The Therapeutic Potential of Migrastatin-Core Analogs for the Treatment of Metastatic Cancer

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Abstract: Tumor metastasis is a complex process in which cells detach from the primary tumor and colonize a distant organ. Metastasis is also the main process responsible for cancer-related death. Despite the enormous efforts made to unravel the metastatic process, there is no effective therapy, and patients with metastatic tumors have poor prognosis. In this regard, there is an urgent need for new therapeutic tools for the treatment of this disease. Small molecules with the capacity to reduce cell migration could be used to treat metastasis. Migrastatin-core analogs are naturally inspired macrocycles that inhibit pathological cell migration and are able to reduce metastasis in animal models. Migrastatin analogs can be synthesized from a common advanced intermediate. Herein we present a review of the synthetic approaches that can be used to prepare this key intermediate, together with a review of the biological activity of migrastatin-core analogs and current hypotheses concerning their mechanism of action.

Keywords: diverted total synthesis; natural products; cancer metastasis

1. Introduction

Cell migration is a physiological process and a central feature in embryonic development, tissue repair and immune cell function. Importantly, cell migration is also responsible for angiogenesis, tumor invasion, and metastasis [1]. The cytoskeleton drives cell migration and a key cytoskeletal component is Actin. Several targets have been proposed for the inhibition of Actin dynamics, including the following: (1) actin (e.g., phalloidin (1) an F-actin stabilizer or cytochalasin D (2), an F-actin destabilizer); (2) tubulin and microtubule (e.g., taxol (3), a microtubule stabilizer or vincristine (4), a microtubule destabilizer); (3) actin-binding proteins (ML-7 (5), a myosin light chain kinase inhibitor); and (4) upstream signaling molecules (e.g., Y27632 (6), a Rho-kinase inhibitor) [2] (Figure 1).

Migrastatin (7) is a natural product that was originally isolated from Streptomyces sp. MK-929-43F1 [3,4] and later found in fermentation broths of Streptomyces platensis [5]. Migrastatin comprises a 14-membered ring macrolactone incorporating two E bonds and one Z double bond, together with three contiguous stereocenters and a pendant alkyl gluturamide side chain. It has been reported that migrastatin inhibits cell migration [3,4], suppresses multi-drug resistance [6], and antagonizes muscarinic acetylcholine receptor [7].
The synthesis and the biological activity of migrastatin (Reymond and Cossy in 2008 [15]. The aim of this review is to discuss the recent synthetic approaches used for the preparation of migrastatin (Figure 2). In 2003, Danishefsky and co-workers described the first total synthesis of migrastatin [8]. One year later, the same group demonstrated that ablation of the glutaramide side chain increases the biological activity of migrastatin analogs 1000-fold with respect to natural migrastatin [9]. They found that simpler analogs at nanomolar concentrations were able to inhibit cell migration in 4T1 mouse mammary tumor cells. Danishefsky demonstrated that migrastatin and simplified migrastatin analogs could be easily synthesized from a common advanced intermediate (8). This result highlights the potential of diverted total synthesis drug discovery (Figure 2).

Several groups have been involved in the preparation of 8 (Figure 3 and Table 1) [8–14]. The synthesis and the biological activity of migrastatin (7) and migrastatin analogs were covered by Reymond and Cossy in 2008 [15]. The aim of this review is to discuss the recent synthetic approaches...
used for the preparation of 8, together with new findings that provide insight into the anti-metastatic potential of migrastatin analogs and their targets.

**Figure 3.** Synthetic strategies for the preparation of advanced intermediate 8.

**Table 1.** Synthetic key aspects for the preparation of advanced precursor 8.

| Year | Authors          | n^0 Steps | Overall Yield | Key Steps | Notes                                      | Reference |
|------|------------------|-----------|---------------|-----------|--------------------------------------------|-----------|
| 2004 | Danishefsky et al. | 10        | 22%           | LACDAC reaction, Ferrier rearrangement | Multi-gram scale synthesis | [8,9,16] |
| 2006 | Cossy et al.     | 11 (+4) * | 11%           | Stereoselective crotylation, RCM    | -                           | [10]      |
| 2007 | Lqbal et al.     | 12        | 8%            | Evans aldol condensation, Still-Gennari olefination | Entry to isomigrastatin analogues, gram scale | [17]      |
| 2010 | Dias et al.      | 14        | 1.2%          | Upjohn dihydroxilation, Horner-Wadsworth-Emmons olefination | Entry to migrastatin-core epimers, gram scale | [12]      |
| 2013 | Lqbal et al.     | 11        | 5.9%          | Pd(II) catalyzed intramolecular C-H oxidation | Entry to isomigrastatin analogues, gram scale | [13]      |
| 2014 | Murphy et al.    | 9         | 30%           | Brown alcoxyallylation, HWE, Zinc catalyzed asymmetric desymmetrization | Entry to isomigrastatin analogues, gram scale | [14,18,19] |

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2. Results

2.1. Synthesis of Advanced Intermediate 8

2.1.1. Synthesis of the Protected Migrastatin-Core (Danishefsky et al.) [16]

The first synthesis of the migrastatin-core was reported by Danishefsky and co-workers in 2002 [16]. MGSTA-1 was synthesized as its MOM derivative through a precursor of advanced intermediate 8. The synthesis began with the selective silylation of the primary hydroxyl group of (S)-3-benzyloxy-1,2-propanediol 9 [20,21], followed by methylation of the secondary hydroxyl group. Regioselective deprotection of benzyl ether gave 10, which was subsequently oxidized under Swern conditions. A Lewis acid-catalyzed diene-aldehyde cyclocondensation (LACDAC) [22] reaction between aldehyde 11 and diene 12 gave dihydropyrone 13, which contains the three contiguous stereocenters of the migrastatin core fragment. Dihydropyrone 13 was treated with NaBH₄ and
CeCl$_3$·7H$_2$O (Luche reduction) [23], followed by Ferrier rearrangement in acidic media, to give lactol 14 [24]. Reductive opening of lactol 14 using LiBH$_4$ gave compound 15, which contains the desired (Z) olefin. Diol 15 reacted with (E)-hepta-2,6-diynyl chloride in the presence of DMAP to give ester 16. The secondary hydroxyl group of 16 was protected as MOM ether, and the TBDPS group was cleaved using HF-py to afford intermediate 17. Oxidation of the primary hydroxyl group using Dess-Martin periodinane (DMP), followed by olefination using the Tebbe reaction [25], gave metathesis precursor 18. Finally, RCM reaction of 18 with Grubbs II catalyst [26] gave protected migrastatin core compound 19 in 50% yield (Scheme 1).

Scheme 1. Danishefsky’s synthesis of protected MGSTA-1 (19). Reagents and conditions: (a) TBDPSCI, imidazole, DMF; (b) MeI, NaH, THF; (c) H$_2$, Pd(OH)$_2$, EtOAc, 73% over three steps; (d) (COCl)$_2$, Et$_3$N, DMSO, CH$_2$Cl$_2$; (e) TiCl$_4$, CH$_2$Cl$_2$; (f) CSA, PhMe, 71% over three steps; (g) NaBH$_4$, EtOH, CeCl$_3$·7H$_2$O; (h) CSA, H$_2$O, THF; (i) LiBH$_4$, H$_2$O, THF, 55% over three steps; (j) (E)-hepta-2,6-dienoyl chloride, DMAP, CH$_2$Cl$_2$, 65%; (k) MOMCl, Bu$_4$NI, DIPEA, CH$_2$Cl$_2$; (l) HO; (m) DMP, CH$_2$Cl$_2$; (n) Tebbe reagent, pyridine, THF, 60% over two steps; (o) Grubbs II catalyst, PhCH$_3$, reflux, 50%.

2.1.2. First Total Synthesis of Migrastatin (Danishefsky et al.) [8]

In 2003, Danishefsky and co-workers publish the first total synthesis of migrastatin (7) [8]. In this regard, migrastatin was obtained from advanced intermediate 8, which was prepared using a similar strategy as that used for the synthesis of 15. A slightly optimized procedure for the preparation of 8 was reported one year later [9] and is illustrated in Scheme 2.

The synthesis [9] began with commercially available dimethyl 2,3-O-isopropylidene-L-tartrate (20), which was reduced using DIBAL. Diastereoselective divinylzinc addition afforded the desired vinyl carbinol 21 [27]. The two free hydroxyl groups were then methylated and the acetone protecting group was removed to afford diol 22 [28]. Glycol cleavage using Pb(OAc)$_4$ led to vinyl aldehyde 23, which was then used for the LACDAC sequence. Aldehyde 23 was reacted with diene 12 in the presence of TiCl$_4$ affording dihydropyrone 24 as a single diastereoisomer [22]. As above, the reduction of 24, followed by Ferrier rearrangement [24], gave lactol 26, which, after reductive opening using LiBH$_4$ and protection of secondary alcohol as TBS ether, afforded advanced intermediate 8. With 8 in hand, migrastatin was finally synthesized (Scheme 2). The synthetic procedure leading to migrastatin has already been reviewed elsewhere [15].
Scheme 2. Danishefsky’s synthesis of 8. Reagents and conditions: (a) DIBALH, then ZnCl₂, H₂C=CHMgBr, PhCH₃, −78 °C to RT, 75% (dr > 90%); (b) (i) Mel, NaH, DME, RT, (ii) 2 M HCl, MeOH, reflux, 80%; (c) Pb(OAc)₄, Na₂CO₃, CH₂Cl₂, 0 °C to RT; (d) (i) TiCl₄, CH₂Cl₂, −78 °C, (ii) TFA, CH₂Cl₂, RT, 87% from 22; (e) LiBH₄, MeOH, THF, −10 °C; (f) CSA, H₂O, THF, reflux; (g) LiBH₄, H₂O, THF, RT, 53% from 24; (h) TBSOTf, 2,6-lutidine, CH₂Cl₂, RT, (ii) AcOH:H₂O:THF (3:1:1), RT, 80%.

2.1.3. Synthesis of the Migrastatin-Core Library (Danishefsky et al.) [9,29]

After achieving the total synthesis of migrastatin [8], Danishefsky and co-workers reported [9,29] the preparation of a small library of migrastatin-core analogs using advanced intermediate 8 (Scheme 3). Reaction of 8 with (E)-hepta-2,6-dienoic acid in the presence of 2,4,6-trichlorobenzoyl chloride and DIPEA gave acylated compound 27, which, after RCM and protecting group removal, afforded MGSTA-1.

A similar strategy was applied for the preparation of MGSTA-2. Intermediate 8 was coupled to 6-heptenoyl chloride to give 29, which, after RCM and de-protection, afforded MGSTA-2. For the preparation of macroketone analogs, intermediate 8 was converted into allylic bromide 31 using the Appel reaction. Compound 31 was then coupled to β-ketosulfone 32 [30], followed reductive removal of the sulfone group mediated by Na/Hg, to give ketone 33. RCM and de-protection of 33 gave MGSTA-3. For the preparation of macrolactam analogs, compound 8 was converted into allylic azide 35 using diphenylphosphoryl azide under Mitsunobu conditions. Staudinger reduction of 35, followed by coupling with 6-heptenoic acid in the presence of EDC, gave compound 36, which, after RCM and removal of silyl protecting group, afforded MGSTA-4 (Scheme 3).

2.1.4. Synthesis by Cossy et al. [10,17]

In 2006, Cossy and co-workers [10] reported the synthesis of a precursor 46 of advanced intermediate 8 (Scheme 4). The synthesis began from methyl ester 38. Acetonide cleavage of 38 followed by regioselective protection of primary alcohol in 39 as TBDPS ether afforded 40. Methylation of secondary alcohol in 40, followed by DIBAL reduction of the ester moiety, gave primary alcohol 42. After oxidation of 42 under Swern conditions, the corresponding aldehyde was treated with 2-ethyl[tri(n-butyl)]stannane the presence of MgBr₂·OEt₂ to give the syn,syn-stereotriad 43 with good stereocore (dr = 90:10) [31]. The authors claim that the stereochemical outcome of that reaction resulted from an open chair transition state of type A, where the carbonyl and the methoxy group of aldehyde are chelated with MgBr₂·OEt₂ [31]. Compound 43 was treated with methacryloyl chloride in the presence of TEA and DMAP to give diene 44. RCM of 44 using Grubbs II catalyst gave unsaturated lactone 45, which contains the olefin with desired Z configuration and three contiguous stereocenters. Treatment of 45 with LiBH₄ in the presence of CeCl₃·7H₂O, followed by simultaneous protection of the two hydroxyl groups as TBS ether and regioselective de-protection of the primary alcohol, afforded precursor 46. This compound was therefore converted into migrastatin 7 using a similar procedure to that described by Danishefsky and co-workers [8] (Scheme 4).
The aldehyde obtained was treated with Still–Gennari phosphonate \[32,33\] in the presence of KHMDS precursor to give the unsaturated ester and therefore subjected to oxidative cleavage using OsO\(_4\), followed by treatment with NaIO\(_4\).

In 2007, Cossy and co-workers \[17\] reported the synthesis of 46 using a Still–Gennari olefination to control the formation of the (Z)-double bond (Scheme 5). Compound 43 \[10\] was protected as TBS ether and therefore subjected to oxidative cleavage using OsO\(_4\), followed by treatment with NaIO\(_4\). The aldehyde obtained was treated with Still–Gennari phosphonate \[32,33\] in the presence of KHMDS to control the formation of the (Z)-double bond (Scheme 5). Reduction of 48 with DIBAL gave the precursor 46. Following similar steps to those reported by Danishefsky et al. \[9\], this compound was converted to MGSTA-1 in 5 steps (Scheme 5).

Scheme 3. Danishefsky’s synthesis of the migrastatin-core library. Reagents and conditions: (a) (E)-hepta-2,6-dienoic acid, 2,4,6-trichlorobenzoyl chloride, DIPEA, pyridine, PhCH\(_3\), RT, 48%; (b) Grubbs II catalyst (20 mol %), PhCH\(_3\) (0.5 mM), reflux, 55% \((28)\), 76% \((30)\), 81% \((34)\), 60% \((37)\); (c) HF-pyridine, THF, RT, 66% (MGSTA-1), 94% (MGSTA-2), 90% (MGSTA-3), 81% (MGSTA-4); (d) 6-heptenoyl chloride, DMAP, CH\(_2\)Cl\(_2\), RT, 82%; (e) CBr\(_4\), solid-supported PPh\(_3\), CH\(_2\)Cl\(_2\), RT; (f) \(\beta\)-ketosulfone 32, DBU, PhCH\(_3\), RT, (ii) Na/Hg, Na\(_2\)HPO\(_4\), MeOH, RT, 61% from 7; (g) DPPA, DBU, PhCH\(_3\), RT, 87%; (h) PPh\(_3\), H\(_2\)O, THF, 70 °C, (ii) 6-heptenoic acid, EDC, DIPEA, CH\(_2\)Cl\(_2\), RT, 92%.

Scheme 4. Cossy’s synthesis of 46. Reagents and conditions: (a) \(p\)TSA, MeOH/H\(_2\)O (1:1), RT, 83%; (b) TBDPSCI, imidazole, CH\(_2\)Cl\(_2\), 0 °C to RT 81%; (c) Ag\(_2\)O, MeI, MS 4 Å, Et\(_2\)O, 40 °C, 96%; (d) DIBAL, CH\(_2\)Cl\(_2\), –78 °C to RT 90%; (e) (COCl)\(_2\), DMSO, CH\(_2\)Cl\(_2\), –78 °C, then Et\(_3\)N –78 °C to RT; (f) MgBr\(_2\)-OEt\(_2\), CH\(_2\)Cl\(_2\), –20 °C, then but-2-enyl-(tri-n-butyl)stannane, –60 °C, 87% (over two steps); (g) methacryloyl chloride, Et\(_3\)N, DMAP, CH\(_2\)Cl\(_2\), 0 °C to RT 80%; (h) Grubbs II catalyst (16.5 mol %), CH\(_2\)Cl\(_2\) (c = 10\(^{-2}\) M), 40 °C, 144 h, 65%; (i) LiBH\(_4\) (7 equiv.), CoCl\(_2\), H\(_2\)O (1 equiv.), THF/H\(_2\)O (4:1), RT, 74%; (j) TBSOTf, 2,6-lutidine, CH\(_2\)Cl\(_2\), 0 °C to RT, 75%; (k) THF/H\(_2\)O/AcOH (1:1:3), 36 h, RT, 75%.
2.1.5. Synthesis by Iqbal et al. [11]

In 2006, Iqbal and Parthasarati reported the synthesis of advanced intermediate 8 as its PMB derivative 65 in 12 steps. The synthesis started with dibutylboron triflate-mediated Evans aldol condensation of acrolein 52 and (S)-benzyl oxazolidinone 53 to give the desired aldol product 54 [34].

Scheme 5. Synthesis of MGSTA-1. Reagents and conditions: (a) TBSOTf, 2,6-lutidine, CH₂Cl₂, −20 °C, 93%; (b) (i) OsO₄, NMO, t-BuOH/H₂O (1/1), RT; (ii) NaIO₄, THF/H₂O (1/1), RT; (iii) 47, KHMD, 18-crown-6, THF, −78 °C, 80% (over 3 steps); (c) DIBAL, CH₂Cl₂, −78 °C to RT, 94%; (d) (E)-2,4,6-trichlorobenzoic (E)-hepta-2,6-dienoic anhydride, Pyridine, PhCH₃, RT, 67%; (e) NH₄F MeOH, reflux, 77%; (f) Dess-Martin Periodinane CH₂Cl₂, 0 °C to RT; (g) Zn, PbCl₂ cat, CH₂I₂Ti(Oi-Pr)₄, THF, RT (h) Grubbs II catalyst (20 mol %), PhCH₃, reflux, 47%; (i) HF Py, THF, RT, 67%.

The chiral auxiliary was then removed using LiBH₄ in THF to yield diol 55, which was then protected as TBS ether to afford compound 56. Acetal 56 was opened using DIBAL [35], and the corresponding primary alcohol 57 was then protected as TBS ether to afford the orthogonally protected compound 58. Oxidative cleavage of 58 using OsO₄-NaIO₄ in the presence of 2,6-lutidine [36] gave aldehyde 59 in good yield. Lewis acid-mediated distereoselective addition of vinylmagnesium bromide to aldehyde 59 gave the desired compound 60 (dr = 7:1), which was separated from its diastereomer in the next step. Secondary alcohol 60 was methylated using
MeOTf [37] to yield enantiomerically pure 61. TBAF-mediated deprotection of the TBS group in 61, followed by oxidation using Dees-Martin periodinane [38], gave the corresponding aldehyde, which was treated with Ando’s phosphonate 63 [39] in the presence of DBU to afford the (Z)-olefin 64 (Z/E ratio not reported). Reduction of the ester moiety using DIBAL gave the advanced intermediate 65, which was used for the preparation of the migrastatin core MGSTA-1 (Scheme 6).

2.1.6. Synthesis by Dias et al. [12]

The strategy used by Dias and co-workers for the preparation of advanced intermediate 8 involved the formation of stereocenters at C4,5 by means of an asymmetric aldol addition. The methoxy group at C6 was introduced as hydroxyl group using Upjohn dihydroxylation and, finally, the formation of stereocenters at C4,5 by means of an asymmetric aldol addition. The methoxy group was treated with Ando’s phosphonate 70 to yield enantiomerically pure 61. TBAF-mediated deprotection of the TBS group in 61, followed by oxidation using Dees-Martin periodinane [38], gave the corresponding aldehyde, which was treated with Ando’s phosphonate 63 [39] in the presence of DBU to afford the (Z)-olefin 64 (Z/E ratio not reported). Reduction of the ester moiety using DIBAL gave the advanced intermediate 65, which was used for the preparation of the migrastatin core MGSTA-1 (Scheme 6).

After chromatographic separation and recovery of the chiral auxiliary, lactone 68 was reacted with 4-methoxybenzyl 2,2,2-trichloroacetimidate in the presence of 10-camphorsulfonic acid to afford fully protected lactone 69. Reduction of 69 with LiAlH4 gave primary alcohol 70 in good yield. The primary alcohol in 70 was then protected as TBS ether (71), and the free secondary alcohol was methylated using 1,8-bis(dimethylamino)naphthalene (proton sponge) [50] to afford compound 72. The p-methoxybenzyl protecting group was then selectively removed using DDQ/H2O. Ley’s oxidation of the resulting primary alcohol [51] followed by Petasis olefination [52] gave olefin 73 in 44% yield (from 72). The primary TBS group was regioselectively removed using HF-Py-TF to

Scheme 7. Dias’ synthesis. Synthesis of 8. Reagents and conditions: (a) TiCl4, DIPEA, CH2Cl2, −78 °C to 0 °C; 1 h; (b) acrolein, −78 °C to 0 °C to RT, 12 h, 87% over two steps; (c) TBSOTf, 2,6-ludine, CH2Cl2, 0 °C, 20 min, 93%; (d) NMO, OsO4, cat., acetone/H2O, 0 °C, 45 min, 67 (57%), 68 (20%); (e) CSA cat., 4-methoxybenzyl-2,2,2-trichloroacetimidate, CH2Cl2, RT, 12 h, 67%; (f) LiAlH4, THF, −78 °C, 1 h, 75%; (g) TiCl4, imidazole, CH2Cl2, 0 °C, 1 h, 95%; (h) proton sponge, Me3OBF4, CH2Cl2, RT, 12 h, 75%; (i) DDQ/H2O, CH2Cl2, RT, 2 h, 88%; (j) NMO, TPAP cat., CH2Cl2; RT, 1 h; (k) Cp2TiMe2, PhCH3, 70 °C, 12 h, 50% over two steps; (l) HF-Py-TF, THF, RT, 12 h, 80%; (m) NMO, TPAP cat., CH2Cl2; 1 h, RT, (n) 75 in THF, NaH, RT, 12 h, 58% over two steps; (o) DIBAL, CH2Cl2, −15 °C, 1 h, 98%.

After chromatographic separation and recovery of the chiral auxiliary, lactone 68 was reacted with 4-methoxybenzyl 2,2,2-trichloroacetimidate in the presence of 10-camphorsulfonic acid to afford fully protected lactone 69. Reduction of 69 with LiAlH4 gave primary alcohol 70 in good yield. The primary alcohol in 70 was then protected as TBS ether (71), and the free secondary alcohol was methylated using 1,8-bis(dimethylamino)naphthalene (proton sponge) [50] to afford compound 72. The p-methoxybenzyl protecting group was then selectively removed using DDQ/H2O. Ley’s oxidation of the resulting primary alcohol [51] followed by Petasis olefination [52] gave olefin 73 in 44% yield (from 72). The primary TBS group was regioselectively removed using HF-Py-TF to
afford the corresponding primary alcohol 74. Ley’s oxidation followed by HWE reaction using Ando’s phosphonate ester 75 [39,53,54] gave compound 76 (Z/E = 85:15) in 58% yield (~100 mg scale) from 74. Finally, reduction of the ester moiety using DIBAL afforded the advanced intermediate 8 in 14 steps with 1.2% overall yield. Using 8, Andricopulo et al. prepared the macrolactone of migrastatin, namely MGSTA-1. The coupling of 8 to (E)-2,6-heptadienoic acid using DCC and DMAP [55–57], followed by ring-closing metathesis (RCM) [8,9,29] and removal of TBS protecting group, afforded MGSTA-1.

Since undesired lactone 67 was prepared on multi-gram scale, the authors undertook the preparation of the C-8 epimer of the migrastatin core, MGSTA-7. Lactone 67 was converted to compound 77 using the same strategy as that described above for the preparation of 8. Coupling of 77 to 6-heptadienoic acid gave 78, which underwent RCM. After removal of the TBS protecting group, 78 afforded MGSTA-7. Interestingly, 77 was coupled to (E)-2,6-heptadienoic acid, and the resulting compound 79 was submitted to RCM using Grubbs II catalyst. However, no metathesis product was observed (Scheme 8).

Scheme 8. Synthesis of C8 epimer of MGSTA-2. Reagents and conditions: (a) DCC, DMAP, 6-heptanoic acid, CH2Cl2, RT, 12 h, 92%; (b) Grubbs II catalyst, PhCH2, reflux, 30 min, 80%; (c) HF, CH2Cl2/CH3CN, RT, 24 h, 40%; (d) DCC, DMAP, (E)-2,6-heptadienoic acid, CH2Cl2, RT, 98%.

2.1.7. Synthesis by Iqbal et al. [13]

Iqbal’s synthesis of advanced intermediate 8 began with an Crimmins modified Evans aldol reaction [43,58] of Evan’s chiral auxiliary 53 and 3-butenal in the presence of (−)-spartein and TiCl4 to give aldol adduct 80. The secondary alcohol was protected as TBS ether (81), and the chiral auxiliary was removed using sodium borohydride to afford primary alcohol 82. Hence, oxidation of 82 using Dess-Martin periodinane gave the corresponding aldehyde, which reacted with ethyl 2-(diphenyl-phosphono)acetate (63) [59] in the presence of DBU to give the corresponding α,β-unsaturated ester 83 with Z configuration. The attempt to promote intermolecular allylic C-H oxidation on ester 83 using White’s catalyst [60] gave two regioisomers 84 and 85 in equal ratio [61]. Compound 84 was characterized and appeared as a single product (1H-NMR evidence); however, no further studies were performed to assign the stereochemistry of the newly formed stereocenter.

With the aim to achieve high regio- and diastereo-selectivity, the authors hydrolyzed ester 83 to the corresponding carboxylic acid 86, which was then submitted to intramolecular lactonization via C-H allylic activation using White’s catalyst [62–64]. After a brief optimization, the authors found that the reaction of 86 with White’s catalyst in the presence of DDQ and Cr(III)salenCl gave lactone 87 as a single diastereoisomer in 40% yield with 50% recovery of the starting material. Reduction of lactone 87 with DIBAL gave the corresponding diallylic alcohol 88. Regioselective protection of primary alcohol was achieved using TBSCl in the presence of imidazole to afford 89. Methylation of the
secondary alcohol using MeOTf gave intermediate 90. Finally, regioselective deprotection of primary alcohol with CSA in methanol gave advanced intermediate 8 (Scheme 9).

2.1.8. Synthesis by Murphy et al. [14]

In their synthesis of fragment 8, Murphy and co-worker used Ando’s phosphonate ester [11–13,39,59] to introduce the C2,3 olefin with Z configuration. A diastereoselective Brown alkoxylallylation [63] was used to introduce the two contiguous stereocenters at C5,6. The synthesis began with desymmetrization using MeOTf gave intermediate 90. Finally, regioselective deprotection of primary alcohol with CSA in methanol gave advanced intermediate 8 (Scheme 9).

Scheme 9. Iqbal’s synthesis of 8. Reagents and conditions: (a) TiCl4, 3-butenal, (−) spartein, CH2Cl2, 0 °C, 1 h, 83%; (dr 20:1); (b) TBSOTf, DIPEA, CH2Cl2, 0 °C, 90%; (c) NaBH4, THF:H2O, 90 h, 80%; (d) DMP, CH2Cl2, 2 h, 90%; (e) NaI, DBU, −78 °C to 0 °C, THF, 3 h, Z:E, 95:5, 60%; (f) White catalyst 10%, p-benzoquinone, p-nitrobenzoic acid, 45 °C, 72 h, 84 (24%) and 85 (20%); (g) LiOH, THF/MeOH/H2O, 4 h, 55 °C, 90%; (h) White catalyst 10%, p-benzoquinone, Cr(III)salenCl, 1,4-dioxane, 45 °C, 72 h, 40% (78% based on recovered starting material); (i) DIBAL, −78 °C to 0 °C, CH2Cl2, 2 h, 90%; (j) TBSCI, Imidazole, DMF, 2 h, 90%; (k) DTBMP, CH2Cl2, MeOTf, 6 h, reflux, 70%; (l) CSA, MeOH 2 h, 90%.

Oxidation of 93 using TEMPO and BAIB gave aldehyde 94. Hence, allyl methyl ether was treated with sBuLi at low temperature. Addition of (+)-B-methoxydiisopino-campheylborane gave the corresponding borane, which reacted with chiral aldehyde 94 to yield 96 with high diastereoselectivity. The benzoate protecting group was then removed [67] to give diol 97 as a single diastereoisomer in good yield. Both hydroxyl groups were protected as TBS ethers using TBSOTf in the presence of triethylamine (73). Regioselective de-protection of the primary TBS protecting group was achieved by treating the fully protected 73 with catalytic p-toluenesulfonic acid in methanol to give 74. Oxidation of 74 using Dess-Martin periodinane afforded aldehyde 98 in excellent yield. Horner-Wadsworth-Emmons reaction was used to introduce the Z olefin at C2,3. Treatment of 98 with Ando’s phosphonate 63 [11,13] gave the desired alkene 76. The reaction proceeded with high Z selectivity (Z/E = 97:3) on a 1.0 g scale. The authors reported some erosion of the Z selectivity when the reaction was scaled up to ~4.0 g (Z/E = 90:10). Finally, reduction of ester moiety using with diisobutylaluminum hydride
(DIBAL-H) afforded intermediate 8 in 9 steps with an overall yield of 30% from 91. Using 8, the authors prepared Macrolactone MGSTA-3 in 100-mg scale, which was used for in vivo studies [14], and the natural product migrastatin (7) (Scheme 10). Interestingly, MGSTA-3, which was contaminated by hydrocarbon and silicon grease [68], was purified by distillation under reduced pressure using a Kugelrohr apparatus (0.05 mbar, T = 90 °C, 18 h).

![Scheme 10. Murphy's synthesis of 8. Reagents and conditions: synthesis of 8, migrastatin 7 and MGSTA-3.](image)

In 2015 [18], Murphy and co-workers reported the preparation of several migrastatin-core analogs with variations in the macrocycle ring size and functionality. Therefore, advanced intermediate 8 was used for the preparation of compounds MGSTA-8 to MGSTA-16.

The coupling of advanced intermediate 8 with carboxylic acids 99a–c under Mitsunobu conditions gave the corresponding esters 29 and 100a,b which underwent ring-closing metathesis (RCM) using Grubbs II catalyst to give macrolactones 30 and 101a,b. Removal of the TBS group was achieved using HF-pyridine to afford MGSTA-2 and the 13- and 15-membered analogs MGSTA-8 and MGSTA-11 (Scheme 11).
Scheme 11. Synthesis of macrolacton migrastatin-core analogs. Reagents and conditions: (a) 99a–c, Ph3P, DIAD, PhCH3, RT; 100a (69%), 100b (76%); (b) Grubbs II catalyst, PhCH3, reflux, 101a (68%), 101b (73%); (c) HF·py, THF, RT; MGSTA-8 (61%), MGSTA-11 (54%).

Furthermore, migrastatin-core-based macroketones were prepared by the conversion of allylic alcohol 8 into the allylic bromide 31 using an Appel reaction [9]. This was subsequently reacted with β-ketosulphones 32-102a,b [9] to give ketones 33 and 103a,b. Ring-closing metathesis reaction using Grubbs II catalyst afforded macroketones 34 and 104a,b, which, after TBS removal using HF·Py, gave MGSTA-3, and the 13- and the 15-membered analogs MGSTA-9 and MGSTA-12. In addition, protected macroketone 34 underwent Saeugusa Ito oxidation using LHMDS and TMSCl, followed by treatment with Pd(OAc)2, to give the α,β-unsaturated macroketone MGSTA-13 after TBS de-protection (Scheme 12).

Scheme 12. Synthesis of macroketone migrastatin-core analogs. Reagents and conditions: (a) CBr4, Ph3P polymer-bound, CH2Cl2; (b) 32-102a,b, DBU, PhCH3 then 31, RT; (c) Na/Hg, MeOH, RT; (103a (51%)) 103b (51%), from 8; (d) Grubbs II catalyst, PhCH3, reflux, 104a (99%), 104b (60%); (e) (i) 34, TMSCI, LHMDS, THF, 0 °C, 2 h; (ii) Pd(OAc)2, CH3CN, RT, 2 h, 78% from 34; (f) HF·Py, THF, RT, MGSTA-13 (90%); (g) HF·Py, THF, RT, MGSTA-9 (84%), MGSTA-12 (82%).

Macrothiolactones were also prepared using Mitsunobu reaction of thioacids 106a,b with allylic alcohol 8. Thioacids 106a and 106b were prepared by treating the corresponding carboxylic acids 105a,b with Lawesson’s reagent under microwave irradiation [69]. As described above, RCM and removal of the TBS group afforded macrothiolactones MGSTA-10 and MGSTA-14 (Scheme 13). Interestingly,
silicon grease impurity in MGSTA-8 to MGSTA-14 was removed after distillation under reduced pressure (Kugelrohr apparatus: 0.05 mbar, T = 90 °C, 2 to 24 h). Since MGSTA-3 was synthesized in 100-mg scale, the authors undertook the preparation of glucuronidated migrastatin-core analogs.

Scheme 13. Synthesis of macrothiolactone migrastatin-core analogs. Reagents and conditions: (a) Lawesson’s reagent, CH2Cl2, mw, 100 °C, 10 min; (b) 105a,b, Ph3P, DIAD, PhCH3, RT; 106a (42%), 106b (39%); (c) Grubbs II catalyst, CH2Cl2, mw, 100 °C, 30 min, 107b (49%); (d) Grubbs-II catalyst, PhCH3, reflux, 107a (88%); (e) HF·Py, THF, RT; MGSTA-3 (85%), MGSTA-7 (63%).

The glucuronidation of small molecules is a physiological process that leads to highly water-soluble compounds that are therefore excreted through the kidney [70]. These compounds are generally biologically inactive. However, it has been found that glucuronidated small molecules can display increased biological activity through a direct or indirect mechanism [70–72]. In addition, β-glucuronidases are generally overexpressed in tumor tissue and glucuronidation strategy has been exploited for the preparation of pro-drugs based on SN-38 or taxol [72,73]. Murphy and co-workers demonstrated that reaction of MGSTA-3 with trichloroacetimidate 109 [75] in the presence of TMSOTf leads to the protected β-glucuronide 110. The resulting compound was subsequently treated with TiCl4 [76–82] to give α-glucuronide 111 as a single product. Finally, removal of benzoate groups and hydrolysis of methyl ester led to migrastatin analogs MGSTA-15 and -16 (Scheme 14).

Scheme 14. Reagents and conditions: (a) 109 TMSOTf, MS (AW300), CH2Cl2, −78 °C, 5 h, 60%; (b) TiCl4, CDCl3, 4 °C, 69%; (c) NaOH aq., MeOH, RT, 18 h, MGSTA-16 (61%), MGSTA-17 (73%).
2.2. Biology

2.2.1. Preliminary Findings [3,6,83]

The biological activity of migrastatin was first reported in 2000 by Imoto et al. [3] These authors showed that migrastatin inhibits the migration of EC17 (mouse esophageal cancer cells), with an IC$_{50}$ of 6 $\mu$M in a wound-healing assay (WHA) and of 2 $\mu$M in a chemotaxis cell chamber assay. However, the same authors reported [83] that a migrastatin sample was contaminated with teleocidin-related impurities, which are known for their anti-migratory activity. After careful purification, they showed an inhibition of cell migration in EC17 cells, pretreated with pure migrastatin for 24 h, with an IC$_{50}$ of 20.5 $\mu$M (WHA). In addition, they showed that migrastatin inhibits the growth of EC17 cells, with an IC$_{50}$ of 167.5 $\mu$M. These results indicate that inhibition of cell migration was not due to cytotoxicity [83].

In 2006, the same authors reported that migrastatin inhibits the function of P-glycoprotein and is therefore capable of suppressing multidrug resistance (MDR) [6]. They demonstrated that migrastatin increases the intracellular concentration of anticancer drugs vinblastine, vincristine and taxol in P-glycoprotein-overexpressing VJ-300 (vincristine-resistant human epidermoid carcinoma) [84] and P388/VCR (vincristine-resistant mouse leukemia) cells [85]. The cytotoxicity of vincristine and taxol in VJ-300 cells treated with migrastatin (61 $\mu$M) increased 40- and 53-fold respectively (migrastatin not toxic up to 102 $\mu$M).

2.2.2. Danishefsky’s Work [9,86–88]

An important breakthrough with respect to the biological activity of migrastatin was made by Danishefsky et al. in 2005 [9]. Through a diverted total synthesis approach (Figure 2), a series of truncated analogs of migrastatin were prepared and tested as inhibitors of cell migration in 4T1 cells (mammary mouse cancer) and HUVECs (human healthy endothelial cells). Migrastatin-core analogs MGSTA-2 to 4 were $\approx$ 1000 more potent that migrastatin itself (Figure 4b) and were not cytotoxic up to 20 $\mu$M. Interestingly, while migrastatin was stable in mouse plasma, macrolactone MGSTA-1 and MGSTA-2 were not. On other hand, macroketone MGSTA-3 and macrolactam MGSTA-5 displayed higher stability, as revealed by an unchanged HPLC signal over 60 min of incubation (Figure 4c).

MGSTA-3 and MGSTA-4 also inhibited the migration of highly invasive and metastatic cancer cell lines, such as MDA-MB-231 (human breast tumor), Lovo (human colon tumor) and PC-3 (human prostate tumor) in WHAs [86]. Importantly, MGSTA-3 and MGSTA-4 did not affect the migration of normal human mammary-gland epithelial cells (MCF-10A), mouse embryonic fibroblasts, or primary mouse leukocytes in WHAs [86].

For in vivo studies [86], the 4T1 mouse mammary model was chosen. In this model, the tumor closely mimics human breast cancer with respect to immunogenicity, metastasis, anatomy and growth characteristics [89]. 4T1 tumors spontaneously metastasize to the lung, bone, brain and liver [90]. MGSTA-3 and MGSTA-4 were administrated daily for 20 days at 10 mg/kg or 20 mg/kg. MGSTA-3 and MGSTA-4 reduced the metastasized 4T1 cells in the lungs by 91%–99% (measured by 6-thioguanine clonogenic assay) (Figure 4d). Tumor growth was not affected by MGSTA-3 or MGSTA-4, and no obvious side effects were observed. MGSTA-2, which was not stable in plasma (Figure 4c), was considerably less effective at inhibiting metastasis (Figure 4). In addition, the authors showed that MGSTA-3 and MGSTA-4 block the activation of RAC (Ras-related C3 botulinum toxic substrate 1), a protein involved in lamellipodia formation and therefore in cell migration.
while that treated with MGSTA-5 (Figure 5d). After 9 weeks, a metastatic tumor was detectable in the former group but not in the latter. Interestingly, a higher concentration of MGSTA-5 of mice treated with MGSTA-5 (three times/week; 40 mg/kg) from day 1 (pre-treatment). Another group of mice was treated starting from day 15 (post-treatment). At the time of surgical resection, 50% of control mice treated starting from day 15 (post-treatment) showed an 87% reduction in metastases, with an IC\textsubscript{50} in the nanomolar range (Figure 5c). Interestingly, a higher concentration of MGSTA-5 was necessary to inhibit the migration of MDA-MB-231 cells (extrapolated from graph) \[87\] of around 1.5–2 µM. LM2 cells are aggressive and highly metastatic and are derived from lung metastasis of MDA-MB-231 \[90\]. The ability of MGSTA-5 to inhibit tumor metastasis in vivo was assessed using luciferase-based noninvasive whole animal bioluminescent imaging in a xenograft breast cancer model in NOD/SCID mice transplanted with MDA-MB-231 cells stably expressing the HSVTK-eGFP-luciferase (TGL) reporter protein \[92\]. After inoculation of MDA-MB-231 cells, a group of five mice were treated with MGSTA-5 (three times/week; 40 mg/kg) from day 1 (pre-treatment). Another group of mice was treated starting from day 15 (post-treatment). At the time of surgical resection, 50% of control mice had metastasis and 85% had tumors invading the muscle layer and peritoneal membrane. The group of mice treated with MGSTA-5 from day 1 (pre-treatment) showed an 87% reduction in metastases, while that treated with MGSTA-5 from day 15 (post-treatment) showed a 47% reduction (Figure 5c). Pre-treatment with MGSTA-5 also had an important effect on overall survival. Mice were pre-treated with MGSTA-5 at 40 mg/kg and 200 mg/kg. After 50 days, all the control mice had died; however, the overall survival of the groups treated with 40 and 200 mg/kg was 30% and 50%, respectively (Figure 5d). After 9 weeks, a metastatic tumor was detectable in the former group but not in the latter. Importantly, treatment with MGSTA-5 did not attenuate the growth of the primary tumor \[87\].
The capacity of MGSTA-5 and 6 to inhibit tumor metastasis generated from human primary small lung carcinoma cells (SLCL) was also evaluated. Primary tumors were obtained from patients, and cells were stably transduced with a triplefusion protein reporter construct (AC3-TGL) and then monitored by serial noninvasive bioluminescent imaging (BLI). At day 55, mice were killed, and the spread of metastasis in lungs, liver, heart, kidneys and spleen was evaluated using BLI (Figure 6c). Neither analog affected the inhibition of tumor growth. No toxicity was detected in mice treated with MGSTA-6 (12 mg/kg) was approximately four times more potent than MGSTA-5 at the same dose (10 mg/kg).

The capacity of MGSTA-5 and 6 to inhibit tumor metastasis generated from human primary small lung carcinoma cells (SLCL) was also evaluated. Primary tumors were obtained from patients, and cells were stably transduced with a triplefusion protein reporter construct (AC3-TGL) and then monitored by serial noninvasive bioluminescent imaging (BLI). At day 55, mice were killed, and the spread of metastasis in lungs, liver, heart, kidneys and spleen was evaluated using BLI (Figure 6c). Neither analog affected the inhibition of tumor growth. No toxicity was detected in mice treated with MGSTA-6. In addition, treatment with the low dose of MGSTA-6 (12 mg/kg) was approximately four times more potent than MGSTA-5 at the same dose (10 mg/kg).
2.2.3. Mechanism of Action [93]

In 2010, Chen and co-worker [93] attempted to elucidate the mechanism of action of MGSTA-3. The authors claimed that the target of MGSTA-3 was fascin. Fascin is an actin-bundling protein responsible for cell migration and therefore for cell invasion and metastasis. Fascin mRNA transcript and protein levels are elevated in aggressive tumors [94,95], and overexpression of fascin is also linked to increased cell migration and invasion [96,97]. Using an affinity protein purification approach, they demonstrated that a MGSTA-3 biotin-labeled analog [9] (Figure 7a) binds to fascin in cancer cell extracts. F-actin pelleting assays [98] revealed that MGSTA-3 significantly decreases fascin-induced bundling of F-actin polymers. The authors also published an X-ray crystal structure of fascin co-crystalized with MGSTA-3 and claimed that MGSTA-3 binds at the same site of actin. However, this X-ray structure was found to be incorrect and was therefore retracted [93], since the chemical structure of MGSTA-3 bound to fascin shown in the X-ray picture was not MGSTA-3 but rather its isomer [99]. Furthermore, the authors showed that selective mutation of fascin in the proposed region of binding reduced the actin-bundling activity of fascin (mutation on H392, Lys471, Ala488, F-actin pelleting assay, Figure 7b). On other hand, mutation of His 474 to Ala did not reduce the activity of fascin but rendered fascin resistant to MGSTA-3 treatment (Figure 7c). These results were also validated in 4T1 cancer cells in a boyden chamber assay (Figure 7d).

**Figure 7.** (a) Structure of Biotin-conjugated MGSTA-3; (b) Quantification of actin bundling assay for the wild-type fascin and mutants; results are means and ± SD (n = 3); * p < 0.05 (c) Mutant sensitivity to MGSTA-3. Wild-type fascin and the E391A and H474A mutants of fascin were assayed for their actin-bundling activity in the absence or presence of MGSTA-3 (10 µM); results are means and ±SD (n = 3). * p < 0.05; (d) Boyden chamber cell migration assay of mouse fascin shRNA-treated 4T1 cells transfected with various mutants of GFP-human fascin (h-fascin) in the presence or absence of MGSTA-3 (10 µM); results are means and ±SD (n = 5) p < 0.05; (e) Tumor metastasis assay with mouse fascin shRNA-treated 4T1 cells overexpressing wild-type human fascin or fascin (H474A) mutant in the presence or absence of MGSTA-3 (10 mg/kg). Comparison of the fascin shRNA group with the control shRNA group. Results are means and ±SD (n = 5–6). * p < 0.05 Adapted by permission from Macmillan Publishers Ltd: Nature, 2010, 464, 1062, copyright (2010) http://www.nature.com/.

Knockdown of fascin using short-hairpin RNAs (shRNA) decreased cell migration. This effect was rescued by transfection of wild-type human fascin cDNA or H477A/H477K human fascin cDNA. Importantly, rescued migration of WT fascin was sensitive to MGSTA-3 while rescued migration by H477A or H477K fascin was not (Figure 7d). Similar results were obtained in vivo using various fascin mutants in mouse fascin shRNA-treated 4T1 cells in the presence or absence of MGSTA-3 (Figure 7e). In 2013, Król and co-workers [19] examined the effect of MGSTA-2 to 3, MGSTA-8 to
MGSTA-13 in canine mammary cell lines. The study was carried out using CMT-W1 and CMT-W2 cells and their corresponding lung metastasis CMT-W1M and CMT-W2M cells [100,101]. A preliminary screen using the wound-healing assay (WHA) showed that MGSTA-3 and MGSTA-13 were the most promising analogs. Further single concentration studies (Boyden chamber assay) showed that MGSTA-13 was the most promising compound. MGSTA-13 inhibited cell migration of CMT-W1, CMT-W1M, CMT-W2 cells, with an IC$_{50}$ in the molar range (Figure 8b). Surprisingly, no effect was observed in CMT-W2M cells. They next examined the invasive phenotype [102,103] of CMT-W1, CMT-W1M, CMT-W2 cells cultured on reconstituted basement membrane (Matrigel™) in the presence or the absence of MGSTA-13. After 24 h, cells cultured in control conditions showed typical branching formation. In contrast, all cells treated with MGSTA-13 showed a remarkable inhibition of branch formation (Figure 7c). This observation indicates that this compound has an effect on actin machinery, which is responsible for filopodia formation and thus for cell migration. Given these findings, the authors focused their attention on fascin, an actin-bundling protein responsible for the development and maintenance of straight and tight F-actin bundles [104–106]. Using confocal microscopy, they demonstrated that actin strongly co-localized with fascin1 in CMTW1 cells, while after treatment with MGSTA-3 this co-localization decreased dramatically (Figure 8d). In addition, CMT-W1 cells in control conditions showed several filopodia and protrusions, which disappeared after treatment with MGSTA-13 (Figure 8e). Similar results were obtained with CMT-W1 and CMT-W2 cells. With these results in hand, the authors analyzed the expression of phospho-fascin1 protein (phospho-FSCN1(Ser39)) in the four cell lines. The expression of fascin1 in CMT-W2M cells, which were not sensitive to MGSTA-3 and 13, was lower than in the other cell lines (Figure 8f).

Figure 8. (a) Structure of MGSTA-13; (b) Chamber cell migration assay with canine mammary cancer cell lines; (c) Growth characteristics of CMT-W1, cell lines cultured on Matrigel and treated with MGSTA-13 100 µM for 24 h; (d) Quantification of fascin1 and F-actin co-localization at merge images; the unpaired t-test was applied. p < 0.01 (e) Representative confocal microscopy images of cytoskeletal protein F-actin and fascin1 in CMT-W1M canine carcinoma cell line; (f) Expression of phospho-FSCN1(Ser39) in canine mammary cancer cell lines; the unpaired t-test was applied. * p < 0.05. Adapted from PLoS ONE, 2013, 8, e76789.

In 2014, Murphy and Anderson [14] studied the effect of MGSTA-3 on epithelial cadherin (E-cadherin) dynamics in vivo and in vitro. Misregulation of epithelial E-cadherin is associated with the ability of cancer cells to detach from the primary tumor and to become invasive and metastatic [107–109]. E-Cadherin is a cell-cell adhesion protein involved in the maintenance of the epithelial architecture [110]. Reduced levels of E-cadherin have been found in colon, breast, prostate and ovarian cancer [111–114]. Pancreatic ductal adenocarcinoma (PDAC) cells were treated with MGSTA-3, and E-cadherin dynamics was studied using fluorescence recovery after bleaching (FRAP). These cells are highly invasive because...
of expression of a mutant form of the tumor suppressor p53 [115]. **MGSTA-3** had no effect on the immobile fraction of E-cadherin, a measure of the amount of E-cadherin immobilized at cell-cell junctions. PDAC cells were therefore injected subcutaneously into nude mice, and tumors were allowed to grow for seven days. The mice were treated with **MGSTA-3** (20 mg/kg) for three days, and E-cadherin dynamics were studied using FRAP. **MGSTA-3** treatment increased the immobile fraction, an effect expected to strengthen cell-cell adhesion and therefore impede metastasis.

In 2015, Murphy and co-workers reported the biological activity of several migrastatin core analogs. **MGSTA-8** to 16 were tested against three breast (MCF7, MCF7-Dox, MDA-MB361) and one pancreatic (HPAC) cancer cell lines in WHA. All analogs inhibited cell migration, and none were toxic up to 100 µM. Selected analogs (**MGSTA-3, MGSTA-12 to 13, MGSTA-15 to 16**) were therefore tested in transwell assays in MCF7, MDA-MB-361 and HPCA cells (Figure 9). All these compounds inhibited cell migration in the nanomolar range, with the exception of **MGSTA-13** in MCF-7 cells. Since **MSTA-13** inhibited cell migration in the highly metastatic MDA-MB-361 cell but not in the less invasive MCF7 cells, the authors claimed that this effect could be related to cytoskeleton proteins targeted by migrastatin analogs [14,93]. Interestingly, **MGSTA-13** was tested against a panel of 55 targets known to be related to adverse drug reactions (ADRs). **MGSTA-13** showed only weak inhibitory capacity over adenosine receptor A2A (27%) and prostanoid EP4 receptor (39%). It has been reported that a promiscuity index (percentage of targets giving more than 50% inhibition at 10 µM in a set of at least 50 targets) of more than 20% is linked to market withdrawal and clinical trial failure. Unsaturated macrolactone **MGSTA-13** registered a promiscuity index of between 0% and 5%, and is therefore a promising candidate for further biological studies.

![Figure 9. Structures of selected migrastatin analogs and effect on human breast cancer cell lines MCF7 and MDA MB-361 assessed with Boyden chamber assay.](image)

| IC50 (nM) | MCF7 | MDA MB-361 |
|----------|------|------------|
| MGSTA-3  | 13   | 974        |
| MGSTA-12 | 153  | nd         |
| MGSTA-13 | >1 µM| 410        |
| MGSTA-15 | 205  | nd         |
| MGSTA-16 | 66   | nd         |

The biological activity of migrastatin-core analogs has attracted the attention of cancer biologists in recent years. Several synthetic approaches have been developed for these compounds, and their relatively simple structure has allowed the preparation of small libraries and scale-up for in vivo studies.

Migrastatin analogs act as anti-metastatic agents by inhibition of the cell motility machinery and they have no effect in tumor growth or proliferation. The mechanism by which migrastatin analogs interfere with cell migration is still debated and further studies are necessary in order to confirm their primary target. Preliminary data suggest that migrastatin analogs selectively reduced cell migration in cancer cells without interfering with non-transformed cells. New findings in this direction will definitely help medicinal chemist to design new analogs with enhanced biological activity and stability.

In addition, this class of compound displayed low cytotoxic activity and recently it have been demonstrated that **MGSTA-13** present low affinity for several targets related to adverse drug reactions (ADRs). Since high affinity of targets related with ADRs result in preclinical/clinical or market withdrawal, migrastatin analogs could be valuable molecules for further preclinical development. Efforts in this direction could lead to the identification of preclinical candidates with enhanced safety profile.
We also believe that the preparation of synthetic migrastatin analogs with enhanced water solubility and plasma stability is also necessary. It has been demonstrated that free hydroxyl group in migrastatin core could be modified with the introduction of a carboxymethyl moiety or sugar moiety without drastically affect the biological activity. Moreover, modification of hydrophobic left hand side of migrastatin-core analogs has not be explored.

From the clinical point of view, migrastatin analogs could act as prophylactic agents that prevent tumor cells to metastasize. Safe and efficient migrastatin analogs could be administrated after liver or lung metastasis resection and this could prevent metastasis relapse. Alternatively, migrastatin analogs could be administrated during or after chemotherapy standard treatment.

We hope that this review serves to inspire the preparation of novel analogs with enhanced pharmacological properties, thus paving the way for their application in the treatment of patients with metastatic cancer.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

List of Abbreviations Used

| Abbreviation | Full Form |
|--------------|-----------|
| 2,6-lutidine | 2,6-dimethylpyridine |
| BAIB | (Diacetoxyiodo) benzene |
| CSA | camphorsulfuric acid |
| DCC | N,N’-dicyclohexylcarbodiimide |
| Dibal | diisobutylaluminium hydride |
| DIPEA | N,N-Diisopropylethylamine |
| DMP | Dess-Martin periodinane |
| DMAP | 4-dimethylaminopyridine |
| DPPA | diphenylphosphoryl azide |
| DTBMP | 2,6-di-tert-butyl-4-methylpyridine |
| DBU | 1,8-Diazabicyclo[5.4.0]undec-7-ene |
| EDC | 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide |
| Grubbs II | (1,3-Bis(2,4,6-trimethylphenyl)-2-imidazolidinylidene)dichloro(phenylmethylene) ruthenium |
| (+)Ipc2BOME | (+)-8-methoxydiisopinocampheylborane |
| MS | molecular sieves |
| MeOTf | methyl trifluormethanesulfonate |
| MOMCl | chloromethyl methyl ether |
| NMO | N-methylmorpholine-N-Oxide |
| Proton sponge | 1,8-bis(dimethylamino)napththalene |
| TBDPSi | tert-butyl diphenylsilyl chloride |
| TBAF | tetrabutylammonium fluoride |
| TBSI | tert-butyl(dimethyl)silyl chloride |
| TBSCl | tert-butyl(methyl)silyl chloride |
| TBSPf | tert-butyl(dimethyl)trifluormethanesulfonate |
| Tebbe reagent | Bis(cyclopentadienyl)-μ-chloro-(dimethylaluminum)-μ-methylenetitanium |
| TEMPO | 2,2,6,6-tetramethyl-1-piperidinoxy |
| TPAP | tetrapropylammonium perruthenate |
| p-TSA | p-toluenesulfonic acid |
| White catalyst | 1,2-bis(phenylsulfinyl)ethane palladium (II) acetate |
| WHA | wound-healing assay |
List of Cell Lines

- 4T1 (mammary mouse cancer)
- A549 (lung carcinoma)
- CMT-W1 (canine mammary cancer)
- CMT-W2 (canine mammary cancer)
- CMT-W1M (canine lung metastasis)
- CMT-W2M (canine lung metastasis)
- EC17 (mouse esophageal cancer)
- H1299 (lung)
- H1975 (lung adenocarcinoma)
- HPAC (human pancreas adenocarcinoma)
- HUVECs (human healthy endothelial cells)
- LM2-4175 (lung metastatic cells derived from MDA-MB-231)
- Lovo (human colon cancer)
- MCF7 (human breast cancer)
- MDAB-MB-361 (human breast cancer)
- MCF-10A (normal human mammary-gland epithelial cells)
- MDA-MB-231 (human breast cancer)
- MDA-MB-435 (human breast cancer)
- P388/VCR (vincristine-resistant mouse leukemia)
- PC-3 (human prostate cancer)
- PDAC (pancreatic ductal adenocarcinoma)
- VJ-300 (vincristine-resistant human epidermoid carcinoma)

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