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Confirmation of the Structures of Synthetic Derivatives of Migrastatin in the Light of Recently Disclosed Crystallographically Based Claims

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Abstract

In the light of recently disclosed crystallographic studies on migrastatin ketone **2** complex with fascin, the structures of migrastatin ketone **2** and migrastatin ether **4** have been re-evaluated by NMR and X-Ray crystallographic techniques. The results of these studies established the correctness of the previously reported structural assignment and confirm that the “small molecule” co-crystallized in complex with fascin is not the migrastatin ketone, which was provided for the infusion experiment, but rather its stereoisomer.

The devastation and lethality of many cancers are enhanced by the migration of the elements of the tumor from its primary site to distal sites, which then become new launching areas for progression.¹ One way to counter the deadly phenomenon of metastasis would be through inhibition of such migration. Accordingly, we took a keen interest following disclosure of the natural product migrastatin **1** (Figure 1) isolated from *Streptomyces* sp. MK929-43F1. ² As its name implies, this compound inhibits the migration of cancer cells, albeit with IC₅₀s in the μM range.

These properties of **1** prompted us to pursue its total synthesis. ³ ⁴ Having accomplished that goal and having confirmed the activity claimed for migrastatin, we undertook an intensive program of diverted total synthesis.⁵ Indeed, we succeeded in exploiting the total synthesis route **1**, as a platform for preparing structurally simpler analogs including ketone **2**, lactam **3** and core ether **4**.⁵ Remarkably, these compounds exhibited far greater potency than migrastatin itself, suppressing the migration of cancer both *in vitro* and *in vivo*. For instance, as suggested in the mouse models, compounds **2** and **3** achieve reduction of 4T1

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murine breast cancer metastasis^{5c} while migrastatin ether **4** significantly reduces the metastatic spread of human MDA231 and LM2 breast cancers.^{5d} Following our recent and intensive *in vivo* study with compound **4**, it was theorized, though certainly not proven, that its impressive activity might reflect its ability to inhibit fascin-1 dependent migratory tendencies.

In this connection, we took particular note of a disclosure by Chen *et al.* of studies demonstrating that migrastatin ketone **2** does indeed target fascin.⁶ These studies were conducted on compounds, which were designed and synthesized in our program and furnished to Huang and coworkers as a part of a mechanism-directed collaboration. Indeed, the Huang paper did herald that the ketone analog, **2**, binds to one of the actin bundling sites of fascin. In support of this claim, Chen *et al.* reported X-ray crystal structures for both fascin, in its native state, as well as in a complex with **2** (Figure 2).⁷

Given this context, we were particularly surprised upon closer inspection of the migrastatin ketone (**2**) complex with fascin, that the “small molecule” binding to the protein emerged not as **2**, which was furnished, but rather as a stereoisomer of **2**, i.e. compound **5** (Figure 3). This structure is different from the compound we had supplied in both the geometry of the 4,5-double bond (*Z* in **2**; *E* in **5**) and the configuration at C6 (*R* in **2**, *S* in **5**). While we had been quite confident of our structural assignments of **2–4** based on their linkage to our eventual total synthesis of **1** by rational chemistry and supportive characterization, the crystallographically-based disclosure by Chen *et al.* depicting structure **5**, rather than **2**, obliged us to re-visit those assignments. Accordingly, we re-analyzed samples of **2** (and **4**) routinely furnished to Huang and coworkers at purity levels of > 98%.

First, we addressed the issue of the olefin geometry by obtaining the corresponding NOESY spectra for **2** and **4** (*cf.* Figure 4).⁸ Thus, if the isomerization of the tri-substituted olefin from *Z*- to *E*-geometry indeed took place during the elaboration of **10** to **2** or **4**, one would observe no NOE signal between the C4 methyl group and C5 hydrogen of **2** (and C3 methyl group and C4 hydrogen of **4**). Also, there would not be any NOE correlation between C3 or C2 methylenes and C6 methine of **2** (and C2 methylene and C5 methine of **4**). The examination of the NOESY spectra of **2** and **4** did not provide any support for the presence of the *E*-trisubstituted olefins in these compounds (*cf.* Figure 4). Quite contrary, the observed NOE correlations are only consistent with the *Z*-olefin geometry for both **2** and **4**, as originally assigned.⁵ Specifically, for migrastatin ketone **2** there are observed strong NOE correlations between C4 methyl group and C5 hydrogen, and C2, C3 methylenes and C6 methine, and no correlations between C4 methyl group and C6 methine or C5 hydrogen and C2, C3 methylenes. These experiments are further supported by X-ray crystallographic analysis of **4** (Figure 4), which unambiguously confirmed the structure of migrastatin ether originally disclosed by our group.

Given the fact that we gained access to **2** and **4** in almost identical fashion from a very well-characterized intermediate, and that the NOESY NMR experiments serve as a reliable instrument in establishing the olefin geometry of **4**, we remain confident that isomerization had not occurred during our synthesis of **2** and that the “small molecule” bound to fascin as described by Chen *et al.* is not migrastatin ketone **2**, but rather its isomer.

The significance of these serious chemistry level discrepancies on the structural biology based picture of “small molecule”-to-fascin binding remains to be sorted out and is the subject of an ongoing study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

- 1(a). Hedley BD, Winqvist E, Chambers AF. *Expert Opin. Ther. Targets*. 2004; 8:527–536. [PubMed: 15584860] (b) Perez L, Danishefsky SJ. *ACS Chem. Biol.* 2007; 2:159–162. [PubMed: 17373763]
- 2(a). Nakae K, Yoshimoto Y, Sawa T, Homma Y, Hamada M, Takeuchi T, Imoto M. *J. Antibiot.* 2000; 53:1130–1136. [PubMed: 11132958] (b) Nakae K, Yoshimoto Y, Ueda M, Sawa T, Takahashi Y, Naganawa H, Takeuchi T, Imoto M. *J. Antibiot.* 2000; 53:1228–1230. [PubMed: 11132973] (c) Takemoto Y, Nakae K, Kawatani M, Tahahashi Y, Naganawa H, Imoto M. *J. Antibiot.* 2001; 54:1104–1107. [PubMed: 11858668] (d) Nakamura H, Takahashi Y, Naganawa H, Nakae K, Imoto M, Shiro M, Matsumura K, Watanabe H, Kitahara T. *J. Antibiot.* 2002; 55:442–444. [PubMed: 12061554]
- 3(a). Gaul C, Danishefsky SJ. *Tetrahedron Lett.* 2002; 43:9039–9042. (b) Gaul C, Njardarson JT, Danishefsky SJ. *J. Am. Chem. Soc.* 2003; 125:6042–6043. [PubMed: 12785819]
4. For the review of the recent work on the synthesis of migrastatin and its analogs refer to: Reymond S, Cossy J. *C. R. Chimie.* 2008; 11:1447–1462.
- 5(a). Njardarson JT, Gaul C, Shan D, Huang X-Y, Danishefsky SJ. *J. Am. Chem. Soc.* 2004; 126:1038–1040. [PubMed: 14746469] (b) Gaul C, Njardarson JT, Shan D, Dorn DC, Wu K-D, Tong WP, Huang X-Y, Moore MAS, Danishefsky SJ. *J. Am. Chem. Soc.* 2004; 126:11326–11337. [PubMed: 15355116] (c) Shan D, Chen L, Njardarson JT, Gaul C, Ma X, Danishefsky SJ, Huang X-Y. *Proc. Natl. Acad. Sci. U.S.A.* 2005; 102:3772–3776. [PubMed: 15728385] (d) Oskarsson T, Nagorny P, Krauss I, Perez L, Mandal M, Yang G, Ouerfelli O, Xiao D, Moore M, Massague J, Danishefsky SJ. *J. Am. Chem. Soc.* 2010; 132:3224–3228. [PubMed: 20155906]
6. Chen L, Yang S, Jakoncic J, Zhang JJ, Huang X-Y. *Nature.* 2010; 464:1062–1066. [PubMed: 20393565]
7. The structures of the migrastatin ketone/fascin complex were accessed through Protein Data Bank: <http://www.pdb.org/pdb/explore/explore.do?structureId=3LNA>
8. The ^1H NMR signal assignment, and ^1H , COSY and NOESY NMR spectra of **2** and **4** are provided in the supporting information.

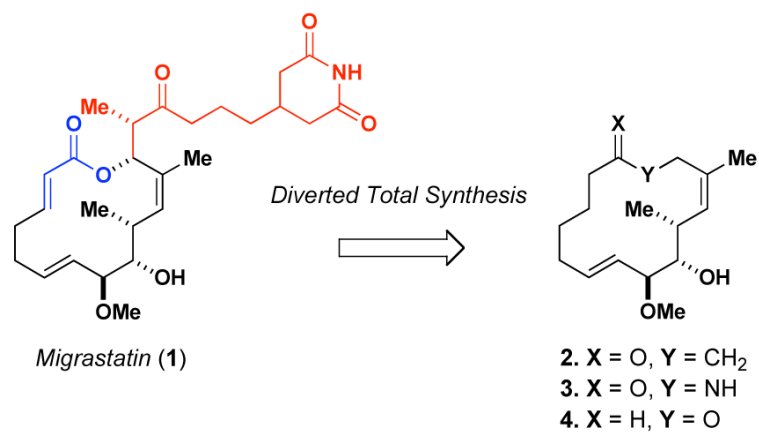


Figure 1.

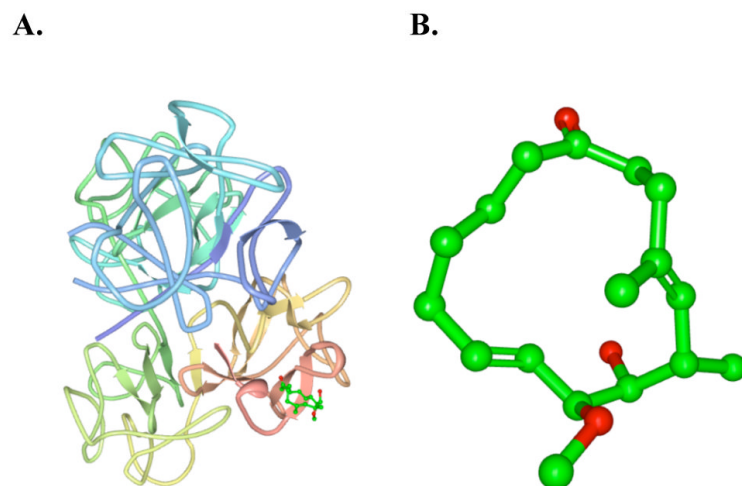
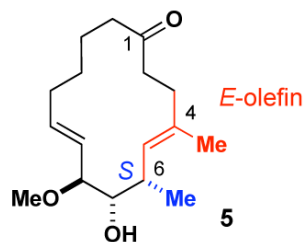


Figure 2. (A) Crystal Structure of Migrastatin Ketone complex with Fascin. (B) Magnified structure of Migrastatin Ketone in complex with Fascin.

^a These images were obtained from the Protein Data Bank (PDB) website and visualized and processed with Protein Workshop software.⁷

Structure of Migrastatin Ketone
From Crystallographic Studies
by Chen et al.⁶



Previously Disclosed
Structure of Migrastatin
Ketone

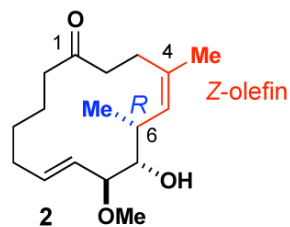


Figure 3.
Structural Features of Migrastatin Ketone (2) and Small Molecule Bound to Fascin (5).

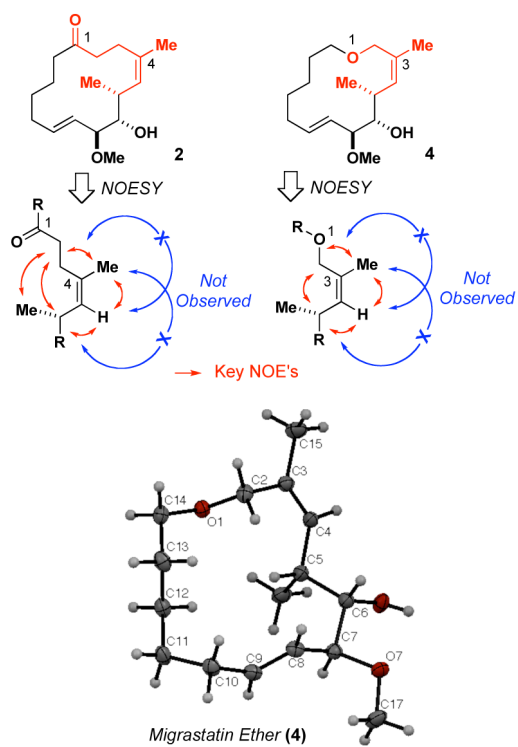


Figure 4. Observed NOE's for the Trisubstituted Olefins of **2** and **4** and X-Ray Structure of **4**.