validate a small molecule, identified as NSC23766, that is a highly soluble and membrane-permeable compound. More importantly, in principle, it is a specific inhibitor of a subset of guanine nucleotide exchange factors

*Corresponding author, s-danishefsky@ski.mskcc.org.

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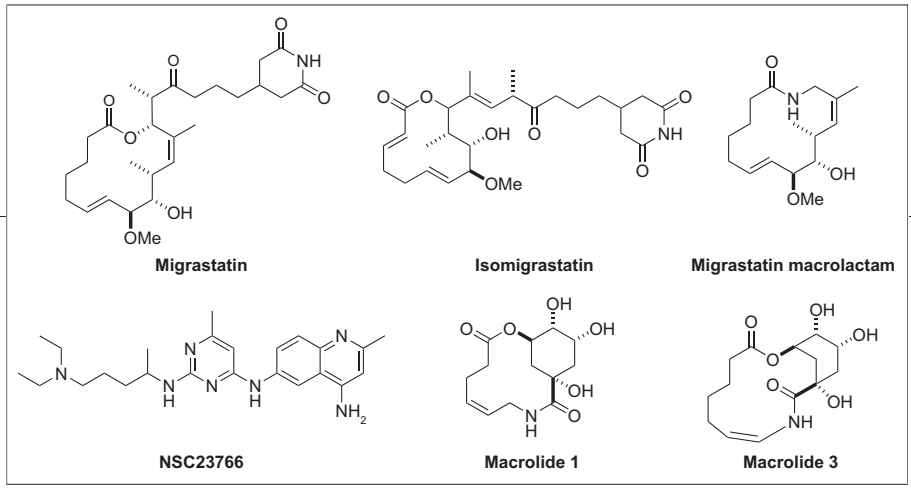

Figure 1. Structures of natural products, natural product analogues, and synthetic small molecules that are inhibitors of murine 4T1 breast cancer cell migration *in vitro*.

binding to Rac (*8*). This is the first small-molecule selective inhibitor of Rac activation. NSC23766 (Figure 1) modulates Rac-mediated functions in diverse cellular systems, including tumor cell transformation, invasion, platelet aggregation, and hematopoietic stem/progenitor cell mobilization (*9*).

A different molecule that is believed to affect Rac activation is migrastatin, a natural product. In 2000, Imoto and coworkers (*10, 11*) reported the isolation of migrastatin (Figure 1), a macrolide from a cultured broth of *Streptomyces* with the potential to suppress metastasis by inhibiting tumor cell migration (*10*–*14*). Migrastatin features a 14-membered macrolactone ring that contains two olefins and three contiguous stereocenters, as well as a glutarimide-containing side chain. Prompted by our keen interest in fully exploring the cell-migration-inhibiting activity of migrastatin, as well as by the challenges raised by its chemical structure, our group engaged in a program aimed at developing an efficient and versatile synthesis of this compound. The realization of the total synthesis resulted in significant amounts of migrastatin for further biological study (*15*).

The rather modest IC_{50} of migrastatin in the chamber cell migration assay (*vide infra*) with mouse 4T1 breast cancer cells (29 μM) provided another opportunity and challenge for organic chemistry. The hope was that by taking advantage of the breadth and versatility of chemical synthesis, we might gain access to migrastatin-inspired, but actually non-natural, agents whose performance level would be advantageous compared with the parent natural product. It was upon examination of structures not available from migrastatin itself that major advances in potency were realized in the context of our studies (*16, 17*). Furthermore, some of these early analogues, the core macroketone and the core macrolactam (Figure 1), were shown to interfere with the formation of lamellipodia in mouse 4T1 breast cancer cells, an indication that the target of these molecules could well be a Rac-related GTPase, or possibly, be upstream of Rac activation. Remarkably, these assays showed that the structures of the most promising compounds of this series were less complicated than that of migrastatin itself; more importantly, these compounds were active *in vivo*, but they were very different structurally from NSC23766 (*18*).

The recent work by Metaferia *et al*. (*2*) expands on the previous structure–activity relationship work on migrastatin analogues, and it adds to the inventory of small molecules that can inhibit the migration of breast cancer cells *in vitro*. These new synthetic 12- and 14-membered macrolides are composed of two fragments. One is derived from pentenoic or heptenoic acid, as was the case for earlier migrastatin analogues (*13*), and one contains quinic acid. The most potent compound identified from the quinic acid analogue series is macrolide 1 (Figure 1), a 12-membered macrocycle that contains a cis double bond. It has an IC_{50} of 77 nM in murine 4T1 breast tumor cells and is reminiscent of the natural product isomigrastatin, which Shen and coworkers (*19*) had earlier identified as the *bona fide* natural product from which migrastatin and the dorrigocins are shunt metabolites.

Isomigrastatin (Figure 1) is a 12-membered macrolactone with a glutarimide side chain that can rearrange in the presence of water to yield migrastatin. Interestingly, the work by Metaferia *et al*. (*2*) indicates that in the presence of the quinic acid moiety, the ring-closing metathesis favors the formation of the cis-isomer over the trans-isomer. This result is quite different from that observed in our syntheses of migrastatin core analogues (*13*). These also utilized Grubbs' second-generation catalyst. It is likely that the preference for formation of the *Z*-isomer in these cases of ring-closing metathesis reflects the instability of the *E*-variant of the smaller macrolide. It would be of interest to map the conformations of these synthetic macrolides to the natural products on which they are based, for a better understanding of the similarities and differences in the 3D arrangement of functional groups in both systems. Thus, organic synthesis can provide insights into how changes in stereochemistry and conformational constraints affect the macrocyclization of a substrate, while allowing the chemist access to new chemical space through diverted total synthesis.

A more extensive knowledge of structure–activity relationships furnishes chemists and biologists with sharper tools to connect a chemical entity to its biological target. The introduction of planned access points (handles) to facilitate the incorporation of affinity-labeled and radiolabeled probes should assist in target identification and efforts to verify that the proposed target of these new synthetic macrolikes is actually part of the Rac-mediated signaling pathway, as suggested by the authors.

A recent report described the interaction between the small GTPase Rac and the second messenger cGMP in a signaling pathway involving platelet-derived growth factor-induced fibroblast cell migration and lamellipodium formation (*20*). Further dissection of this and related pathways with small molecules such as the ones designed

by Bewley and coworkers (*2*) can be helpful to our understanding of the roles played by different gene products in cytoskeleton rearrangement during cell migration.

It is not yet known whether the macrolides synthesized by Bewley and coworkers (*2*) are stable in plasma or have *in vivo* activity at levels appropriate to eventual drug development. However, they already serve to stimulate ideas about viable structures for the study of the migration of breast cancer cells *in vitro*. Breast cancer is a heterogeneous disease with diverse metastatic behavior, and recent reports have shed light on the use of genetic markers to delineate the role of genes in specific steps of the metastatic process (*21*). Such research should lead to a better understanding of the biology of metastasis and the susceptibility of metastases to treatment by small molecules. Continued efforts to gain access to new, pertinent, and informative small molecules that are stable, water-soluble, orally bioavailable, and can be used for dissecting the signaling pathways involved in cell migration are well-justified.

Target identification continues to be one of the main challenges of phenotype-based assays, such as the wound-healing assay utilized to study cell migration, and small molecules can play a pivotal role in these endeavors. Small molecules provide the advantage of offering the temporal and reversible control over biological function that classical genetics does not (*22–24*). Over the years, organic chemistry has made large contributions to human health through the development of new therapeutics. However, the many challenges and unsuccessfully addressed therapeutic areas that still remain ensure continued vitality for this line of research.

To date, a large number of small-molecule probes used to explore life processes are derived from natural product extracts or synthetic analogues of natural products. For decades, synthetic chemists have been inspired by the complexity of natural products and their exquisite affinity for their protein targets (*25, 26*). Recognizing that natural products can have remarkable properties, medicinal chemists have traditionally synthesized analogues of natural products, looking to improve their properties by minor modifications of their chemical structure. As it becomes more difficult to locate new natural products that themselves "make it" as drugs, the need for synthetic organic chemistry, inspired by both natural products and other inventive channels, becomes increasingly clear.

Over the years, small molecules that exhibit unique biological activity have been discovered on a case-by-case basis (*27–29*). Evolutionary advantage has served as an assay for natural products to identify substrates that allow organisms to compete for survival. With the sequencing of genomes leading to the identification of numerous novel gene products with unknown functions (www.ornl.gov/sci/techresources/Human_Genome/home.shtml), small-molecule partners that activate or inhibit such gene products are at a particular premium. As the sciences move toward a more integrated "systems/genomics" approach, the pace of small-molecule discovery must be accelerated if the goal of discovering small-molecule partners for increasing numbers of identified gene products is to be realized. Chemistry is thus ideally positioned to serve as an important link between biology and medicine.

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