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Development of 5-hydroxypyrazole derivatives as reversible inhibitors of lysine specific demethylase 1

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ABSTRACT

A series of reversible inhibitors of lysine specific demethylase 1 (LSD1) with a 5-hydroxypyrazole scaffold have been developed from compound **7**, which was identified from the patent literature. Surface plasmon resonance (SPR) and biochemical analysis showed it to be a reversible LSD1 inhibitor with an IC_{50} value of 0.23 μM. Optimisation of this compound by rational design afforded compounds with K_d values of <10 nM. In human THP-1 cells, these compounds were found to upregulate the expression of the surrogate cellular biomarker CD86. Compound **11p** was found to have moderate oral bioavailability in mice suggesting its potential for use as an *in vivo* tool compound.

The many functions of the histone demethylase lysine specific demethylase 1 (LSD1) have unravelled over the past decade to reveal a complex network of interactions with a number of protein complexes,[1,2] transcription factors,[3–5] and nucleosomes[6] in a multitude of cell types. In cancer, Harris and co-workers found that LSD1 plays a key function in maintaining the oncogenic potential of acute myeloid leukaemia (AML) cell lines by preventing differentiation.[7] Subsequently, research has shown LSD1 expression to be linked to a number of cancers, most notably small cell lung cancer (SCLC).[8] The development of irreversible inhibitors of LSD1 from the monoamine oxidase (MAO) inhibitor tranylcypromine (TCP) has been well documented,[9] and several compounds are now in clinical trials for AML and SCLC, either as a monotherapy, or in combination with other pro-differentiation agents such as all-trans retinoic acid (ATRA).[10] Results from a Phase I study of AML patients with resistant or refractory disease showed that a blast cell differentiation effect was observed in the majority of treated patients.[11]

Commercial interest in the development of irreversible TCP LSD1 inhibitors has increased rapidly in the past decade. Despite this, the rate of optimisation of tranylcypromine derivatives has not been matched by reversible inhibitors, as evidenced by the paucity of clinical trials involving reversible agents.[12] The large size and polarity of the LSD1 active site presents significant challenges to drug discovery. However, in the past two years, the patent literature has seen a convergence towards optimisation of derivatives of GSK-690 (also known as GSK-354),[13] a potent reversible LSD1 inhibitor compromised by a significant hERG liability.

Various bicyclic and monocyclic scaffold-hops have been disclosed (Fig. 1) in numerous patents. We became interested in a hydroxypyrazole scaffold template disclosed, but not claimed in a patent from Quanticel Pharmaceuticals.[19] The replacement of the tolyl- (**1**) or other aryl or heteroaryl-groups (**2**–**6**) with an ether linkage offers a new vector to investigate. Whilst, the patent only disclosed three pyridylmethyl isomers, the 2-pyridyl isomer (**7**) was preferred, and was reported to have activity of <100 nM in biochemical assays. This compound was resynthesised via the disclosed procedure, and profiled in our biochemical time-resolved fluorescence resonance energy transfer (TR-FRET) assay against LSD1 where it displayed an IC_{50} value of 0.23 μM (SD 0.08 μM). Surface plasmon resonance (SPR) analysis indicated **7** was a reversible inhibitors of LSD1 with a K_d value of 0.042 μM. It also displayed activity in our previously reported CD86 cellular biomarker assay

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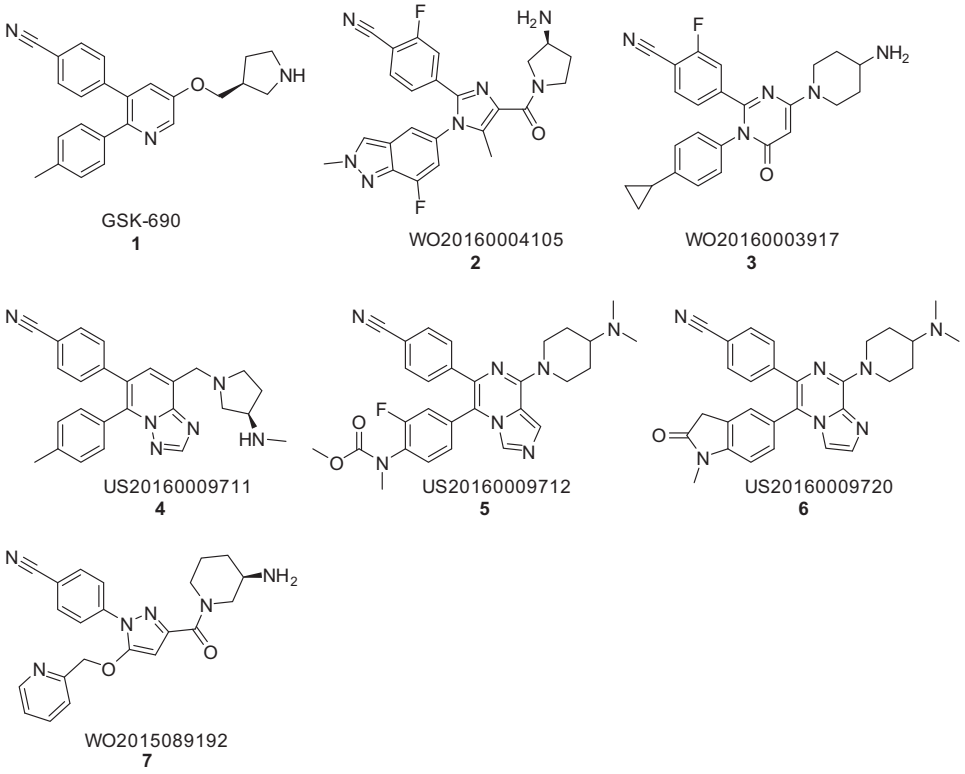
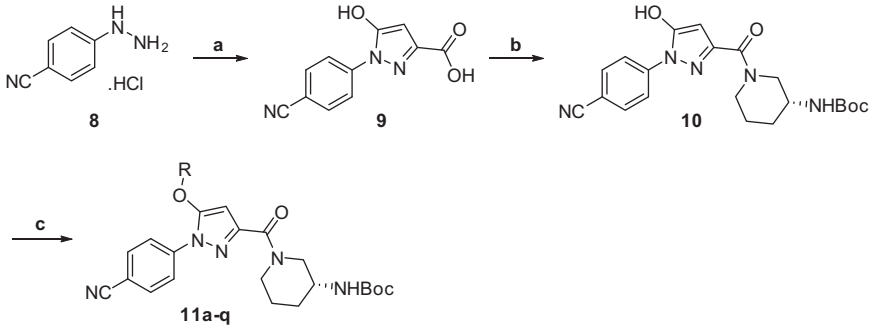


Fig. 1. Structure of GSK-690 (**1**) and examples of recently disclosed reversible inhibitors of LSD1 from the patent literature (**2**–**7**).[14–19]

(EC_{50} = 2.3 μM).[20] These values suggested **7** was an attractive start point for further development.

To explore the SAR around the ether functionality, we synthesised hydroxypyrazole **9** from 4-cyanophenylhydrazine (**8**) via reaction with oxaloacetic acid (Scheme 1). The amide was then installed under standard coupling conditions to give Boc-protected amide **10**. The hydroxypyrazole could be alkylated under basic conditions with a variety of alkylating agents, and then deprotected under acidic conditions to afford compounds **11a**–**q**. From the limited SAR disclosed in the patent, we envisaged that the pyridyl nitrogen may be acting as a H-bond acceptor, hence we focused on replacement of the pyridyl with groups containing functionalities which have hydrogen-bonding groups analogous to the pyridyl nitrogen (Table 1). While the primary amide **11a** had only modest affinity for LSD1 by SPR, methylation at either carbon (**11b**) or nitrogen (**11c**) resulted in a significant improvement in potency in both assay formats, with compound **11c** achieving sub-micromolar affinity by SPR. Extending the dimethylamide further to a morpholine (**11d**) improved potency as well as resulting in decreased lipophilicity. More promising were alcohols **11g**–**i**. Compound **11h** achieved a biochemical IC_{50} of 50 nM and a K_d value of 6 nM (Fig. 2), a significant improvement over **7**, and one of the most potent reversible inhibitors of LSD1 reported to date. The acidifying effect of the trifluoromethyl group appears important, giving a 32-fold improvement in potency over **11g**. These compounds demonstrated that a variety of alcohol side chains could be tolerated. Finally, we incorporated a number of different 5-membered heterocyclic groups to investigate how the presence of different heteroatoms in varying positions would affect the activity (**11i**–**q**). Most notably, methylisoxazole **11o** displayed excellent affinity for LSD1 by SPR, with the extension of the methyl group to ethyl (**11p**) and isopropyl (**11q**) also well tolerated.



Scheme 1. Synthesis of compounds **11a**–**q**. Reagents and conditions: (a) oxaloacetic acid, acetic acid, reflux, 1 h, 93%; (b) COMU, (*R*)-3-Boc-aminopiperidine, DIPEA, DMF, 30 min, 84%; (c) R-X, K_2CO_3, DMF, 100 °C, 1 h, then 4 M HCl/dioxane, rt, 1 h, 24–71%.