Monoenomycin: A Simplified Trienomycin A Analogue that Manifests Anticancer Activity

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Abstract

Macrocyclic natural products are a powerful class of lead-like chemical entities. Despite commonly violating Lipinski's "rule of 5", these compounds often demonstrate superior drug-like physicochemical and pharmacokinetic attributes when compared to their acyclic counterparts. However, the elaborate structural architectures of such molecules require rigorous synthetic investigation that complicates analogue development and their application to drug discovery programs. To circumvent these limitations, a conformation-based approach using limited SAR and molecular modeling was implemented to design simplified analogues of trienomycin A, in which the corresponding analogues could be prepared in a succinct manner to rapidly identify essential structural components necessary for biological activity. Trienomycin A is a member of the ansamycin family of natural products that possesses potent anticancer activity. These studies revealed a novel trienomycin A analogue, monoenomycin, which manifests potent anticancer activity.

Keywords

Trienomycin A; Conformation; Anticancer; Ansamycin synthesis; Structure-activity relationship

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The macrocycle is an important structural motif amongst bioactive natural products, and imparts favorable physicochemical and pharmacological attributes. Macrocyclic geometry maintains a delicate balance between flexibility and rigidity that commonly affects solubility, permeability, and binding to biological targets. Despite their inherent drug-like properties, the complicated architecture exhibited by macrocyclic natural products provides a significant challenge toward their utility in drug development. While the discovery of novel chemical methodologies has led to the *de novo* synthesis of numerous macrocyclic natural products, approaches toward simplified analogues remains underinvestigated. In this letter, a strategy utilizing molecular modeling combined with a conformation-based approach was used to develop simplified ansamycin analogues that maintain biological activity.

Mycobacteria produce the ansamycin family of natural products, which manifest diverse biological activities. Structural characteristics of this family include a macrocyclic polyketide bridged by an aromatic core. Several ansamycins display significant clinical

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SUPPORTING INFORMATION. Full experimental procedures and characterization for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

attributes, including rifampicin, which manifests antimicrobial activity for the treatment tuberculosis,[1] and geldanamycin, derivatives of which have entered phase III clinical trials for the treatment of cancer.[2] In general, the ansamycin family exhibits a high degree of broad inhibitory activities that include antiviral, antifungal, and immunosuppressant activity.[3, 4]

Trienomycin A (Figure 1, **1**) is a member of the ansamycin family and was first isolated from *Streptomyces* sp. No. 83-16 by Umezawa and coworkers.[5] In contrast to other members of the ansamycin family that possess a *p*-quinone or *p*-hydroquinone moiety within the aromatic bridge, trienomycin A contains a non-redox active phenol. In addition, trienomycin A displays a biological profile that contrasts the activity manifested by other ansamycins. For example, mycotrienin II, which is structurally identical to trienomycin A with the exception of a *p*-hydroquinone moiety, is a potent antifungal agent as well as a promising anticancer agent.[3, 6] In contrast, trienomycin A manifests potent anticancer activity (IC_{50} of 128 nM against HeLa cervical cancer cells),[7] but displays no antifungal activity, nor does it display any significant antimicrobial, antiviral, or immunosuppressant activity.[3, 5, 7–9]

A previous report indicated that while trienomycin A exhibits potent anticancer and antitumor activity, it was found to be significantly less toxic to non-transformed cells.[8] Thus, the unique biological activity manifested by trienomycin A poses this natural product as an attractive lead compound for cancer chemotherapeutic development. Unfortunately, limited structure-activity relationships (SAR) for trienomycin A have been reported and the mechanism of action remains unknown. Furthermore, significant quantities of the natural product are not available and only one total synthesis has been reported, which produces the natural product in 31 linear steps.[10] Although elegant in nature, this route does not afford a succinct method for evaluation of SAR. Therefore, a generalized method for the production of synthetically useful trienomycin A analogues employing a conformation-based approach was pursued.

Previously reported semi-synthetic modifications to the natural product including acetylation of the 13-OH (**2**), saturation of the triene motif (**3**), and deletion of the *N*-cyclohexylcarbonyl D-Ala (NCxDA) side chain (**4**), resulted in almost complete ablation of anticancer activity (Figure 3, **2**–**4**).[3] However, methylation of the free phenol did produce an analogue equipotent to the natural product (Figure 2, **5**). Without apparent SAR trends, and the lack of activity for semi-synthetic derivatives, no obvious hypothesis for further exploration was available. Therefore, an alternate approach was pursued based on the overall conformation of the macrocyclic compounds, and the lowest energy conformations of such analogues.

The natural product (**1**) and four semi-synthetic derivatives (**2**–**5**) were constructed in the lowest energy conformations using SYBYL. The three-dimensional geometries of these compounds were then analyzed using the Surflex Ligand Similarity tool (default parameter settings).[11] Rigid superposition maintained low energy conformations and provided informative differences in ligand similarity (Figure 2). Semi-synthetic derivatives **2**–**4** exhibited significant conformational perturbations in macrocycle geometry and placement of NCxDA side chain, but methyl-phenyl ether **5** retained the native geometry. Because these semi-synthetic derivatives maintain most of the hydrogen bonding capabilities of trienomycin A, we hypothesized that orientation of the NCxDA side chain and projection of the phenol are critical to the biological activity manifested by trienomycin A.

The efficiency by which simplified analogues of trienomycin A could overlay with the natural product was evaluated *in silico,* also *via* Surflex calculations. Sequential removal of

functionality and subsequent comparison of the energy-minimized derivatives to the natural product were analyzed. The 13-OH, 12-CH$_3$, and 3-OCH$_3$ functionalities appeared to exhibit little effect on the overall conformation, which suggested they could be omitted without significantly affecting biological activity (Figure 3).

The triene motif was evaluated next, with the aim of generating the most synthetically accessible derivatives. Twelve energy minimized "monoene" derivatives, containing all possible olefin isomers and the methyl ether of the reported derivative, were evaluated based upon their ability to adopt a conformation that allowed both the phenol and NCxDA side chain to occupy the same conformational space as the natural product. Olefin geometry was critical in these structures and was responsible for orienting both the NCxDA side chain and the phenol into conformations that were similar to the natural product. Most of the monoene derivatives exhibited significant differences in macrocycle geometry compared to trienomycin A. However, derivatives **7**–**10** manifested macrocycle geometry similar to the natural product and produced relatively high similarity scores (Figure 4). Monoene A, which contains *trans* olefins at both C8 and C14, occupied the closest three-dimensional orientation to trienomycin A and exhibited the highest similarity score (0.7815).

Since monoene A (**7**) exhibited the highest similarity score to trienomycin A, it was the target for chemical synthesis. Synthesis of **7** was envisioned retrosynthetically to occur through a Wittig olefination between salt **12** and ketone **13**,[12] followed by Mitsunobu coupling of the D-Ala side chain **14**,[13] and subsequent ring-closing metathesis (Figure 5).[14] This synthetic route would also provide access to monoene E (**11**), which exhibited the lowest similarity score and could be used as a negative control to evaluate our conformation-based approach.

Synthesis of Wittig salt **12** commenced with nucleophillic aromatic substitution of methyl 3,5-dinitrobenzoate **15** by lithium methoxide to provide methyl-phenyl ether **16**,[15] which was then reduced to aldehyde **17** by diisobutylaluminum hydride.[16] Aldehyde **17** was subjected to Horner–Wadsworth–Emmons conditions to furnish **18**,[17] which was subjected to diimide mediated reduction to provide **19**.[18] The ethyl ester of **19** was reduced to the alcohol using diisobutylaluminum hydride to yield **20**, the nitro moiety of which was reduced to the aniline **21** by palladium on carbon under a hydrogen atmosphere. Selective amidation of compound **21** with non-8-enoic acid was accomplished using (1-Cyano-2-ethoxy-2-oxoethylidenaminooxy)dimethylamino-morpholino-carbenium hexafluorophosphate (COMU) to afford amido-alcohol **22**.[19] Until this point, no chromatographic separation was required, enabling the preparation of large quantities. Amido-alcohol 22 was then iodinated and converted to Wittig salt **12** (Scheme 1).

Synthesis of ketone **13** was similarly straightforward. Oxidation of ketol **23** to the ketoaldehyde and treatment of the dicarbonyl species with 0.5 eq. of Brown's allylborane furnished optically active ketol **24**.[20] Synthesis of ketone **13** was completed following silyl ether formation (Scheme 2).

N-Cyclohexylcarbonyl D-alanine acid **14** was constructed in one step by treating D-alanine with cyclohexylcarbonyl chloride in the presence of tribasic potassium phosphate in tetrahydrofuran (Scheme 3).

With the synthons in hand, Wittig olefination reaction between **12** and **13**, followed by silyl deprotection, and Mitsunobu coupling between **25** and **14** led to metathesis precursors **26** (*trans*) and **27** (*cis*) in a 1:1 mixture, which was separable by chromatography. Originally, it was desired to obtain the macrocyclic alcoholafter ring closing metathesis, which could subsequently undergo diversification at a later stage. However, the macrocyclic alcohol failed to efficiently undergo esterification *via* Mitsunobu conditions or direct acylation, due

to steric constraints resulting from the macrocyclic conformation. Therefore, the NCxDA side was appended prior to cyclization. Each isomer was subjected to ring-closing metathesis with Grubbs first generation catalyst to yield products **7** and **11** as a 2:1 and 5:1 (*trans:cis*) mixture of olefin isomers, respectively (Scheme 4). The isomeric mixtures were evaluated for anti-proliferative activity against MCF-7 and HeLa cancer cells. As predicted in our conformation based design approach, compound **7** retained anti-proliferative activity ($IC_{50} = 0.47 \mu M$) while compound **11** was inactive.

The anticancer activity exhibited by compound **7** prompted investigations into the amino acid side chain. Specifically, the effects of increasing steric bulk in lieu of the methyl substituent and alterations of the amide to include small alkyl groups were pursued. The various side chains were synthesized from the corresponding amino acids and appropriate acyl chlorides similar to compound **14**. Once synthesized, the side chains were coupled with compound **25** using Mitsunobu conditions and subsequently cyclized with Grubbs first generation catalyst (Figure 6, 28–**34**).

These compounds were evaluated for anti-proliferative activity against HeLa and MCF-7 cancer cells (Table 1). From the data presented, deviation in steric bulk, with functionalities that exhibit smaller or larger groups than methyl, is not tolerated at the *alpha* position of the D-Ala side chain. Similarly, decreasing steric bulk of the alkyl amide decreases biological activity.

In conclusion, molecular modeling was used to predict simplified derivatives of trienomycin A that were energetically predisposed to adopt a conformation similar to the natural product. The analogues manifested potent anti-proliferative activities against MCF-7 and HeLa cancer cell lines and provide the first SAR for these natural product analogues. Since trienomycin A is synthetically complex, simplified derivatives provide a method that enables rapid elucidation of SAR and provides a potential tool by which the biological target may be discovered through subsequent studies. Further investigation into the SAR and mechanism of action for monoenomycin is ongoing and will be reported in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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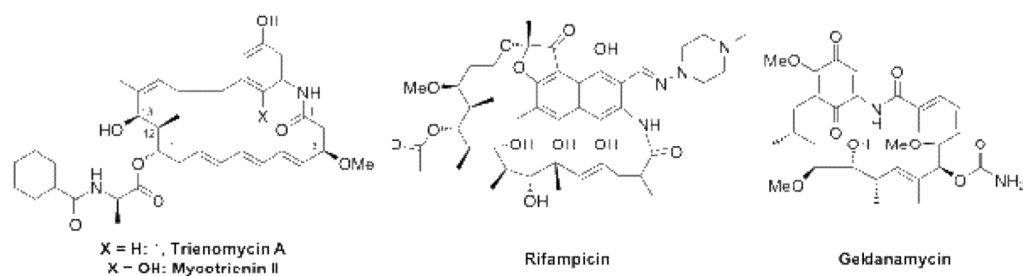
Figure 1.
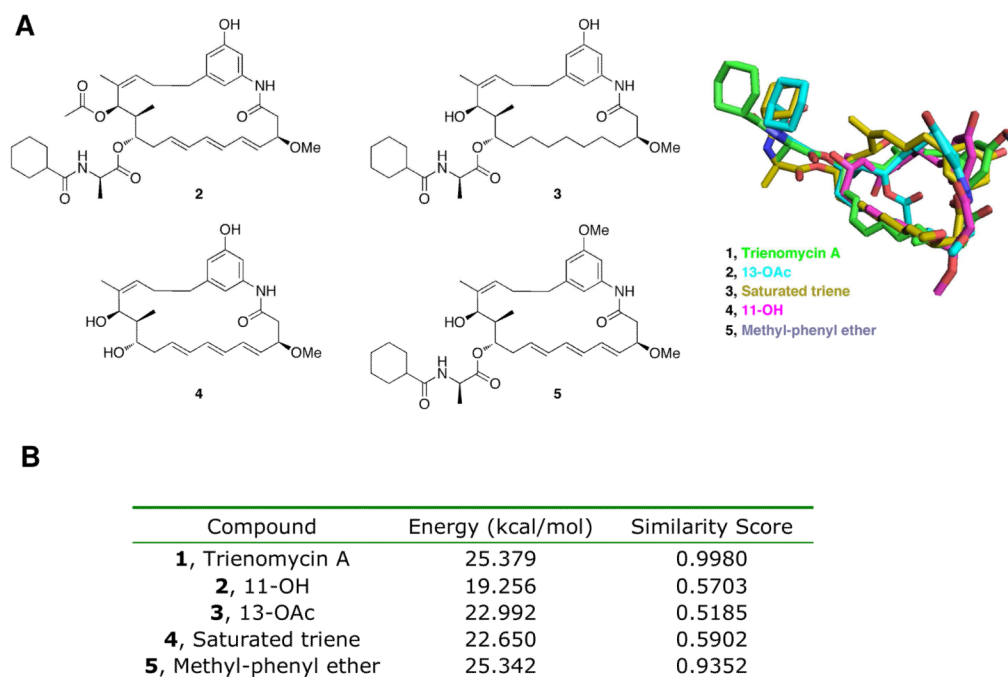Structures of rifampicin, geldanamycin, trienomycin A, and mycotrienin II.

Figure 2.
A) Overlay of energy minimized trienomycin A and select semi-synthetic derivatives using the Ligand Similarity tool (rigid superposition) from the Surflex software suite. B) Relative energies and similarity score from the Ligand Similarity tool.
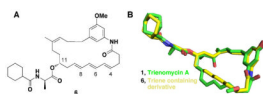
Compound	Energy (kcal/mol)	Similarity Score
1, Trienomycin A	25.379	0.9980
2, 11-OH	19.256	0.5703
3, 13-OAc	22.992	0.5185
4, Saturated triene	22.650	0.5902
5, Methyl-phenyl ether	25.342	0.9352

Figure 3.
A) Structure of theoretical trienomycin A analogue lacking the 13-OH, 12-CH$_3$, and 3-OMe functional groups. B) Overlay of trienomycin A and the theoretical derivative lacking the 13-OH, 12-CH$_3$, and 3-OCH$_3$ functional groups (**6**). Theoretical analogue exhibited an energy value of 21.527 kcal/mol and a similarity score of 0.7935 with the trienomycin A (**1**).
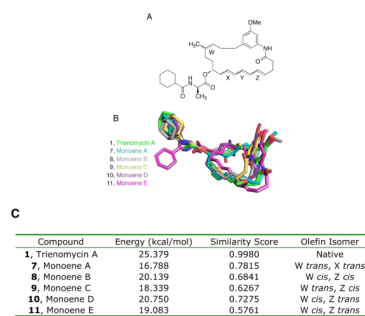
Figure 4.
A) Labeled monoene derivative key. B) Comparison of monoene derivatives and trienomycin A. C) Relative energies and similarity score from the Ligand Similarity tool.
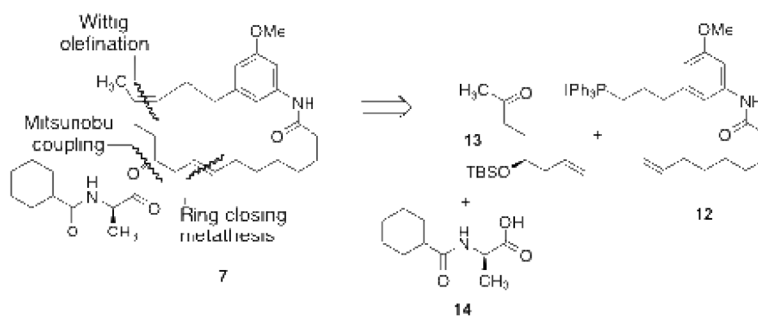
Figure 5.
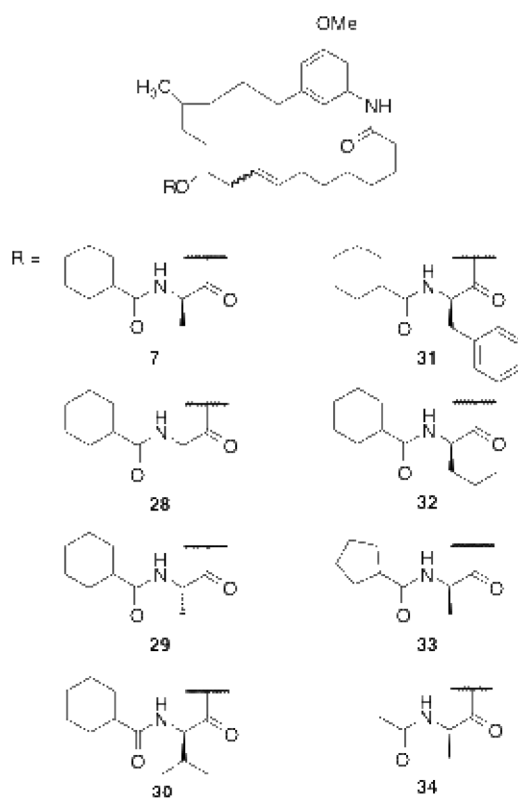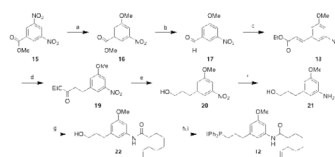Retrosynthetic analysis of compound **7**, monoene A.
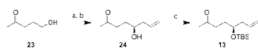
Figure 6.
Structures of monoenomycin analogues **28**–**34**.

Scheme 1.
Synthesis of Wittig salt **12**.[a]

[a] Reagents and Conditions: (a) LiOMe, MeOH, reflux, 97%. (b) DIBAl-H, DCM, −78°C, 93%. (c) triethylphosphonoacetate, NaH, THF, 0°C, 94%. (d) Potassium azodicarboxylate, AcOH, DME, 50°C, 97%. (e) DIBAl-H, PhMe, 0°C. (f) 10% Pd/C, H_2, EtOAc. (g) Non-8-enoic acid, COMU, DIPEA, DMF, 93% over two steps. (h) PPh_3, imidazole, I_2, DCM, 83%. (i) PPh_3, CAN, 90%.
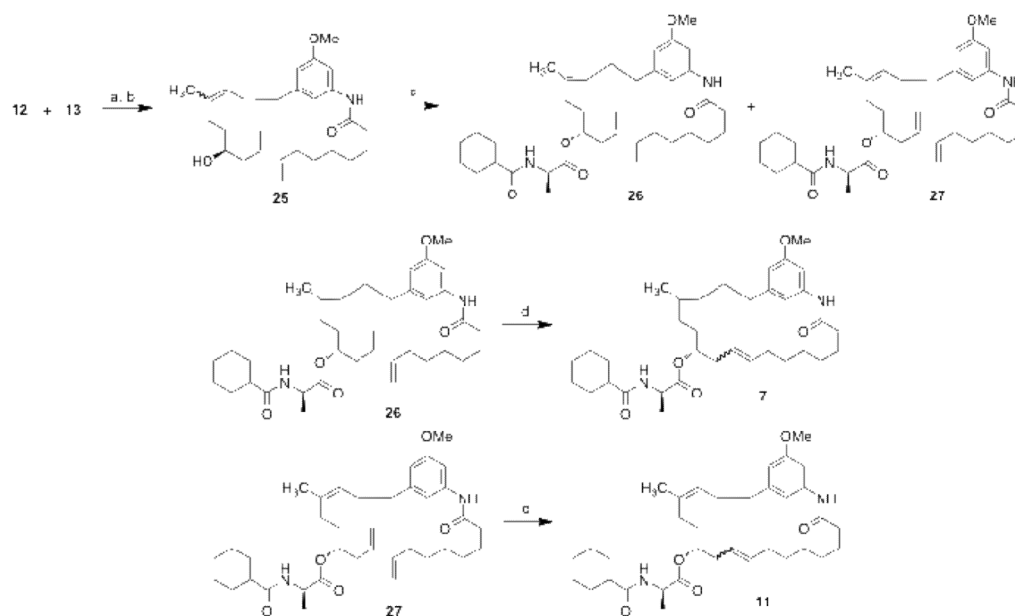
Scheme 2.
Synthesis of ketone **13**.[a]
[a] Reagents and Conditions: (a) PCC, DCM. (b) Allyl magnesium bromide, (+)-*B*-methoxydiisopinocampheylborane, Et$_2$O, *ee* > 95%, 82% (BRSM) over two steps. (c) TBSCl, imidazole, DMF, 79%.
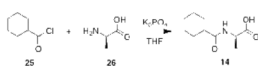
Scheme 3.
Synthesis of *N*-cyclohexylcarbonyl D-alanine acid **14**. [a]
[a] Reagents and Conditions: (a) K$_3$PO$_4$, THF, 76%.

Scheme 4.
Synthesis of monoene derivatives of trienomycin A.[a]

[a] Reagents and Conditions: (a) KHMDS, toluene, 78%. (b) TBAF, THF, 97%. (c) Diethyl azodicarboxylate, PPh$_3$, **14**, 37% (**26**), 39% (**27**). (d) Grubbs first generation catalyst, DCM, 83%.

Table 1

Anti-proliferative effects of Trienomycin A compound library against HeLA and MCF-7 cancer cell lines.[a]

Compound	HeLa IC$_{50}$ (µM)	MCF-7 IC$_{50}$ (µM)
7	0.47 ± 0.04	0.53 ± 0.02
11	>100	>100
28	13 ± 0.5	12 ± 0.5
29	1.7 ± 0.03	1.9 ± 0.05
30	9.1 ± 0.09	1.0 ± 0.04
31	4.6 ± 0.09	5.1 ± 0.07
32	>100	>100
33	1.6 ± 0.09	0.72 ± 0.5
34	2.5 ± 0.9	1.7 ± 0.4
Geldanamycin	>5	0.053 ± 0.001

[a]IC$_{50}$ value based on MTS assay run in triplicate (See Supp. Info).