Organic & Biomolecular Chemistry

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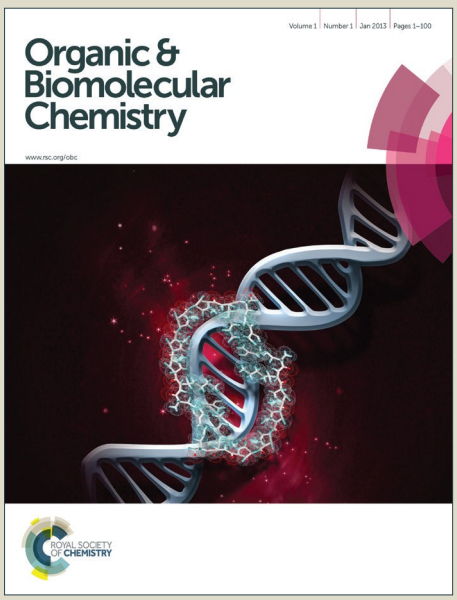

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COMMUNICATION

Structure-Based Design of 3-Carboxy-Substituted 1,2,3,4-Tetrahydroquinolines as Inhibitors of Myeloid Cell Leukemia-1 (Mcl-1)

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Mcl-1 has recently emerged as an attractive target to expand the armamentarium in the war on cancer. Using structure-based design, 3-carboxy-substituted 1,2,3,4-tetrahydroquinolines were developed as a new chemotype to inhibit the Mcl-1 oncoprotein. The most potent compound inhibited Mcl-1 with a K_i of 120 nM, as determined by a fluorescence polarization competition assay. Direct binding was confirmed by 2D ^{1}H-^{15}N HSQC NMR spectroscopy with ^{15}N-Mcl-1, which indicated interactions with R263 and T266, and occupation of the p2 pocket are likely responsible for the potent binding affinity. The short and facile synthetic chemistry is expected to mediate future compound optimization.

Introduction

The intrinsic apoptosis pathway is activated when a cell undergoes stress, which leads to the homodimerization of the pro-apoptotic Bcl-2 proteins Bak and Bax at the outer mitochondrial membrane, and, in turn, a caspase cascade ensues that results in the formation of apoptosomes. The net result of this process is programmed cell death, or apoptosis.[1,2] In many human cancers, anti-apoptotic members of the Bcl-2 family, which include Bcl-x_L, Bcl-2 and Mcl-1, are over-expressed, immortalizing the cancer cells.[3,4] Whilst inhibitors of Bcl-x_L and Bcl-2 have advanced to clinical trials (dual Bcl-x_L/Bcl-2: ABT-263 (navitoclax); Bcl-2 specific: ABT-199), progress in the development of specific Mcl-1 inhibitors has been less successful and there currently exists no drug to inhibit this protein.[5,6] Upregulation of Mcl-1 specifically has been associated with the development and progression of several cancers that include acute myeloid leukaemia,[7] melanoma,[8] non-small-cell lung,[9] pancreatic,[10] prostate,[11] and ovarian cancers.[12] Moreover, it is known that cancers dependent on Bcl-x_L can exhibit resistance to the Bcl-xL inhibitor ABT-737 (a variant of ABT-263) through upregulation of Mcl-1.[13,14] Therefore, the development of inhibitors of Mcl-1, either as single agents or as adjuvant therapies, represents an unmet medical need.[15,16]

By virtue of a hydrophobic grove on its surface, Mcl-1 directly antagonizes the pro-apoptotic Bcl-2 proteins, which include Bak, Bax and Bim, through capturing their BH3 α-helical "death" domains, effectively "neutralizing" the cell-killing role of these proteins.[17] More specifically, BH3 domains project four conserved hydrophobic side chains from one face of the α-helix that recognize sub-pockets on the surface of Mcl-1, which are termed p1 through p4. Additionally, a conserved aspartate residue on the opposite face of the helix recognizes R263.[18] We, and others, have developed α-helix mimetics of the BH3 "death" domains to inhibit Mcl-1, as well as the family members Bcl-2 and Bcl-x_L.[5,19–24] Similarly, a complementary strategy to inhibit Mcl-1 through a more traditional small-molecule approach has begun to emerge.[25–31] However, the discovery of clinical candidates remains elusive, and so new Mcl-1 inhibitors fashioned from novel scaffolds are essential. Furthermore, synthetic routes to access these inhibitors should be as short and simple as possible to expedite compound synthesis and keep costs to a minimum. In light of these considerations, we present our progress on the discovery of novel Mcl-1 inhibitors based on a simple and synthetically-accessible 3-carboxy-substituted 1,2,3,4-tetrahydroquinoline (THQ) scaffold.

Design

Fesik et al. recently described the fragment-based design of potent Mcl-1 inhibitors based on indoles, benozthiophenes and benzofurans.[26,29] In each case, a carboxylic acid is presented from the 2-position, which engages R263 in a salt

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bridge, whilst various phenoxyalkyl groups tethered to the 3-position probe deeply into the p2 pocket. To enhance the diversity of Mcl-1 inhibitor chemotypes further, we speculated that a similarly functionalized THQ might also block Mcl-1's protein–protein interaction (PPI) with the BH3 domain of its partner pro-apoptotic Bcl-2 proteins. Specifically, a carboxylic acid projected from the THQ ring, and substitutions off the ring nitrogen atom are predicted to achieve interactions with R263 and the p2 pocket, respectively. Since the nitrogen may be readily functionalized by alkylation, sulfonylation and acylation, this provides a potentially safer and broader avenue for diversification compared to the recently described BuLi/alkylation step.[29]

Transposing the 4-chloro-3,5-dimethylphenyl moiety and 2-carboxylic acid from compound **1** onto a THQ scaffold thus generated novel compound ±-**2** (Figure 1). The reason for the use of a 4-substituted-phenylsulfonyl moiety to tether the THQ scaffold to the 4-chloro-3,5-dimethylphenyl group was simply for ease of synthesis. It is known that the hydrophobic crevice on the surface of Mcl-1 is somewhat plastic.[29] For example, a crystal structure (PDB ID: 4HW3) reveals the p2 pocket substantially opens up in the presence of **1** relative to that in the crystal structure of Mcl-1 binding a native BH3 peptide (PDB ID: 4HW4). Thus, we performed GOLD docking solutions of the *S*-enantiomer of **2** with Mcl-1 extracted from three different PDB files (4HW3, 4HW2 and 3WIX). Excellent shape complementarity of *S*-**2** with Mcl-1 in 3WIX was observed, and a low energy docked solution is given in Figure 2. The carboxylate anion is predicted to form a salt bridge with R263 and an hydrogen bond to T266 (black dashed lines in Figure 2). The 4-chloro-3,5-dimethylphenyl moiety is buried deep in the p2 pocket, interacting with residues that include M250, V253 and F270. The benzene ring of the THQ scaffold possibly engages in π-π stacking with H224, and is near the p3 pocket. Interestingly, the docking experiments with the *R*-enantiomer of **2** generated unreasonable results, indicating that the chiral centre of **2** may have an impact on binding affinity.

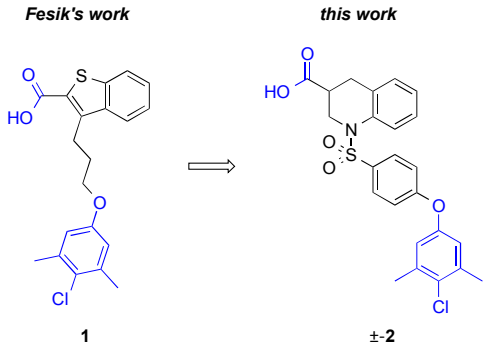


Figure 1: The development of a new chemotype to inhibit Mcl-1 based on the previously reported benzothiophene 1.[29]

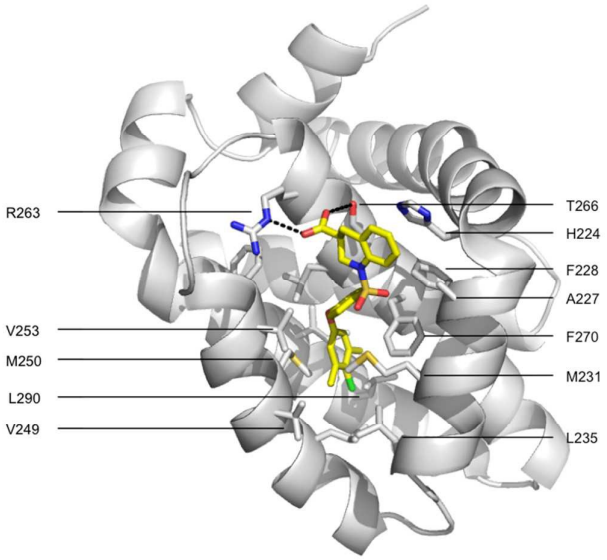
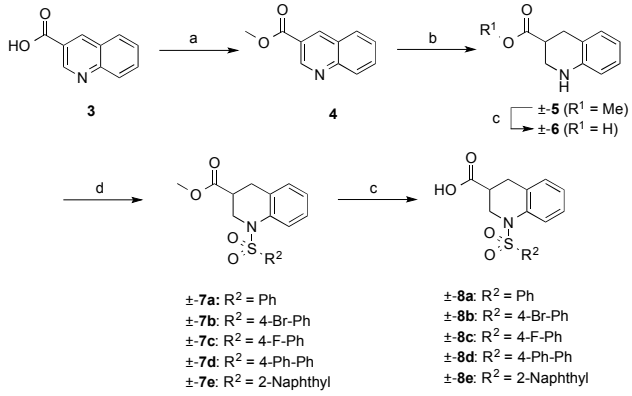


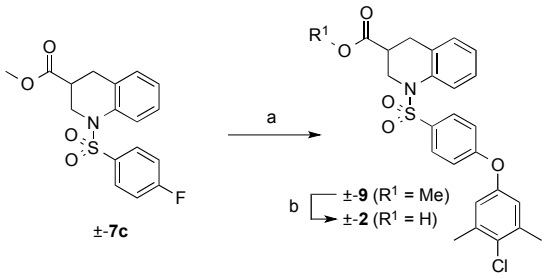
Figure 2: A high-scoring GOLD docked solution of *S*-**2** using Mcl-1 extracted from PDB ID: 3WIX. The binding site was defined as 10 Å about Met231. Image rendered using PyMOL.

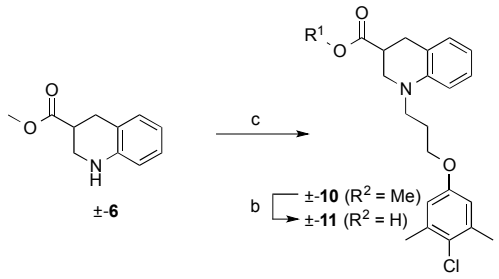
Synthesis

As depicted in Scheme 1, quinoline-3-carboxylic acid **3** was esterified with thionyl chloride in methanol to yield ester **4**. Reduction of the pyridine ring of **4** with pyridine-borane complex in glacial acetic acid then delivered racemic THQ **5** whose methyl ester was saponified to yield ±-**6**. Alternatively, sulfonylation of ±-**5** furnished compounds ±-**7**, which were also saponified with lithium hydroxide to afford the 3-carboxy target compounds ±-**8**. Further elaboration of the phenylsulfonyl group in ±-**7c** was accomplished by an S_NAr reaction with 4-chloro-3,5-dimethylphenol followed by ester hydrolysis to afford compound ±-**2**, as shown in Scheme 2. In addition, the phenylsulfonyl moiety in ±-**2** was replaced with a more flexible propylene group through a reductive amination-saponification sequence to yield ±-**11**.



Scheme 1: (a) $SOCl_2$, MeOH, 0 °C to reflux, overnight; (b) Pyr–BH_3, AcOH, RT, overnight; (c) LiOH.H_2O, THF–MeOH–H_2O, 3:1:1, RT, overnight; (d) R^1SO_2Cl, DIPEA, cat. DMAP, $CHCl_3$, reflux, overnight.



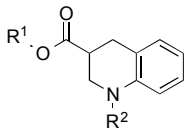


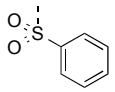
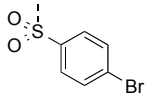
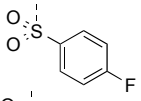
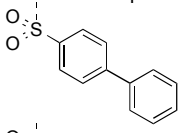
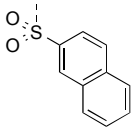
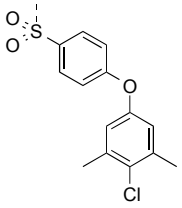
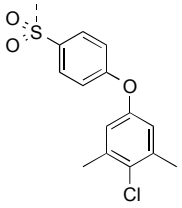
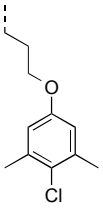
Scheme 2: (a) 4-Chloro-3,5-dimethylphenol, K_2CO_3, DMF, 100 °C, 48 h; (b) LiOH.H_2O, THF–MeOH–H_2O, 3:1:1, RT, overnight; (c) 4-chloro-3,5-dimethylphenoxypropaldehyde, $NaBH(OAc)_3$, DCE, 35 °C, overnight.

Results and Discussion

The target molecules were assayed for their abilities to disrupt the Mcl-1–Bak-BH3 PPI in a fluorescence polarization competition (FPC) assay with Mcl-$1^{172\text{-}327}$ and FITC-labeled Bak-BH3 peptide. Further details on the FPC assay can be found in the Supporting Information. As expected, unsubstituted racemic 1,2,3,4-tetrahydroquinoline-3-carboxylic acid (±-**6**) did not show any inhibitory effect (K_i > 500 μM) likely due to an inability to reach into the p2 pocket. Substitution of the THQ nitrogen with a phenylsulfonyl group (±-**8**) resulted in the discovery of a weak inhibitor of Mcl-1 (K_i = 193 μM), whose activity was further enhanced by the addition of a bulky, hydrophobic bromine atom in the *para* position of the phenyl ring (±-**8b**: K_i = 117 μM). However, there appears to be a geometrical constraint on the nature of the *para* hydrophobic group, since a phenyl ring here was not tolerated ((±-**8d**: K_i > 500 μM). On the other hand, a 2-naphthylsulfonyl group (±-**8e**) afforded a two-fold increase in affinity to Mcl-1 over ±-**8b** indicating that large groups can be accommodated in the pocket binding the sulfonyl substituent. Gratifyingly, the originally designed molecule ±-**2** exhibited the most potent binding of the series with a K_i of 120 nM. We surmise this dramatic improvement in binding affinity is due to efficient and deep occupation of the p2 pocket by the 4-chloro-3,5-dimethylphenyl moiety that is facilitated by the ether oxygen providing increased structural flexibility not available to biphenyl ±-**8d**. A similarly impressive enhancement in the K_i

Table 1: Fluorescence polarization competition assay with Mcl-$1^{172\text{-}327}$ and FITC-labeled Bak-BH3 peptide ("FITC-Bak"). IC_{50} data, which refers to the concentration of inhibitor that results in 50% displacement of FITC-Bak from Mcl-1, were converted to K_i values using the Nikolovska-Coleska equation.[30]



Compd	R^1	R^2	K_i (μM)
±-**6**	--H	--H	>500
±-**8a**	--H		193 ± 24
±-**8b**	--H		117 ± 19
±-**8c**	--H		176 ± 115
±-**8d**	--H		>500
±-**8e**	--H		59.0 ± 6.0
±-**9**	--Me		>500
±-**2**	--H		0.120 ± 0.053
±-**11**	--H		7.77 ± 1.39

value of about three orders of magnitude (from ±-**8a** to ±-**2**) has been reported elsewhere.[29] Compound ±-**11**, which is analogous to **1** as they carry the same propyl linker that tethers the acid-containing core to the 4-chloro-3,5-dimethylphenyl moiety, was a weaker inhibitor than ±-**2** by over an order of magnitude, demonstrating the positive contribution made by the phenylsulfonyl group likely achieving favourable interactions at the top of the p2 pocket. Finally, the methyl ester of ±-**2**, i.e. compound ±-**9**, had no appreciable affinity to Mcl-1 indicating the significance of the carboxylic acid, which presumably binds R263 as proposed.

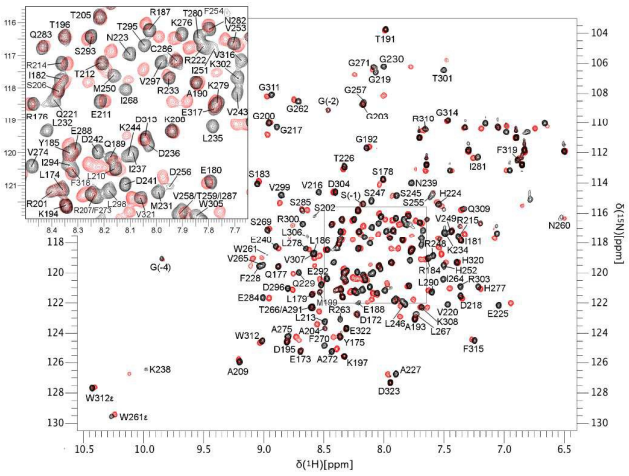


Figure 3: ^{1}H, ^{15}N HSQC spectra overlay of apo-Mcl-1 (black) and ±-**2**-bound Mcl-1 (red). Purified protein was concentrated to 91.4 μM in buffer containing 20 mM HEPES, pH 6.8, 50 mM NaCl, 0.34 mM NaN_3, 3 mM DTT, 5% DMSO. Concentrated ±-**2** was added in excess to a final protein:ligand ratio of 1:1.1. NMR datasets were acquired with 200 indirect points and 32 scans at 299K on a Bruker Avance 800 MHz spectrometer equipped with a z-gradient cryogenic probe. Data were processed using NMRPipe and analyzed with CCPN.[32,33]

Confirmation of the direct binding of ±-**2** to Mcl-1 was afforded by heteronuclear NMR studies. 2D ^{1}H-^{15}N HSQC spectra of Mcl-1 were collected in the absence (black) and presence (red) of ±-**2** (Figure 3). An overlay of the two spectra revealed significant chemical shift changes and negligible peak broadening, characteristics of a fast exchange regime, and consistent with the nanomolar affinity observed in the FPC assay. Chemical shift perturbations were calculated for each amino acid and the resonance variation was mapped onto the Mcl-1 crystal structure, 3WIX in Figure 4. Residues that experienced significant chemical shifts in the presence of ±-**2** are shown in red and cluster around the p2 pocket, in support of the docking model presented above. More particularly, considerable shifts were observed among residues predicted to bind the carboxylate anion (R263 and T266) and 4-chloro-3,5-dimethylphenyl moiety (M250 and F270). On the other hand, the NMR data argues against a model in which H224 participates in π-π ring stacking with the benzene ring of the THQ.

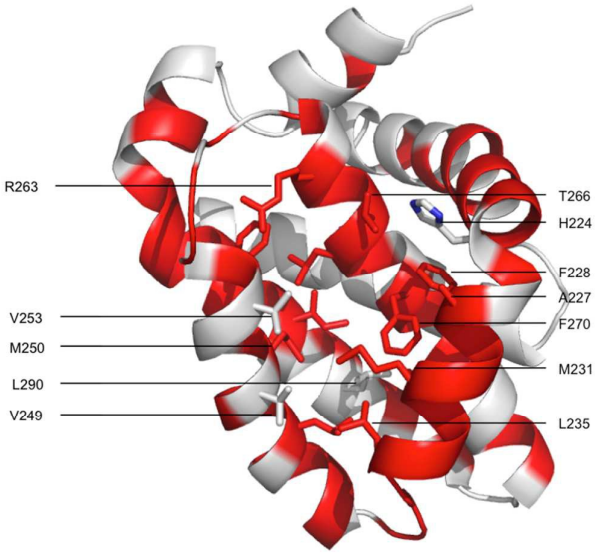


Figure 4: NMR chemical shift perturbations of the Mcl-1/±-**2** complex mapped onto Mcl-1 crystal coordinates (PDB ID: 3WIX) in PyMOL.[34] Residues experiencing chemical shifts of at least 0.3 ppm are shaded red. Combined ^{1}H and ^{15}N resonance variations were calculated using the following equation:

$$\Delta \text{ ppm} = \sqrt{\Delta\delta_{\text{HN}}^2 + (\Delta\delta_{\text{N}} \cdot \alpha_{\text{N}})^2} \qquad \text{Equation 1}$$

with spectral dimensions normalized by the a 0.14 ^{15}N scaling factor, α_N (0.17).[35]

We next investigated the activity of our most potent inhibitor ±-**2** on the viability of human A375 melanoma cells that demonstrate increased expression of Mcl-1.[36] A modest GI_{50} of 50 μM for ±-**2** was observed (Table 2), which is more than two orders of magnitude less potent than the in vitro FP data. We reasoned that the moderate cellular activity of ±-**2** might be due to limited cell penetration owing to the charged carboxylic acid. Thus, we prepared acetoxymethyl ester ±-**12** (R^1 = CH_2OCOCH_3) as a neutral prodrug of ±-**2**. Like methyl ester ±-**9**, ±-**12** was inactive in the FPC assay (K_i > 500 μM), consistent with the requirement of the carboxylic acid function to engage R263. Surprisingly, however, the highly labile ester ±-**12** did not yield a more potent cellular agent suggesting that the charged carboxylic acid of ±-**2** might not be a limiting factor in the translation of in vitro to cellular activity. Whilst this work was in progress, Leverson *et al.* described an especially potent Mcl-1 inhibitor (A-1210477: K_i = 0.454 nM) and stated that such exquisite potencies (sub-nanomolar) in vitro are required to ensure on-target cellular activity.[37] Therefore, the triple-digit nanomolar inhibitor described herein is not expected to achieve unequivocal on-target effects in cells, and further analysis in cells should be reserved until the discovery of more potent inhibitors.

Compd	K_i (μM)[a]	GI_{50} (μM)[b]
±-**2**	0.120 ± 0.053	50
±-**12**	>500	73

Table 2: Biological activity of select compounds. [a]In vitro activity determined as described in Table 1. [b]Viability of A375 cells as determined by a CellTiter-Blue® assay.

In conclusion, a new chemotype to inhibit Mcl-1 has been discovered based on a THQ core. Our most potent inhibitor ±-**2** has a K_i of 120 nM. The direct interaction of ±-**2** with Mcl-1 was confirmed with 2D ^{1}H-^{15}N HSQC NMR data. Unlike the binding modes of the BH3 peptides with Mcl-1 in which all four hydrophobic pockets are bound, GOLD docking studies indicated that, whilst ±-**2** interacts with the p2 pocket, no significant interactions with the p3 or p4 pockets were observed, which was largely substantiated by the HSQC NMR data. Thus, future analogues of ±-**2** will focus on targeting the p3 and p4 pockets in addition to further exploration of the p2 pocket. Furthermore, docking studies suggested that the *S*-enantiomer is likely a stronger binder than its *R* counterpart, and so both enantiomers of ±-**2** will be synthesized and evaluated to interrogate the impact of the chiral centre.

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