

Development and evaluation of 4-(pyrrolidin-3-yl)benzotrile derivatives as inhibitors of lysine specific demethylase 1



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ABSTRACT

As part of our ongoing efforts to develop reversible inhibitors of LSD1, we identified a series of 4-(pyrrolidin-3-yl)benzotrile derivatives that act as successful scaffold-hops of the literature inhibitor GSK-690. The most active compound, **21g**, demonstrated a K_d value of 22 nM and a biochemical IC_{50} of 57 nM. In addition, this compound displayed improved selectivity over the hERG ion channel compared to GSK-690, and no activity against the related enzymes MAO-A and B. In human THP-1 acute myeloid leukaemia cells, **21g** was found to increase the expression of the surrogate cellular biomarker CD86. This work further demonstrates the versatility of scaffold-hopping as a method to develop structurally diverse, potent inhibitors of LSD1.

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Lysine specific demethylase 1 (LSD1) has been found to have roles that go far beyond its initially discovered function of removing methyl marks from histone tails via an oxidative demethylation mechanism.^{1–3} The clinical potential of this epigenetic eraser in cancer and associated disease areas has been unravelled, largely as a result of experiments exploiting the use of irreversible LSD1 inhibitors developed from tranylcypromine. In a subset of acute myeloid leukaemia (AML), LSD1 has been shown to be important in the maintenance of leukaemia stem cells and the promotion of a block on differentiation.⁴ Additionally, LSD1 inhibition has been shown to reactivate the all-trans retinoic acid (ATRA) pathway by increasing the expression of myeloid-differentiation genes, suggesting that a combination of LSD1 inhibitor and ATRA may offer a new therapeutic option for AML subtypes that are generally insensitive to ATRA therapy alone.⁵ While tranylcypromine derivatives have facilitated a great deal of research, there is still an unmet need for a variety of reversible tool compounds that display no off-target pharmacology.⁶

GSK-690 (**1**, Fig. 1) is a versatile start-point for the development of reversible LSD1 inhibitors, however the development of this

compound was hindered by a human ether-a-go-go related gene (hERG) liability.⁷ Aromatic scaffold replacements are an attractive and logical way to retain potency while moving into novel intellectual property space.⁸ To that end, patents that disclose a number of mono- and bicycle heterocyclic scaffolds that replace the central pyridine ring have recently been disclosed.^{9–14} We instead looked to focus on scaffold-hops that featured ring systems that were partly or fully saturated. We hypothesised that the decreased lipophilicity and increased flexibility of a saturated core structure may be a valid strategy to try and overcome the persistent hERG liability of **1** and related derivatives.^{15,16}

An increase in sp^3 character has the effect of increasing the molecules 3-dimensional (3D) shape and reducing the aromatic ring count, which have both been noted as important factors in improving the developability of compounds as potential drugs.^{17,18} On the other hand, this approach brings the added complications of potential stereocenters and synthetic challenges of transformations that would be trivial on an aromatic scaffold but remain complex in the timeframe of hit-to-lead development.¹⁹

Herein, we describe our efforts to identify series of reversible inhibitors with scaffolds containing multiple sp^3 centres, and their elaboration and development into potent inhibitors of LSD1 as confirmed by biochemical, surface plasmon resonance (SPR) and

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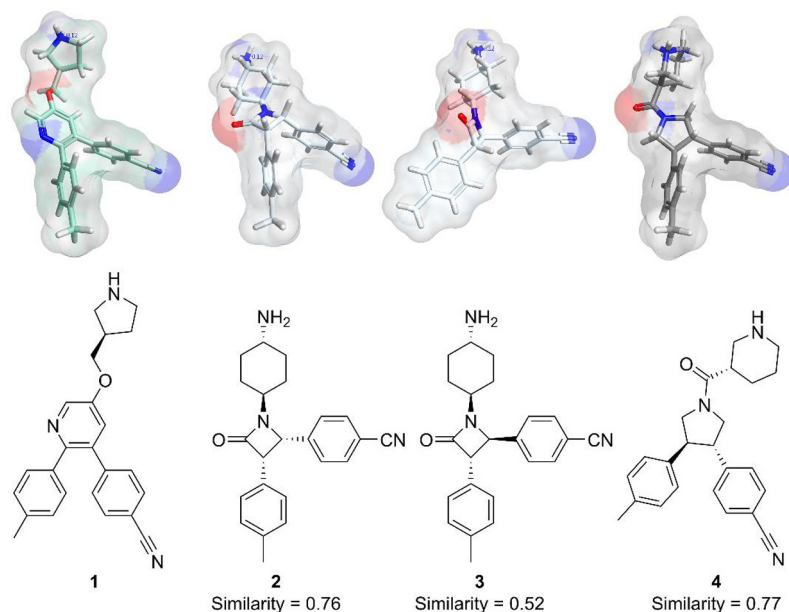
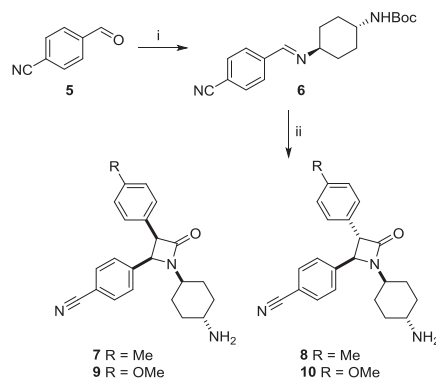


Fig. 1. Cresset Torch alignment between **1** and new scaffold designs **2–4**. A similarity score of >0.7 was considered a good score. Absolute stereochemistry indicated.

cellular biomarker assays with significantly reduced activity against the hERG ion channel.

To investigate the potential of saturated scaffold-hops from compound **1**, we begin by using Cresset Spark (Welwyn Garden City, UK) software to identify potential replacements of the pyridine core incorporating sp^3 centres at the attachment points of the two aryl groups. This identified two potential isosteric replacements that appeared to be synthetically accessible via cycloaddition reactions, namely β -lactams and pyrrolidines. From there, we refined the spatial overlap between the scaffold-hops and **1** using Cresset Torch to design potential ligands (Fig. 1). The *cis*- β -lactam diastereomers (**2**) were predicted to be far more favourable than the *trans*- (**3**), as this allows the two aryl groups be oriented in the same plane as modelled by **1**, whereas in the *trans*-diastereoisomers the aryl groups are forced out of the plane. Modelling potential pyrrolidine scaffolds on the other hand, the increased flexibility of the fully saturated ring system means that it is possible for a *trans*-diastereoisomer (**4**) to adopt a conformation where the two hydrogens sit axially, with the aryl rings oriented only slightly out of plane.

The β -lactam core can be constructed via a Staudinger [2+2] cycloaddition reaction between an imine and a ketene which can optionally be formed *in situ*. We used the method developed by Coulthard and co-workers,²⁰ which employs propylphosphonic anhydride (T3P) under basic conditions to afford a mixture of diastereoisomers, with the *trans*- β -lactam being formed as the major diastereoisomer on similar substrates to the target molecule. After forming imine **6**, T3P-mediated cycloaddition with 2-(*p*-tolyl)acetic acid or 2-(4-methoxyphenyl)acetic acid afforded a mixture of diastereometric products in an almost 1:1 ratio (Scheme 1). These were separable by preparative HPLC to afford the *cis*- and *trans*- products **7–10** as a mixture of enantiomers. The characteristic vicinal coupling of the lactam protons by NMR (*cis*- $J = 5.2$ Hz; *trans*- $J = 2.3$ Hz) allowed for identification of the structure of each product.²⁰ These enantiomeric mixtures were tested for their inhibitory activity against LSD1 in biochemical time resolved fluorescent resonance energy transfer (TR-FRET) and biophysical surface plasmon resonance (SPR) assays, as previously reported.¹⁶ While the *trans*-diastereoisomers (**8–10**) were inactive in both assay formats ($IC_{50} > 30 \mu\text{M}$ and $K_d > 100 \mu\text{M}$), we were encouraged to



Scheme 1. Synthesis of compounds **7–10**. Reagents and conditions: i) *N*-Boc-*trans*-1,4-cyclohexanediamine, MeOH, reflux, 1 h, 78%. ii) T3P, DIPEA, (*p*-tolyl)acetic acid (**7**, **9**) or 2-(4-methoxyphenyl)acetic acid (**8**, **10**), chloroform, reflux, 2 h, then TFA, DCM, 1 h, 24–58% (total of both isomers).

find that the *cis*-diastereoisomers, which were predicted to adopt a more favourable conformation to act as an isostere of **1**, did show modest activity and reversibility against LSD1 by SPR (**8** $K_d = 38 \mu\text{M}$, **10** $K_d = 53 \mu\text{M}$).

Historically 3,4-disubstituted pyrrolidines have been constructed by the reaction of alkenes most commonly with azomethine ylide sources such as *N*-(methoxymethyl)-*N*-(trimethylsilylmethyl)benzylamine in the presence of trifluoroacetic acid.²¹ However, we found this combination of reagents was unable to effect conversion of the stilbene precursors to the cycloaddition product. The *trans*-pyrrolidines were synthesised via 1,3-dipolar cycloaddition of *trans*-stilbene precursors with trimethylamine oxide in the presence of excess LDA, as developed by Davoren and co-workers.²² This afforded the *N*-methyl products, which were deprotected by reaction with 1-chloroethyl chloroformate followed by refluxing in methanol. Amide coupling, followed by Boc-deprotection, afforded amines **21a–h**, **22a–b** and **22a–e** (Scheme 2).

Pyrrolidines **21a–h** were screened against LSD1 by biochemical TR-FRET assay, and we were gratified to observe that this series transferred the spatial and electrostatic similarity to **1** into

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