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Diversity-oriented synthesis of a cytisine-inspired pyridone library leading to the discovery of novel inhibitors of Bcl-2

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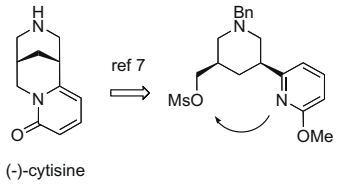
ABSTRACT

Four enantiopure cytisine-inspired scaffolds can be accessed via a versatile pyrrolidine template derived from a stereocontrolled [3+2] azomethine ylide–alkene cycloaddition. Differential ester protection allows for the selective formation of either a bridged bicyclic or tricyclic scaffold via pyridone cyclization. Solid-phase diversification of the pyridone scaffolds yielded a diverse library of 15,000 compounds enabling the discovery of a novel class of Bcl-2 inhibitors.

In light of their structural complexity and inherent biological activity,[1] natural products can serve as useful starting points for diversity-oriented synthesis (DOS).[2] Indeed, screening of natural product-based DOS libraries has led to the discovery of numerous small molecules possessing biological activity unrelated to that of the parent compound.[3] In designing a DOS library for the purpose of drug discovery, we drew inspiration from the natural product cytisine (Fig. 1), which contains a fused pyridone ring within a bridged bicyclic scaffold.[4] Cytisine is a well-known nicotine agonist, which led in part to the development of the smoking cessation agent Chantix®.[5] Interestingly, cytisine analogs have also been found to possess anti-phosphatase activity.[6]

Previous work in the total synthesis of cytisine and related analogs made use of an efficient pyridone cyclization step involving the activation of a primary alcohol and subsequent reaction with 2-methoxypyridine.[7] We chose to exploit this reaction for the synthesis of a diverse compound library to be screened against multiple, non-CNS related, biological targets. Below we will highlight the strategy we used to generate an unbiased library of pyridone compounds, which led to the discovery of several low micromolar inhibitors of the anti-apoptotic protein Bcl-2.[8]



(-)-cytisine

Figure 1. Retrosynthesis of the natural product (−)-cytisine.

For the design of our cytisine-based scaffolds we chose to make use of a highly versatile pyrrolidine intermediate (**1**, Fig. 2) substituted with a 2-methoxypyridine unit adjacent to the pyrrolidine nitrogen. We envisioned that both enantiomers of this densely functionalized template could be accessed via a stereocontrolled [3+2] cycloaddition[9] between a glycine-derived imine (**2**) and a chiral *E*-α,β-unsaturated ester (**3**). Cyclization to form the pyridone-ring could be achieved by activation of a primary alcohol masked as an ester to form the methylene bridge. Orthogonal protection of the two ester functionalities, as an allyl or methyl ester, would allow ring closure to occur via alternate pathways (**A** and **B**) providing two distinct skeletons (**I** and **II**). We reasoned that the cytisine-inspired scaffolds would serve as attractive starting points for library synthesis given their low molecular weight (~264) and opportunities for incorporating appendage diversity (ester and amine).

As shown in Scheme 1, the α,β-unsaturated methyl (**3a**) and allyl (**3b**) esters were readily prepared starting from D-glyceralde-

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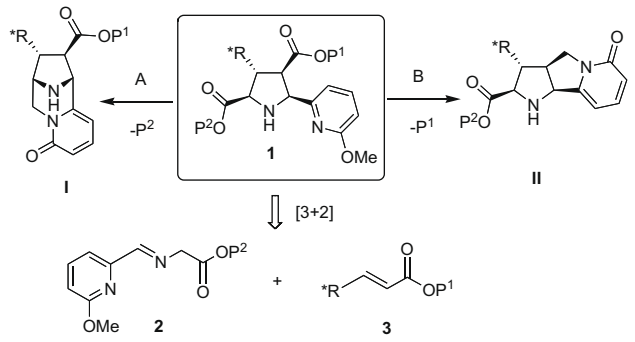
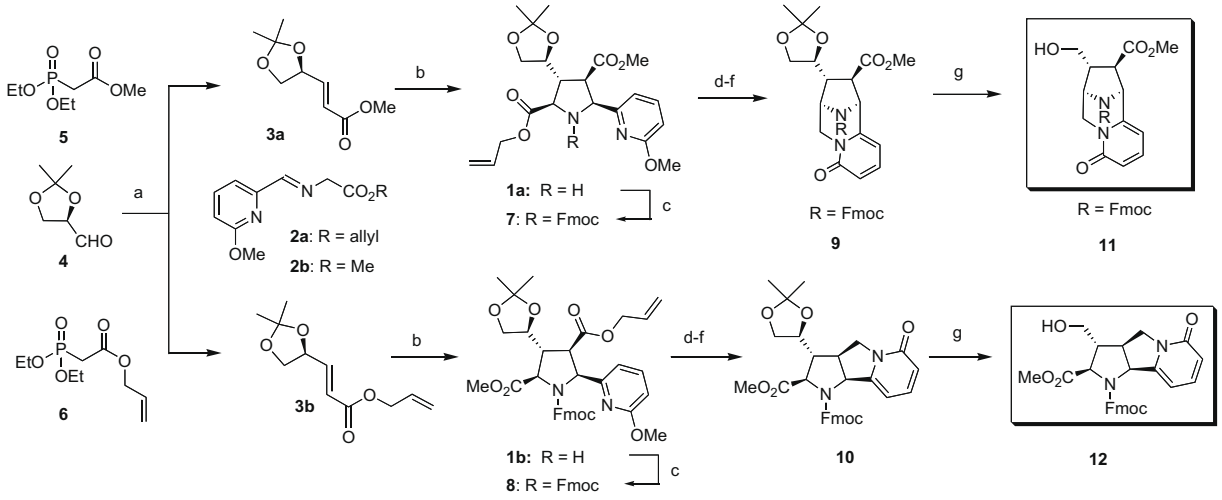


Figure 2. Cyclization strategy to form pyridone scaffolds **I** and **II**.

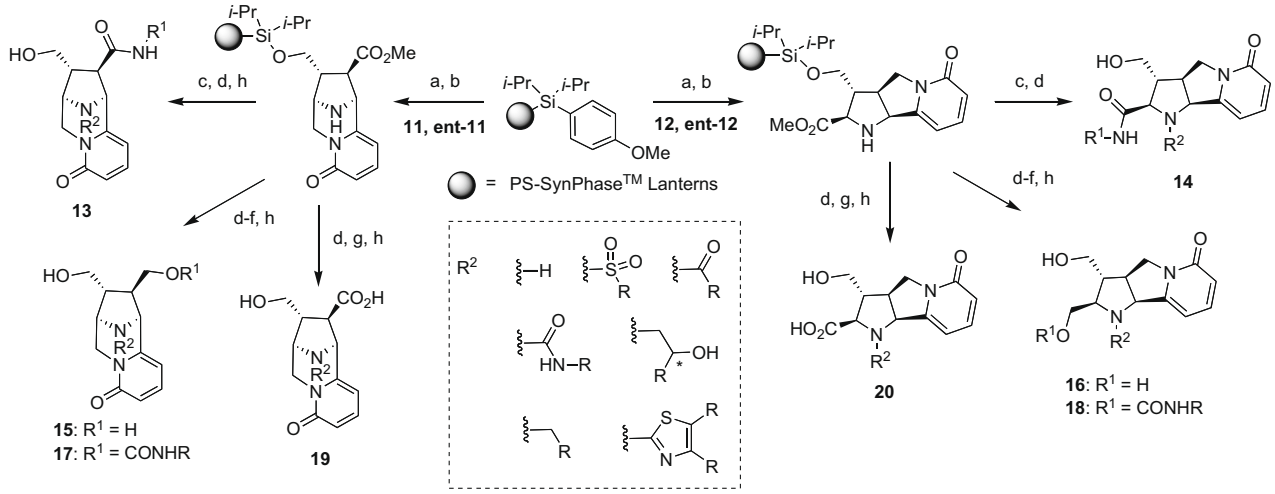
hyde acetonide (**4**) via a Horner–Emmons reaction with commercially available phosphonates **5** and **6**.[10] Meanwhile, imines **2a** and **2b** were prepared starting from 2-methoxypyridine-6-carboxaldehyde and the glycine allyl[11] or methyl ester. Reaction of the glycine-derived azomethine ylides with esters **3a** and **3b** promoted by AgOAc/DBU yielded cycloadducts **1a** and **1b** in high yield with excellent diastereoselectivity (>95:5).[12]

Following protection of pyrrolidines **1a** and **1b** as Fmoc-carbamates **7** and **8**, the allyl ester was removed via treatment with $Pd(PPh_3)_4$ using *N*-methylaniline as the π-allyl scavenger.[13] Initial attempts to reduce the resulting acid with BH_3–DMS were unsuccessful, as were attempts to reduce various mixed anhydrides. Optimal conditions for this transformation were found to be BOP-activation of the acid followed by reduction of the HOBt-ester with sodium borohydride.[14,15]

Having optimized the reduction of the acid, the key cyclization reaction to form the pyridone ring could be carried out. This was achieved via activation of the primary alcohol with mesyl chloride followed by heating in toluene to afford the bridged bicyclic scaffold (**9**) and the tricyclic core (**10**). Isolation of the intermediate mesylate was not required for the success of this reaction thus providing the desired pyridone compounds in 'one-pot'.



Scheme 1. Reagents and conditions: (a) NaH, THF, **5** or **6**; (b) **3a**/**2a** or **3b**/**2b**, AgOAc, DBU, toluene, −78 °C, 86–90%; (c) FmocCl, DIEA DCM, 81–90%; (d) $Pd(PPh_3)_4$, PhMeNH, THF, 82–99%; (e) BOP, $NaBH_4$, THF, 70–75%; (f) (i) MsCl, Et_3N; (ii) toluene, reflux, 70–74%; (g) (i) HCl, THF, H_2O; (ii) $NaIO_4$; (iii) $NaBH_4$, 76–78%.



Scheme 2. Reagents and conditions: (a) (i) TfOH (6.0 equiv), DCM; (ii) **11**, **ent**-**11**, **12** or **ent**-**12** (1.2 equiv), 2,6-lutidine (9.0 equiv), DCM, rt, 24 h; (b) 20% piperidine, DMF, rt, 30 min; (c) $AlMe_3$ (15 equiv), amine (20 equiv), toluene, 40 °C, 3 d; (d) RSO_2Cl (20 equiv), 2,6-lutidine (25 equiv) DCM, rt, 24 h; RNCO (15 equiv), DCM, rt, 24 h; RCOCl (10 equiv), 2,6-lutidine (15 equiv), DCM, rt, 24 h; epoxide (20 equiv), $LiClO_4$ (20 equiv), *i*-propanol, 70 °C, 1–3 d; RCHO (20 equiv), $Na(OAc)_3BH$ (22 equiv), 2% AcOH, DMF, rt, 3 d; FmocNCS (5 equiv), rt, 24 h then 20% piperidine, DMF, rt, 30 min then $RCOCH_2Br$ (10 equiv), rt, 24 h; (e) $LiBH_4$ (5 equiv), THF, rt, 1–2 d; (f) RNCO, toluene, 40 °C, 24 h; (g) LiOH (10 equiv), THF, MeOH, H_2O, rt, 24 h; (h) (i) 15% HF/pyridine, THF, rt, 2 h; (ii) TMSOMe, 10 min.

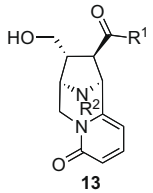
In order to prepare for solid-phase library synthesis, which required a primary alcohol for loading onto solid-support,[16] the dimethyl acetal was hydrolyzed, and the resulting diol was cleaved oxidatively with sodium periodate. Reduction of the resulting aldehyde could be achieved by controlled reaction with sodium borohydride to provide cores **11** and **12** and their corresponding enantiomers (**ent-11** and **ent-12**).

Having established a practical large-scale (>75 g) synthesis of the pyridone scaffolds, the library production was undertaken. Loading of the four scaffolds onto silicon-functionalized Lanterns[16b] was carried out via activation with TfOH to provide an average loading level of 15 mmol of compound per Lantern (Scheme 2). Following Fmoc removal the ester functionality was converted into a variety of amides and pyrrolidine capping was achieved by reaction with sulfonyl chlorides, acid chlorides, epoxides, isocyanates and aldehydes. 2-Aminothiazoles were formed by capping with FmocNCS, followed by Fmoc-removal and treatment with an assortment of bromoketones.[17] Cleavage from the Lantern was achieved via reaction with 15% HF/pyridine to afford amides **13** and **14**. The ester functionality was also reduced to provide the alcohols **15** and **16** and then reacted with isocyanates to afford carbamates **17** and **18**, while hydrolysis of the ester with LiOH provided acids **19** and **20**. In total ~15,000 cytisine-inspired compounds were produced with purities exceeding 75% for over 75% of the library.[18]

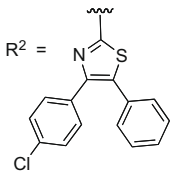
Compounds produced in the pyridone library were screened for binding affinity against Bcl-2 and Bcl-xL in traditional solution-based competition binding assays formatted for HTS analysis. Competition against a fluorescently tagged BH3 peptide was measured in the presence of an inhibitor, and compounds arrayed in a 384-well format were initially screened at a single fixed concentration of approximately 10 mM. Library hits demonstrating inhibition with significant *Z* factors (three standard deviations above assay error) were subsequently assayed in a multiple point dilution series to generate K_i values in order to measure the affinity of the compound for Bcl-2 and Bcl-xL.[19] The hit rate from this library screen was 1.1% and 0.2% against Bcl-2 and Bcl-xL respectively, with the best inhibitors having single digit micromolar activities.

A survey of the current literature in the area of published Bcl-2 small molecule ligands suggests that the library hits identified in this screen represent a significant accomplishment as the majority of the reported ligands are in fact weaker in affinity that those identified from this screen.[20,21]

Table 1



13



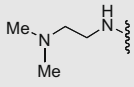
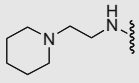
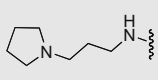
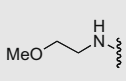
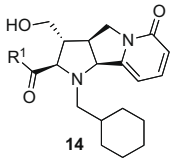
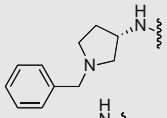
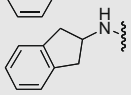
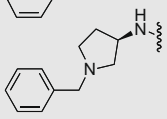
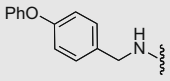
Compound	R^1	Bcl-2 K_i (μM)	Bcl-xL K_i (μM)
13a		2.0	5.7
ent-13a		1.3	6.6
13b		2.5	6.0
ent-13b		1.5	11.0
13c		3.0	5.7
ent-13c		1.6	7.8
13d		4.3	>100
ent-13d		14.0	>100

Table 2



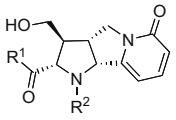
14

Compound	R^1	Bcl-2 K_i (μM)	Bcl-xL K_i (μM)
14a		4.1	>100
14b		5.4	>100
14c		7.8	>100
14d		8.4	>100

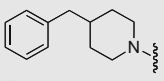
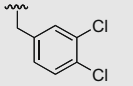
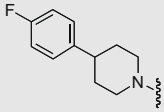
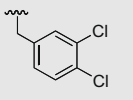
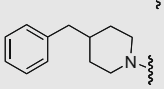
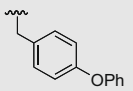
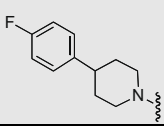
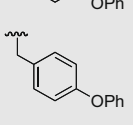
The most potent compounds derived from the bridged bicyclic pyridone scaffold (**11**) were those containing a diamine at R^1 and a chloro-substituted diphenyl 2-aminothiazole at R^2 (**13**, Table 1). Both enantiomers in this series were equally active against Bcl-2, perhaps indicative of non-specific binding. They also displayed inhibitory activity against Bcl-xL except for compounds **13d** and **ent-13d**, which lacked the diamine at R^1.

The most active compounds derived from the tricyclic pyridone core (**12**) were the products of reductive alkylation and Weinreb amidation (**14**). For one enantiomeric series there was a distinct preference at R^2 for cyclohexyl carboxaldehyde in combination with primary amines containing a hydrophobic aromatic ring at R^1 (Table 2). For the enantiomeric series (**ent-14**) 3,4-dichlorobenzyaldehyde as well as 4-phenoxybenzaldehyde were preferred at R^2 in combination with benzyl or phenyl substituted piperidines at R^1 (Table 3). This difference in activity for the two enantiomeric series suggests more specific binding as compared to the 2-amino-

Table 3



ent-14

Compound	R^1	R^2	Bcl-2 K_i (μM)	Bcl-xL K_i (μM)
ent-14e			1.2	>100
ent-14f			2.2	>100
ent-14g			5.2	>100
ent-14h			6.8	>100

thiazole compounds. In both cases the active compounds in this series were selective for Bcl-2 over Bcl-xL.

Given the difficulty in designing inhibitors of protein–protein interactions, the identification of low micromolar inhibitors directly from a primary screen is significant. This work serves to further highlight[2] the potential of screening natural product-inspired DOS libraries in an unbiased manner as a valid approach to drug discovery.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.03.037.

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