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**Paper:**

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Discovery of novel FabF ligands inspired by platensimycin by integrating structure-based design with diversity-oriented synthetic accessibility

Martin Fisher,[a,b] Ramkrishna Basak,[a,b] Arnout P. Kalverda,[b] Colin W. G. Fishwick,[a,b] W. Bruce Turnbull[a,b] and Adam Nelson*[a,b]

An approach for designing bioactive small molecules has been developed in which de novo structure-based ligand design (SBLD) was focused on regions of chemical space accessible using a diversity-oriented synthetic approach. The approach was exploited in the design and synthesis of a focused library of platensimycin analogues in which the complex bridged ring system was replaced with a series of alternative ring systems. The affinity of the resulting compounds for the C163Q mutant of FabF was determined using a WaterLOGSY competition binding assay. Several compounds had significantly improved affinity for the protein relative to a reference ligand. The integration of synthetic accessibility with ligand design enabled focus to be placed on synthetically-accessible regions of chemical space that were relevant to the target protein under investigation.

Introduction

Structure-based ligand design (SBLD) is a powerful approach that can facilitate the discovery of bioactive small molecules (including drugs) when high quality structural information is available. SBLD can facilitate the identification of chemical space that is relevant to a specific protein target.1–6 De novo SBLD allows chemical space to be explored particularly efficiently through linking docked fragments within the context of a binding site, and has been extensively exploited in the discovery of novel ligands for many classes of protein.7–9 De novo SBLD is, however, rarely integrated with an assessment of synthetic accessibility, and the preparation of designed ligands can raise significant synthetic challenges.

In the last decade, diversity-oriented synthesis (DOS)10,11 has emerged as an approach to increase the structural diversity of small molecule libraries.12,13 The approach has facilitated the discovery of a wide range of novel bioactive compounds.14–16 A significant challenge in diversity-oriented synthesis, however, is to target selectively regions within chemical space that are most biologically-relevant.

In the Nelson group, we have previously developed a robust approach for the synthesis of skeletally-diverse small molecules (Scheme 1).17 The approach relies on the synthesis of metathesis substrates through iterative attachment of simple unsaturated building blocks to a fluorous-tagged linker 1 (e.g. → 2 or 3); subsequently, metathesis cascade reactions “reprogramme” the molecular scaffolds, and release the products from the linker (e.g. → 4 or 5). The approach enabled the combinatorial variation of molecular scaffold, and was exploited in the synthesis of a library of natural product-like small molecules with unprecedented scaffold diversity (over 80 distinct scaffolds).18,19 In this paper, we describe how de novo SBLD may be used to identify regions of chemical space that are both synthetically accessible using this diversity-oriented approach and are relevant to a specific protein target.
We began by selecting a suitable protein target, FabF, an enzyme that catalyses the chain elongation in the bacterial (FAS II) fatty acid biosynthetic pathway.20,21 The fatty acid biosynthesis pathway may have potential as an antimicrobial target,22–24 and, as a result, novel inhibitors of this pathway are of interest. Crucially, high quality structural information is available for FabF, a pre-requisite for the discovery of inhibitors using SBLD. FabF possesses a catalytic triad (Cys163, His303, His340) which is critical for catalysis. The formation of a thioester between Cys163 and the growing fatty acid chain results in a conformational change, allowing the malonyl-acyl carrier protein (ACP) to bind in the active site. His303 and His340 then catalyse the decarboxylation of the malonyl-ACP, and carbon-carbon bond formation to extend the fatty acid chain (Figure 1).20,25,26

Platensimycin (Figure 2) is a natural product, known to target FabF, that displays good activity against a range of Gram positive bacteria including those that have developed resistance to common antibacterials.24 Platensimycin inhibits wild type FabF with an IC₅₀ of 160 nM for native E. coli, and 48 nM S. Aureus. Platensimycin inhibits the protein by targeting the malonyl-ACP binding site and thus prevents its binding to FabF. Remarkably, platensimycin has low affinity for wild-type FabF until the enzyme has been primed with the growing fatty acid chain.24 A crystal structure of the C163Q mutant of FabF (native to E. coli.) that mimics the primed enzyme has been determined in complex with platensimycin. The affinity of platensimycin for this C163Q mutant is increased relative to the wild type protein with an IC₅₀ of 19 nM.24 The catalytic histidine residues (His303 and His340) interact with the polar aromatic headgroup of platensimycin. The amide group is almost perpendicular to the plane of this aromatic ring, allowing additional hydrogen bonds to be made with Thr270 and Thr307 (Panel A, Figure 2). Finally, the unusual caged ring system forms hydrogen bonds with Thr270 and Ala309, but is otherwise rather solvent-exposed (Panel B, Figure 2).24

Several total syntheses of platensimycin have been reported. Additionally, analogues of platensimycin have been synthesised, allowing the definition of structure-function relationships. In most cases, even small modifications of the polar aromatic headgroup lead to a dramatic reduction in biological activity.34,36 In sharp contrast, analogues in which the caged ring system has been decorated or modified often retain good biological activity.34,35

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Figure 1. Proposed key intermediate in the mechanism of fatty acid chain elongation catalysed by FabF. Decarboxylation of the malonyl-ACP, and carbon-carbon bond formation, would extend the fatty acid chain.
Figure 2. Platensimycin and its interaction with the C163Q mutant of FabF. Panel A: Key interactions between platensimycin, 6, and FabF (pdb accession code: 2GFX); Panel B: Surface representation showing that only the platensimycin head group protrudes from the binding pocket to sit in close proximity to Ala205 and Arg206.

However, current approaches for analogue generation have largely relied on bespoke syntheses of cage region replacements\(^\text{34,35,37-9}\) and the functionalisation or removal of the enone portion of the molecule.\(^\text{32,35}\) In this paper, we describe an approach to platensimycin analogue design in which \textit{de novo} SBLD is focused on regions of chemical space that are accessible using our diversity-oriented synthetic approach.

Results and Discussion

\textit{De novo} design of structurally-diverse platensimycin analogues

Initially, a \textit{de novo} approach was exploited using the SPROUT suite of software.\(^\text{40-42}\) SPROUT enables the assembly \textit{in silico} of potential ligands from fragments within the context of an active site. We sought to design platensimycin analogues in which the caged ring system was replaced with alternative ring systems that were accessible using a flexible diversity-oriented synthetic approach.

We designed a virtual library of fragments for the \textit{de novo} design of platensimycin analogues using SPROUT (Figure 3). This virtual library comprised three classes of fragment. First, we designed fragments that incorporated the headgroup of platensimycin (Figure 3, Panel A). Second, we identified fragments that were found in skeletally-diverse small molecules that had already been prepared using our diversity-oriented synthetic approach: these fragments were obtained by virtual cleavage of bonds that were generally adjacent either to a ring or to an acyclic alkene (Panel B). Finally, we identified a range of nitrogen capping groups, which included some of the standard SPROUT fragments (Panel C). Most of the fragments had relatively few rotatable bonds, and their accessible conformations were identified using Maestro. An alternative approach was used to identify productive conformations of the more flexible fragments that incorporated the platensimycin headgroup (Figure 3, Panel A). These fragments were docked into the active site of FabF such that the headgroup adopted a similar position to that observed in the structure of the platensimycin–FabF complex; a conformational search using Maestro then allowed the identification of conformers that could be accommodated within the FabF active site (for further details see Supplementary Information).

A range of target sites in the FabF active site were selected for the \textit{de novo} design process using SPROUT (see Figure 2): the residues observed to hydrogen bond with platensimycin in its complex with FabF, and two additional promising sites that lie on the periphery of the binding site (Ala205 and Arg206).
The fragments that incorporated the platensimycin headgroup (Figure 3, Panel A) were docked such that the binding mode observed in the platensimycin-FabF complex was reproduced. Suitable H-bonding fragments (Panels B and C, Figure 3) were docked to the target sites defined by Ala205, Arg206 and Thr270. The docked fragments were then linked with appropriate “spacer” fragments (Figure 2, Panel B).

The designed ligands that were deemed to be likely synthetically accessible using our diversity-oriented approach were docked using Glide,43 and scored using both Glide and SPROUT. To validate the docking process, platensimycin was docked to the C163Q mutant of FabF, and its binding mode was successfully reproduced provided that its carboxylate was forced to interact with His303 (Figure 2 and Supplementary Information).

In addition, to serve as a comparison, the ligand 7, which lacks the caged ring system of platensimycin, was also docked and scored. This scoring process suggested that the caged ring system of platensimycin makes a very significant contribution to binding (compare ligands 6 and 7, Table 1). Scores for selected de novo designed ligands are also summarised in Table 1 (and Supplementary Information), several of which are comparable with that of platensimycin (6). It was concluded that it may be possible to simplify the complex caged ring system of platensimycin, whilst retaining much of its affinity for the C163Q mutant of FabF.

### Synthesis of the de novo designed ligands

Initially, we prepared the fluorous-tagged building block 23 that incorporates the platensimycin headgroup (Scheme 2).

![Scheme 2. Synthesis of the fluorous-tagged building block 23](image)

The aromatic ester 14 was treated with lithium hydroxide in 4:1 THF-H2O; surprisingly, the carboxylic acid 15 was obtained in which one of the methoxymethyl groups had been removed. The carboxylic acid 15 was esterified to give the fluorous-tagged ester 16 that was treated with MOMCl and disopropylethylamine to give the fluorous-tagged ester 17. The nitro group of 17 was hydrogenated to

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**Table 1. Summary of GLIDE and SPROUT scores for selected de novo designed ligands**

<table>
<thead>
<tr>
<th>Ligand</th>
<th>GLIDE score</th>
<th>SPROUT score</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>-13.2</td>
<td>-8.6</td>
</tr>
<tr>
<td>7</td>
<td>-10.7</td>
<td>-6.1</td>
</tr>
<tr>
<td>8 (R=H)</td>
<td>-11.5</td>
<td>-7.6</td>
</tr>
<tr>
<td>9a (R=COPr)</td>
<td>-11.2</td>
<td>-8.7</td>
</tr>
<tr>
<td>9b</td>
<td>-10.7</td>
<td>-8.9</td>
</tr>
<tr>
<td>9c (R=SO2Ph)</td>
<td>-10.7</td>
<td>-7.9</td>
</tr>
<tr>
<td>9d (R=SO2NHBoc)</td>
<td>-11.6</td>
<td>-7.7</td>
</tr>
<tr>
<td>9e (R=SO2NH2)</td>
<td>-12.6</td>
<td>-8.2</td>
</tr>
<tr>
<td>9f (R=CONHiPr)</td>
<td>-12.2</td>
<td>-8.7</td>
</tr>
<tr>
<td>9g</td>
<td>-11.5</td>
<td>-9.2</td>
</tr>
<tr>
<td>10a (R=H)</td>
<td>-11.3</td>
<td>-7.0</td>
</tr>
<tr>
<td>11</td>
<td>-12.0</td>
<td>-7.6</td>
</tr>
<tr>
<td>12 (R=R’=H)</td>
<td>-12.5</td>
<td>-9.0</td>
</tr>
</tbody>
</table>

[a] Score for the pose observed in the X-ray crystal structure [b] R = 5-(1-methyl imidazyl)carbonyl [c] R = 3-pyridyl aminocarbonyl.
give the corresponding aniline 18. The aniline 18 was coupled with the carboxylic acid 19, prepared by hydrolysis of the corresponding ethyl ester,23 by treatment with HATU and diisopropylethylamine in DMF to give the fluorous-tagged amide 20. Finally, a series of functional group manipulations yielded the required fluorous-tagged building block. Thus desilylation (→ 21), Fukuyama–Mitsunobu46 reaction with NsBocNH (→ 22), and removal of the Boc group (by treatment with caesium carbonate and imidazole in acetonitrile47) gave the fluorous-tagged building block 23.

The metathesis substrate 31 was prepared from the known38 allylic alcohol 24 (Scheme 3). Thus, Fukuyama–Mitsunobu reaction between the allylic alcohol 24 and the sulfonamide17 25 gave the allylic sulfonamide 26. Finally, Tamao48 oxidation, and Fukuyama–Mitsunobu reaction with the sulfonamide 23, gave the required metathesis substrate 31.

A series of simpler building blocks was also attached to the fluorous-tagged building block 23 using Fukuyama–Mitsunobu reactions (Scheme 4). The products were purified using fluorous-solid phase extraction49 (F-SPE), followed by elution through a silica plug. The metathesis substrates 27, 28 and 31 were treated with 5 mol% Hoveyda–Grubbs second generation catalyst and 10 mol% 1,4-benzoquinone in MTBE at 50 °C to yield the corresponding metathesis products 29, 30 and 32.

Finally, the metathesis products 29, 30 and 32 were substituted and deprotected (Table 2). First, the metathesis products were treated with LiOH, and then aqueous acid, resulting in removal of the fluorous tag and the MOM groups to yield 9h (R = Ns), 10b (R = Ns) and 13d (R= R’=Ns) respectively; subsequent removal of the α-nitrophenylsulfonyl group gave 8, 10a (R = H) and 12. In contrast, removal of the α-nitrophenylsulfonyl group from 30 was followed by derivitisation and removal of the fluorous tag and the MOM groups to give the derivatives 9c–g. Here, the presence of the fluorous tag allowed facile removal of excess reagents, with purification typically achieved using F-SPE followed by filtration through a silica plug.
Table 2. Synthesis of de novo-designed ligands

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Denosylation Method[a] (Yield)</th>
<th>Derivatisation Method[a] (Yield)</th>
<th>Deprotection Method[a] (Yield)</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>A (50%)</td>
<td></td>
<td></td>
<td>9h (R=Ns)</td>
</tr>
<tr>
<td>9h (R=Ns)</td>
<td>B (80%)</td>
<td></td>
<td></td>
<td>8</td>
</tr>
<tr>
<td>29</td>
<td>B (82%) C (93%) A (36%)</td>
<td></td>
<td></td>
<td>9c (R=SO_JH)</td>
</tr>
<tr>
<td></td>
<td>B (82%) D (70%) A (54%)</td>
<td></td>
<td></td>
<td>9d (R=SOJHBoc)</td>
</tr>
<tr>
<td></td>
<td>B (82%) D (87%) E (46%)</td>
<td></td>
<td></td>
<td>9e (R=SOJH)</td>
</tr>
<tr>
<td></td>
<td>B (82%) F (90%) A (74%)</td>
<td></td>
<td></td>
<td>9f (R=CONHJPr)</td>
</tr>
<tr>
<td></td>
<td>B (82%) G (85%) A (49%)</td>
<td></td>
<td></td>
<td>9g[a]</td>
</tr>
<tr>
<td>30</td>
<td>A (68%)</td>
<td></td>
<td></td>
<td>10b (R=Ns)</td>
</tr>
<tr>
<td>10b (R=Ns)</td>
<td>B (79%)</td>
<td></td>
<td></td>
<td>10a (R=H)</td>
</tr>
<tr>
<td>32</td>
<td>A (49%)</td>
<td></td>
<td></td>
<td>13d (R=R‘=Ns)</td>
</tr>
<tr>
<td>13d (R=R‘=Ns)</td>
<td>B (91%)</td>
<td></td>
<td></td>
<td>12 (R=R‘=H)</td>
</tr>
</tbody>
</table>

[a] Methods: A: (a) LiOH, 4:1 THF–H2O, 45 °C; (b) 2M HCl–THF, 45 °C B: PhSH, DMF, C: PhSO3Cl, DMAP, CH2Cl2; D: i) NMe2CH2HN assured for the negative signal observed in the absence of protein, weak negative signals were observed for the reference ligand 7 (concentration: 600 µM). In sharp contrast, however, addition of the C163Q mutant of FabF (30 µM) resulted in strong positive signals for the reference ligand 7, demonstrating that the ligand binds to the protein under the conditions of the assay. Crucially, it was also demonstrated that the ligand 7 (1.5 mM) could be displaced from the protein by platensimycin (30 µM), suggesting that the ligand 7 targets the platensimycin binding site (see Supplementary Information).

Figure 5: Development of a WaterLOGSY assay. A selected region of spectra recorded at 300 K is shown. The protein concentration was 30 µM and the concentration of the ligand 7 was 1.5 mM. Signals corresponding to protons HA and HB were observed in a 500 MHz 1H NMR spectrum (top); shown to be positive in a waterLOGSY spectrum in the presence of protein (middle); and shown to be negative in a waterLOGSY spectrum in the absence of protein (bottom). DSS = 4,4-dimethyl-4-silapentane-1-sulfonic acid.

The affinity of the reference ligand 7 was determined using the WaterLOGSY assay. Thus, spectra were recorded at a range of ligand concentrations (300, 450, 600, 1500 and 2500 µM) in the presence of the C163Q mutant of FabF (33 µM). The intensity of the signal corresponding to the terminal methyl group, corrected for the negative signal observed in the absence of protein, was determined as a function of concentration, allowing the determination of a dissociation constant (7: Kd = 650 ± 90 µM).

The ability of the de novo designed ligands to displace the reference ligand 7 from the C163Q mutant of FabF (23 µM) was investigated by WaterLOGSY spectroscopy. Thus, the intensity of the signal corresponding to the terminal methyl group of the reference ligand 7, corrected for the negative signal observed in the absence of protein, was determined as a function of concentration (0, 150, 300, 450, 600 µM) of a range of competitor ligands. The
dissociation constants for the \textit{de novo}-designed ligands are summarised in Table 3.

**Discussion**

An integrated strategy, in which \textit{de novo} SBLD was focused on regions of chemical space accessible using metathesis-based diversity-oriented synthesis, was implemented successfully. This modular synthetic strategy facilitated the synthesis of several scaffolds that incorporated the platensimycin headgroup. Crucially, a fluororous-tag allowed rapid purification of the products of several key steps. Finally, a waterLOGSY assay allowed the affinity of all ligands to be determined, and suggested that the ligands selectivity targeted the platensimycin binding site.

<table>
<thead>
<tr>
<th>Table 3. Dissociation constants of \textit{de novo}-designed ligands for the C163Q mutant of FabF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ligand</td>
</tr>
<tr>
<td>-----------------------------------------------</td>
</tr>
<tr>
<td>$8$ (R = H)</td>
</tr>
<tr>
<td>$9c$ (R = SO$_2$Ph)</td>
</tr>
<tr>
<td>$9d$ (R = SO$_2$NH$_2$NHBoc)</td>
</tr>
<tr>
<td>$9e$ (R = SO$_2$NH$_2$)</td>
</tr>
<tr>
<td>$9f$ (R = CONH$_2$iPr)</td>
</tr>
<tr>
<td>$9g$ (R = 3-pyridylaminocarbonyl)</td>
</tr>
<tr>
<td>$10a$</td>
</tr>
<tr>
<td>$12$</td>
</tr>
</tbody>
</table>

The ability of the scoring functions to predict the relative affinity of potential ligands for FabF \textit{in silico} was disappointing. There was a significant discrepancy between the predicted affinities, and the observed affinities, within the series of ligands. Notably, compound $12$, which had a predicted affinity similar to that of platensimycin, actually bound with affinity comparable to the reference ligand $7$.

Notably, differences were observed in the reliability of scoring between SPROUT and Glide. The SPROUT scores (Table 1) correlated relatively poorly with the experimentally obtained affinities (Table 3); whilst those generated using Glide (Table 1) broadly correlated with experimental observations. Thus, Glide predicted correctly that platensimycin has the highest affinity for the protein. Crucially, Glide also predicted that the $N$-substituted ligands $9d$-$g$ would bind to the C163Q mutant of FabF significantly (generally 1-2 orders of magnitude) more strongly than the reference ligand $7$; this prediction is broadly in line with experimental observations (full details of the design parameters used are provided in the Supplementary Information).

In light of these broadly consistent correlations between prediction and experiment, we were surprised to find that the predicted affinities of amines $8$, $10a$, and $12$ to the C163Q mutant of FabF were much higher than the measured values; in fact, these amines displayed affinities similar to the reference ligand $7$. However, in the case of amines $8$, $10a$ and $12$, it is possible that the additional hydrogen bonds predicted to be formed between the amine-containing moiety and specific residues located within the solvent exposed cavity were, in fact, absent, possibly due to these solvent-exposed amines (presumably present as the protonated forms) undergoing extensive hydration and therefore negating their ability to H-bond with the protein. Despite these problems with amine-containing ligands it is notable that based on the data from modelling in Glide and SPROUT, and biophysical data obtained in the waterLOGSY assay, it seems likely that all the analogues $8$, $9e$-$g$, $10a$ and $12$ were able to reproduce the interactions made by the platensimycin headgroup.

Interestingly, it was the Boc-substituted sulfurea $9d$ which had the biggest improvement in affinity relative to the reference ligand $7$. This ligand included a tert-butyl group able to participate in hydrophobic interactions. It is noteworthy that the sulfurea portion of the molecule contains a highly acidic NH proton that may be ionised under the conditions of the assay. Potentially, this group may participate in an electrostatic interaction with a re-orientated Arg206 side chain (Supplementary Information). This additional interaction may account for its increased affinity relative to the H-bonding ligands $9e$-$g$. 
Analysis of the predicted binding poses of the ligands 9a-g shows that the headgroup portion of the molecule is in a very similar position to that of platensimycin. Crucially, all the hydrogen bonds made by the headgroup in platensimycin are vital for its activity. However, it seems reasonable to assume that the observed increases in affinity compared to reference ligand 7 for ligands 9e-g were due to the envisaged additional hydrogen-bonding and hydrophobic interactions.

Conclusion

A strategy, in which de novo design and synthetic accessibility were integrated, was implemented. Crucially, the approach facilitated the synthesis of a range of platensimycin analogues that were predicted to bind tightly to the C163Q mutant of FabF. The design and synthesis of a focused library of ligands relied on the selection of fragments for de novo design that were found in products of reliable diversity-oriented metathesis cascade chemistry. The resulting designed compounds bound in the platensimycin binding site of the C163Q mutant of FabF, and, in several cases, the ligands had higher affinity than the reference compound 7.

While none of the ligands approached the activity of platensimycin, they do provide useful structure-activity information to guide further design of platensimycin analogues. The general synthetic strategy could, through exploitation of other simple building blocks, yield additional diverse platensimycin analogues for further exploration of the binding site. The overall integrated approach is, however, limited by the performance of current scoring functions which can struggle to predict the relative affinities of series of ligands.

The integration of structure-based ligand design with diversity-oriented synthetic approaches can enable the rapid exploration of relevant, yet synthetically-accessible, chemical space. Ultimately, this integrated approach may empower researchers to focus their synthetic resources on the regions of chemical space that are most relevant to a protein of interest.

Acknowledgements

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Notes

a) School of Chemistry University of Leeds, West Yorkshire, LS2 9JT, UK.

b) Astbury Centre for Structural Molecular Biology, University of Leeds, West Yorkshire, LS2 9JT, UK
References