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Tetrahydroisoquinoline amide substituted phenyl pyrazoles as selective Bcl-2 inhibitors

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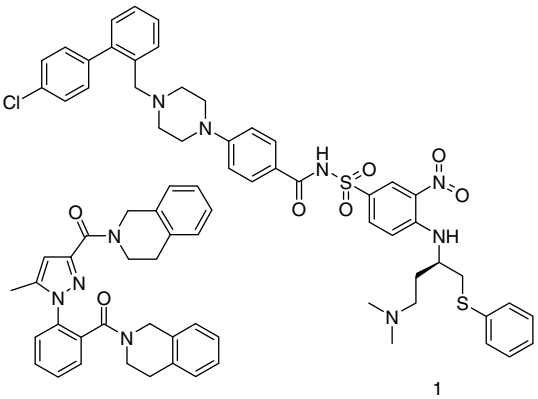
ABSTRACT

Anti-apoptotic Bcl-2 protects cells from apoptosis by binding to pro-apoptotic members of the Bcl-2 family thereby playing a role in tumour survival in response to chemo- or radiation therapy. We describe a series of phenyl pyrazoles that have high affinity for Bcl-2 and rationalise the observed SAR by means of an X-ray crystal structure.

In normal cells Bcl-2, an anti-apoptotic 26 kDa protein, regulates the activity of pro-apoptotic proteins by direct binding and sequestration.[1] Bcl-2 is overexpressed in a wide range of human haematopoietic and solid cancers and serves to prevent apoptosis induced by protective cell-death mechanisms. This is manifested clinically as drug resistance when treating with traditional cytotoxics and can also delay apoptosis in response to radiation therapy.[2] The highly homologous family member, Bcl-xL, also shows significant overexpression in some types of lung and ovarian cancers. The precise mechanism of Bcl-2 mediated apoptosis is the subject of considerable debate.[3]



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The Bcl-2 family of proteins can be divided into three groups based on the presence of Bcl-2 homology domains (BH1–BH4). Anti-apoptotic members such as Bcl-2 and Bcl-xL have a binding groove that accommodates the BH3 domain of pro-apoptotic family members,[4] preventing their oligomerization and the initiation of the apoptosis cascade.[5] Chimaeric Bcl-2/xL NMR and X-ray crystal structures have demonstrated that the binding groove is amenable to small-molecule intervention[6] and further studies have shown that such molecules are able to sensitise tumour cells to apoptosis. It is envisaged that a small-molecule inhibitor of Bcl-2 function would prevent binding and sequestration of pro-apoptotic Bcl-2 partner proteins. Such a molecule would abrogate the anti-apoptotic effects of Bcl-2 expression and chemosensitize a significant proportion of tumours to cell-death by existing cytotoxics and new targeted therapies. This has been most successfully demonstrated with ABT-737, **1**, a small molecule Bcl-2/Bcl-xL inhibitor,[7] an analogue of which has now entered the clinic.[8]

The phenylpyrazole **2** was identified by a HTS campaign as a moderately potent and selective Bcl-2 inhibitor (IC_{50} Bcl-2 1.5 μM; Bcl-xL 16.6 μM). ^{15}N–^{1}H HSQC NMR experiments showed that phenylpyrazole **2** binds to a similar region of Bcl-2 as ABT-737.[6] We now describe some of our attempts to develop **2** and rationalise the SAR observed with reference to an X-ray crystal structure.

The target molecules were prepared by two methods. Initially the pyrazole ring was constructed from the 2-hydrazinebenzoic acid[9] and ethylacetopyruvate, Scheme 1, to give a 4:1 mixture of regioisomers. Amide formation with lithiated amine gave **4**, followed by coupling with the second amine gave the desired product **5**. This method was limiting for the more highly functionalised tar-

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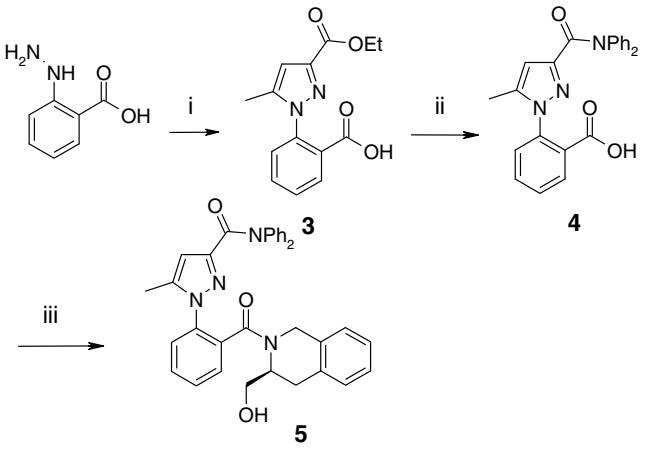


Scheme 1. Reagents and conditions: (i) ethyl acetopyruvate, gAcOH, reflux 2 h, 59%; (ii) *n*BuLi 2 equiv, Ph_2NH, THF, −78 to 25 °C, 98%; (iii) (*S*)-1-(1,2,3,4-tetrahydroisoquinolin-3-yl)-methanol, DIEA, EDC, HOBT, DCM, 25 °C, 16 h, 30%.

get molecules so an alternative method using the base mediated coupling of a pyrazole **6** to an activated phenyl, was employed, Scheme 2.

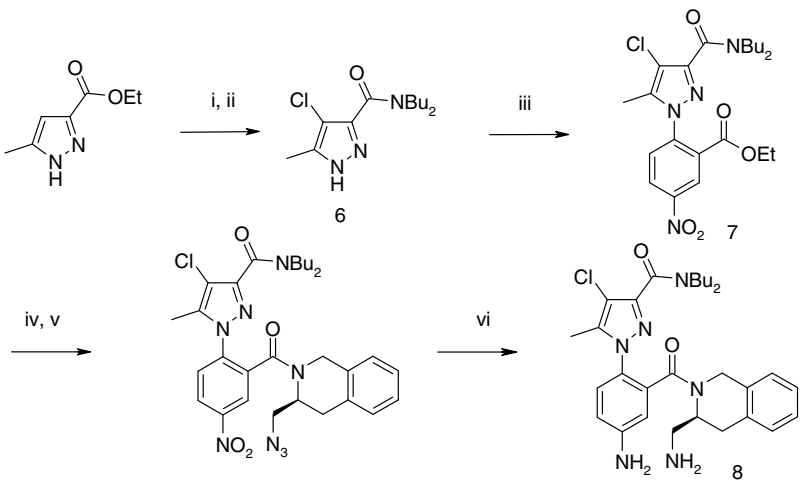
Confirmation that the desired regioisomer **7** had been prepared was obtained by nOe NMR. Hydrolysis of the ester, amide formation and reduction gave the desired product **8**.

3-Substituted tetrahydroisoquinolines (THIQ) were prepared from the commercially available chiral material by standard functional group interconversions.[10] For example, preparation of the aminomethyl analogue was achieved through the azidation of the alcohol using DPPA. The azide THIQ was then coupled to the benzoic acid and subsequently reduced to give the amine. The 3-methyl analogue was prepared as the racemate by reduction of 3-methyl isoquinoline using $NaBH_4$ and $NiBr_2$.[11]

Screening for binding affinity against Bcl-2 was done by means of a LANCE based assay using a 16-residue Bak peptide.[12] As binding affinity improved the peptide substrate was switched to the higher affinity 26-residue analogue, which typically gave an approximate 10-fold drop-off in activity compared to the 16-mer peptide. A similar system was used to screen against Bcl-xL.

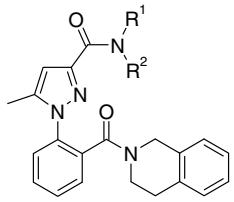
Phenylpyrazole **2** was a moderately potent inhibitor with an IC_{50} of 1.5 μM against Bcl-2 (16-mer) and 16.6 μM against Bcl-xL (16-mer). DMPK studies showed that both THIQ groups were extensively metabolised so initial efforts focused on modifying these groups. Initial SAR studies indicated that while the phenyl THIQ amide appeared essential for activity; the requirement for the pyrazole THIQ amide was less critical. Consequently, we embarked on the systematic replacement of the pyrazole THIQ amide, with the results for some representative examples shown in Table 1. A preference for tertiary over secondary amides (compare **10** with **9**) presumably reflects a conformational preference for the pyrazole amide substituent. The benzyl analogue, **11**, was inactive and para-substituents on the aromatic ring were disfavoured (compare **12** with **13** or **14**). Larger amide N-substituents were tolerated, culminating in the diphenyl analogue **18** being amongst the most potent. Interestingly, this compound showed good levels of selectivity for Bcl-2 over Bcl-xL. Reversal of the amide by switching the nitrogen and carbonyl positions; replacement with sulfonamide, carbamate, ester or carbonyl functionality; or directly attaching a phenyl group to the pyrazole completely abolished activity.

Attempts to replace the phenyl THIQ substituent with a wide range of isosteres were unsuccessful. However, the addition of a methanol group at the 3-position, Table 2, gave a marked improvement in potency. Although the direct attachment of carboxylate functionality, for example **23**, at this position was detrimental, (presumably an unfavourable interaction with the amide carbonyl) a range of homologated substituents were active. In fact, it became clear that size, hydrogen bonding potential or charge of these groups were not critical, and that the observed activity must come from a conformational lock exerted by the 3-substituent. This was confirmed by NMR studies showing the 3-substituted THIQ in an theoretically energetically unfavoured pseudo-axial conformation. This is a manifestation of a special case of the A-1,3 strain which is commonly observed in substituted acylated cyclic amines.[13] All of these compounds were prepared from homochiral starting materials, with the exception of the 3-methyl analogue, HPLC separation of the two enantiomers was accomplished to give the eutomer **27** and the distomer **28**. There is a clear preference for the S-enantiomer at this position, again suggesting a specific conformational requirement for this part of the molecule. Interestingly, the NMR spectra of these 3-substituted THIQ compounds typically show a mixture of rotamers (as determined by integration of the pyrazole proton), with one rotamer conformation predominating. Although these rotamers can make analysis difficult, their presence sug-



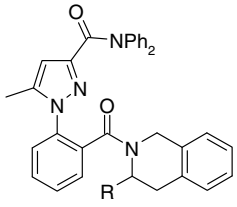
Scheme 2. Reagents and conditions: (i) N-Chlorosuccinimide, CCl_4, benzoyl peroxide, reflux, 88%; (ii) *n*BuLi, nBu_2NH, THF, −78 to 25 °C, 88%; (iii) ethyl 2-fluoro-5-nitrobenzoate, K_2CO_3, DMF, 100 °C, 91%; (iv) NaOH, EtOH, water, 25 °C, 98%; (v) (*S*)-3-azidomethyl-1,2,3,4-tetrahydroisoquinoline HCl, DIEA, EDC, HOBT, DCM, 25 °C, 20%; (vi) H_2, 10% Pd/C, EtOH, 25 °C.

Table 1
Modifications to pyrazole amide substituent.



Compound	R^1	R^2	Bcl-2 (16mer) IC_{50} (μM)	Bcl-xL (16mer) IC_{50} (μM)
9	H	Ph	50	
10	Me	Ph	2.5	
11	Me	CH_2Ph	Inactive	
12	Me	4-F-Ph	10.7	
13	Me	3-F-Ph	2.3	
14	Me	2-F-Ph	3.0	
15	Et	Ph	1.9	
16	*i*-Pr	Ph	7.7	
17	c-Hex	Ph	1.4	4.11
18	Ph	Ph	0.8	12.4

Table 2
Modifications to the THIQ-3-substituent.



Compound	R	Bcl-2 (16mer) IC_{50} (μM)	Bcl-xL (16mer) IC_{50} (μM)
5	(*S*)-CH_2OH	0.19	
19	(*S*)-CH_2NH_2	0.11	11.36
20	(*S*)-CH_2OMe	0.16	5.57
21	(*S*)-CH_2NHAc	0.73	9.69
22	(*S*)-CH_2NHSO_2Me	1.05	40% @ 33 μM
23	(*S*)-CO_2Et	5.61	18.0
24	(*S*)-CH_2CO_2Me	0.28	5.17
25	(*S*)-CH_2NMe_2	0.15	6.79
26	(*S*)-CH_2NHMe	0.14	12.63
27	(*R*)-Me	0.11	5.24
28	(*S*)-Me	27% @ 30 μM	4% @ 30 μM

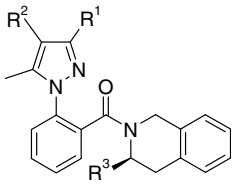
gested a way of determining the bioactive conformation. Our attempts to achieve this will be described elsewhere.

A significant advance came from the observation that bromination at the pyrazole 4-position led to a considerable improvement in potency, Table 3, **29**. Chloro-substitution was equipotent, for example **30**, but other functionality such as phenyl and nitro were, at best, only equipotent with the non-substituted pyrazole. With these modifications in hand we returned to the pyrazole amide substituent. Replacement of the diphenylamide with dialkylamides was relatively successful, Table 3, with the di-*n*-butyl group appearing to be optimum, for example **36**. The dialkylamides also appear to have greater Bcl-xL activity than the phenylamides, compare for example **36** with **31**.

The X-ray crystal structure of **31** complexed to a chimaeric Bcl-2/Bcl-xL protein was solved, Figure 1.[14] Comparison with the published structures shows that **31** binds to part of the same cleft as that occupied by the Bak, Bad, and Bim peptides and small molecules[4,15] and is consistent with the observed SAR. Peptide binding to Bcl-2/Bcl-xL is mediated via hydrophobic interactions of the side-chains (*i*, *i* + 4, *i* + 7, and *i* + 11) in discrete sub-pockets in the cleft. **31** occupies some of these sites (*i*, *i* + 4), but also makes a unique interaction through the THIQ group to a site that is not seen by the peptides or, the Abbott ligand.[15] **31** exhibits the predicted conformation determined from the ^1H NMR derived molecular models of the isolated rotamers of conformationally locked analogues. The key hydrophobic pyrazole amide substituent prefers to be *cis* to the pyrazole which may have implications in the design of suitable amide replacements. There is clearly a hydrophobic 'second-site' binding pocket that is occupied by the peptides and the Abbott compounds. Accessing this pocket would be expected to improve potency by maximising ligand interaction with Bcl-2. The crystal structure suggests that this may be achieved by substituting in the 4-position of the central phenyl ring. Initial studies were performed on the non-substituted THIQ scaffold, Table 4. The most potent examples contained carbonyl functionality and a hydrophobic group also appeared to be preferred. Although these molecules had poor solubility, results were sufficiently encouraging to prepare the 3-aminomethyl THIQ analogues, the most potent being the ether, **42**. Interestingly, the thioether, **45** has much improved Bcl-xL activity compared to the ether analogue **46**, suggesting a key binding interaction with the protein in this binding pocket.

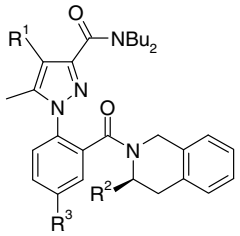
In conclusion we have identified a series of phenylpyrazoles with high binding affinity to Bcl-2 and Bcl-xL. These small molecules have a novel mode of binding and with the appropriate functionality display selectivity for Bcl-2 over Bcl-xL. Access to a small molecule that specifically blocks Bcl-2 function would be of value

Table 3
Pyrazole modifications.



Compound	R^1	R^2	R^3	Bcl-2 16mer IC_{50} (μM)	Bcl-2 26mer IC_{50} (μM)	Bcl-xL 16mer IC_{50} (μM)	Bcl-xL 26mer IC_{50} (μM)
29	$CONPh_2$	Br	CH_2OH	0.04	0.21	2.41	9% @ 30 μM
30	$CONPh_2$	Cl	CH_2OH	0.05	0.48	2.28	29% @ 30 μM
31	$CONPh_2$	Cl	CH_2NH_2	0.03	0.10	1.61	28% @ 30 μM
32	$CONEt_2$	Br	CH_2OH	3.67	17.3		14% @ 10 μM
33	$CON(CH_2CF_3)_2$	Cl	CH_2OH		6% @ 10 μM	13% @ 30 μM	4% @ 30 μM
34	$CON(nBu)_2$	H	H	0.23	2.01		25.20
35	$CON(nBu)_2$	Cl	CH_2OH	0.10	0.78		6.94
36	$CON(nBu)_2$	Cl	CH_2NH_2	0.02	0.16	0.25	5.05

Table 4
Phenyl substituents.



Compound	R^1	R^2	R^3	Bcl-2 16mer IC_{50} (μM)	Bcl-2 26mer IC_{50} (μM)	Bcl-xL 26mer IC_{50} (μM)
37	Cl	H	$-NH_2$	0.09	0.70	7.99
38	Cl	H	–NHCOPh	0.81	5.66	7% @ 30 μM
39	Cl	H	$-NHCOCH_2Ph$	0.04	0.27	2.37
40	Cl	H	$-NHCOCH_2OPh$	0.04	0.13	2.38
41	Cl	H	$-NHSO_2CH_2Ph$	0.250	3.15	19% @ 10 μM
43	H	H	$-OCH_2COPh$	0.02	0.33	3.24
44	H	H	$-OCH_2CO_2H$	0.01	0.13	5.64
45	H	H	$NHCOCH_2SPh$		0.60	0.40
46	H	H	$NHCOCH_2OPh$		0.51	3.05
8	Cl	CH_2NH_2	$-NH_2$		0.38	6.16
42	Cl	CH_2NH_2	$-NHCOCH_2OPh$		0.03	0.808
47	Cl	CH_2NH_2	$NHCH_2$(4-pyridyl)		0.65	17% @ 30 μM

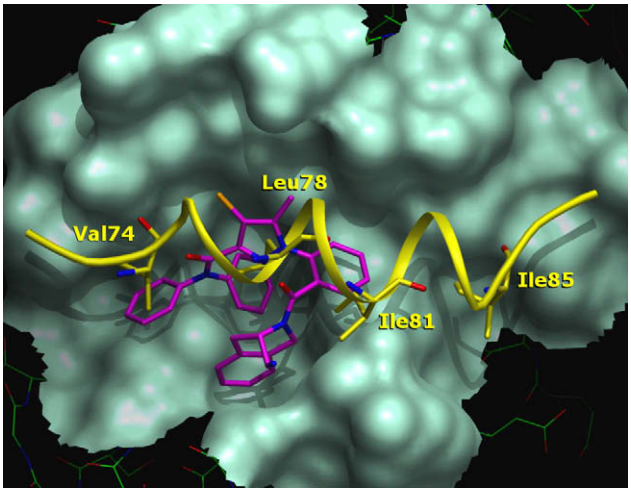


Figure 1. X-ray crystal structure of **31** (magenta) in Bcl-2/Bcl-xL. The BH3 binding cleft of the protein is represented as a cyan Connolly surface, with the 'second-site' (Ile85) binding pocket on the right The 16-mer Bak peptide (PDB ID: 1BXL) shown as a yellow helix is aligned to the structure. Bak residues involved in binding to the hydrophobic cleft (l to r: *i*, *i* + 4, *i* + 7, and *i* + 11) are also shown in yellow.

in revealing the individual roles of this family of anti-apoptotic proteins, particularly as blockade of Bcl-xL has been associated with an apoptosis-like response in platelets that is distinct from platelet activation and results in enhanced clearance of platelets in vivo by the reticuloendothelial system.[16] Solution of the X-ray crystal structure of chimaeric Bcl-2/Bcl-xL with a phenylpyrazole ligand has identified key areas for further optimisation. Inhibitor profiling in cell assays either by themselves or in combination with cytotoxics will be reported elsewhere.[17]

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