Bioorganic & Medicinal Chemistry Letters 19 (2009) 1592–1595

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Deciphering the antitumoral activity of quinacrine: Binding to and inhibition of Bcl-xL

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ARTICLE INFO

Article history:
Received 23 December 2008
Revised 3 February 2009
Accepted 4 February 2009
Available online 8 February 2009

Keywords:
Acridine
Anticancer
Apoptosis
bcl-2
bcl-xL
Fluorescence polarization spectroscopy
Quinacrine

ABSTRACT

From the screening of a unique collection of 880 off-patent small organic molecules, we have found that quinacrine inhibits the interaction between a BH3 domain-derived peptide and the antiapoptotic protein Bcl-xL. Nuclear magnetic resonance spectroscopy confirmed that quinacrine binds to the hydrophobic groove that Bcl-xL uses for interacting with the BH3 domain of proapoptotic proteins. This activity can contribute to the anticancer activity of quinacrine.

Cancer cells are resistant to natural apoptotic signals.[1] Then, pro-apoptotic compounds to treat cancer have been objective of extensive research. The efforts have been directed to inhibitors of protein-protein interactions between pro- and anti-apoptotic members of the Bcl-2 family of proteins and antagonists of apoptosis suppressor XIAP.[2] The family of Bcl-2 proteins falls into three classes.[3] The pro-apoptotic members, such as Bax and Bak, stimulate apoptosis, while the anti-apoptotic members, such as Bcl-2 and Bcl-xL, inhibit the functions of Bax and Bak. A third class is defined by the 'BH3 only' members (Bid) that inhibit the anti-apoptotic proteins. Up-regulation of either Bcl-2 or Bcl-xL is commonly observed in cancerous cells and correlates with resistance to chemotherapy.[4,5] Inhibitors of Bcl-2 or Bcl-xL were early proposed as apoptosis inducers.[6] A strategy for Bcl-xL antagonism is based on mimicking the actions of endogenous inhibitors that bind to Bcl-xL and its relatives via BH3 domains. Several chemicals that occupy the same binding site on Bcl-xL as BH3 peptide have been identified. These compounds promote apoptosis[7] and some of them may enter human clinical trials for cancer treatments. We used a similar strategy to that generally employed for the identification of Bcl-xL (Bcl-2) antagonist by screening of chemical libraries that could compete with a fluorescent-labelled BH3 peptide for binding to Bcl-xL. In some instances, the screening of diversity-based combinatorial libraries has paved the road to the discovery of hit molecules that needed further optimization.[8–13] However, in order to shorten the long way between basic research and clinical applications, we reasoned that it could be of interest to screen compound collections of known pharmacology properties and to explore their side activities. We have analysed 880 off-patent drugs that initially comprised the Prestwick Chemical Library® and found lead compounds that in vitro inhibited the interaction between the BH3-based peptide and Bcl-xL. The most active compound identified from this collection was quinacrine that was found to induce cell death in different tumor cell lines. Nuclear magnetic resonance (NMR) spectroscopy confirmed that quinacrine binds to the hydrophobic groove that Bcl-xL uses for interacting with the BH3 domain of proapoptotic proteins. Then, a direct activation of the apoptotic pathway contributes to the anticancer activity of quinacrine.

The Bcl-xL binding activity of the Prestwick Chemical Library® was evaluated by a fluorescence polarization competitive assay[14] using a soluble form of Bcl-xL[15] and a synthetic fluorescent-labelled peptide derived from the Bak BH3 domain (CF-BH3-Bak)[14] (see Supplementary data). The library compounds (50 μM in 1% DMSO containing PBS) were incubated with Bcl-xL, then the CF-BH3-Bak peptide was added and the fluorescence polarization measured. As a result of the screening, eight compounds, all with Bcl-xL binding activity above the initial threshold, were selected and their IC50 (concentration needed to reduce the fluorescence polarization by 50%) values were determined. For two of these

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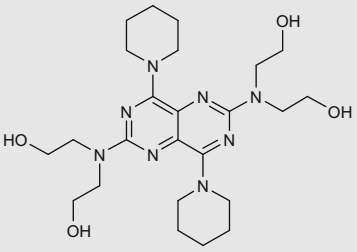
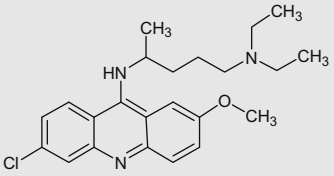
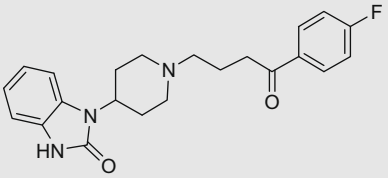
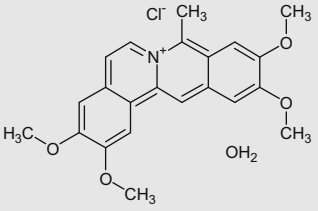
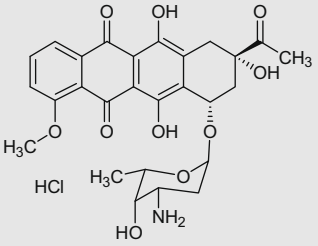
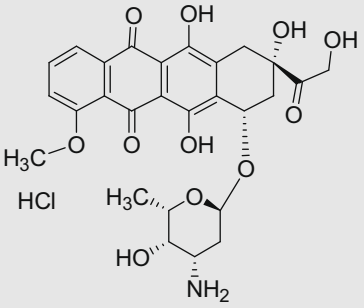
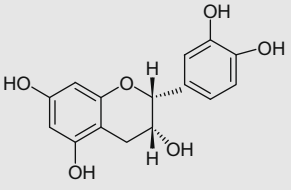
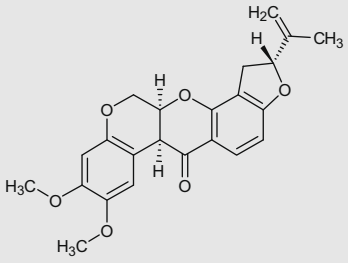
doi:10.1016/j.bmcl.2009.02.020

Table 1
Biological activity of the library compounds identified as Bcl-xL ligands. Data expressed as mean ($n > 3$). In all cases SE < 10%

Compound		Binding[a] IC_{50} (μM)	Induced cell death[b] IC_{50} (μM)
Name	Chemical structure		
Dipyridamole		10	>100
Quinacrine		24	18
Benperidol		>50	>100
Coralyne		>50	–
Daunorubicin		>50	–
Doxorubicin		>50	–
Epicatechin		>50	>100

(*continued on next page*)

Table 1 (*continued*)

Compound		Binding[a] IC_{50} (μM)	Induced cell death[b] IC_{50} (μM)
Name	Chemical structure		
Rotenone		>50	25

[a] The library compounds (50 μM in 1% DMSO containing PBS) were incubated with Bcl-xL, then the CF-BH3-Bak peptide was added and the fluorescence polarization measured in millipolarization units (mP) and adjusted to a two-state binding model.

[b] Jurkat cells were treated with the compounds for 19 h and cell viability was evaluated by MTT.

compounds, namely dipyridamole and quinacrine we obtained acceptable IC50 values of 10 μM and 24 μM, respectively (Table 1). We also analyzed the ability to induce cell death in Jurkat cells and quinacrine was the most effective compound with an IC50 value of 18 μM. A large number of acridine derivatives have been tested as anticancer agents.[16–18] In particular, quinacrine and quinacrine derivatives, can restore p53 function in renal cell carcinomas (RCC) that are characterized by maintaining a wild-type but functionally inactive p53.[19] Thus, quinacrine was found to induce cell death in RCC and non-RCC cells with similar IC50 values while other anticancer agents were more effective in non-RCC cells.[19] The activity of quinacrine was hypothesized to be related with the inhibition of NF-kB.[19] In fact, we have found that quinacrine was also able to induce cell death in the p53 null cell line HL60 (Fig. 1) through activation of the cell death machinery as demonstrated by the activation of caspase-3. These results suggest that the cell death inducing ability of quinacrine could include a proapoptotic effect by a direct intervention in the cell death machinery through the inhibition of Bcl-xL.

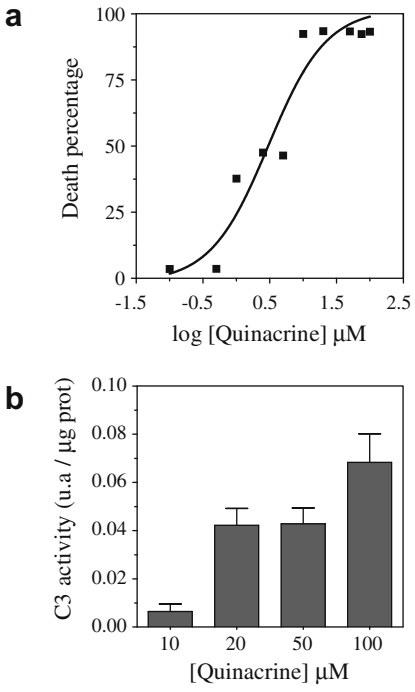


Figure 1. Quinacrine induced apoptosis in the HL60 p53 null cell line. (a) MTT assay of HL60 in the presence of different concentrations of quinacrine. (b) Caspase 3 activity measured in extracts of cells treated with different concentrations of quinacrine.

To map the binding site of quinacrine on Bcl-xL, NMR chemical shift experiments were performed. Several high resolution structures of Bcl-xL, both by X-ray and NMR, have been deposited at the Protein Data Bank (www.rcsb.org/pdb/home/home.do). All of the structures lack the C-terminal putative transmembrane helix (residues Phe210 to Lys224), and most of them have been based on a Bcl-xL deletion mutant lacking a long loop connecting α1 and α2 between residues Met45 and Ala84. The Bcl-xL construct used in these studies correspond to the full length form of the protein, just lacking the transmembrane segment. A ^{15}N-labeled sample of Bcl-xL was mixed with quinacrine (ratio 1:10), and a two-dimensional HSQC spectrum was acquired to monitor the changes in the chemical shifts of the backbone amides induced by the binding to quinacrine. Residues exhibiting the largest chemical shift changes upon binding to quinacrine were Trp57, Leu59, Ala85, Phe97, Gln121, Asp133, His177 and Gly196. Interestingly, the first two residues (Trp57 and Leu59) are located at the most distal position of the long loop and are probably interacting with quinacrine in a non-specific manner. It is also plausible that such a loop could be involved in a quinacrine-dependent conformational change that would induce local modifications on elements of the protein.[20,21] However, the rest of amino acids involved in the interaction are all located on one side of the protein (Fig. 2). They form a coherent patch that somehow resembles the interaction described between Bcl-xL and the BH3 domains of Beclin-1, Bad and Bak.[22,23] In fact, most of these amino acids had been previously reported to be interacting with BH3 domains and the existence of additional amino acids involved in the interaction with quinacrine could be due to the different molecular structure exhibited by both the quinacrine and the BH3 domains. Thus, Bcl-xL residues involved in the interaction with quinacrine belong to α2 (Ala85, Phe97), α4 (Gln121, Asp133), α6 (His177) and α8 (Gly196), and are surrounded by other residues located in α2 (Val86), α3 (Leu108) and α4 (Ser122, Val127) also implicated, but at a lower extent as indicated by their chemical shift changes, in the binding to quinacrine. Furthermore, these results are in agreement with those obtained[24] for novel inhibitors of Bcl-xL using SAR by NMR. In particular, they point out the important role that Phe97 and Gly196 play in the interaction between Bcl-xL and small molecule compounds able to modulate its activity.

In conclusion, we have identified quinacrine as a weak inhibitor of the antiapoptotic protein Bcl-xL. Quinacrine has been long used in clinic as antimalarial agent,[25] it was controversially proposed as an effective nonsurgical method for female sterilization[26] and more recently, although with some debate, it was claimed as a useful agent for treatment of prion-related diseases.[27,28] Furthermore,

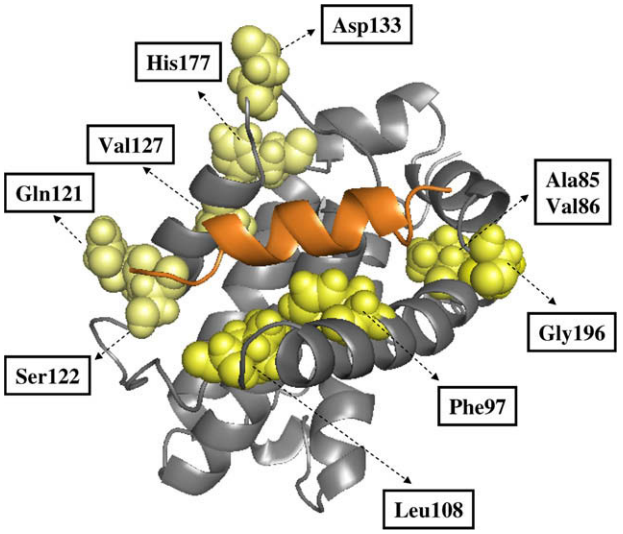


Figure 2. Ribbon diagram of the Bcl-xL/BH3 Bak peptide complex (PDB code: 1BXL). The BH3 helix of Bak is shown in orange and Bcl-xL residues undergoing chemical shift changes after quinacrine addition are represented by yellow spheres.

in a recent screening campaign, quinacrine was identified as a molecule capable of restoring p53 activity in RCC that contain wild-type but functionally inactive p53, then these cells, that were found resistant to several anticancer agents, are more tractable in the presence of quinacrine.[19] p53, as a sensor of cellular stress, is the protein responsible in coordinating the cell response to DNA-damage. These results enhanced previous interest in the anticancer properties of quinacrine[29] and stimulated the study of the molecular mechanism of action. Acridines have the ability to form both binary drug/DNA complexes and ternary drug/DNA/topoisomerase enzymes complexes.[16–18] The later has been proposed to be the mechanism of their anticancer activity.[18] In fact, it has been recently shown that defined acridine derivatives act as dual topoisomerase II and proteasome inhibitors.[30] Furthermore, it has been also proposed that the anticancer properties of quinacrine by some means rely on the activation of apoptosis through the proapoptotic protein Bax that, in turn, is a p53 target.[31]

Despite intense research during the last decade, the molecular mechanism of action of the proteins of the Bcl-2 family is still not well understood. The prevalent mechanism considers that the proapoptotic members (like Bak) are bound to the antiapoptotic Mcl-1 and Bcl-xL through the Bak-BH3 domain thus blocking its action and ensuring cell survival. In the presence of apoptotic stimuli, the BH3-only proteins are stimulated and bind to the antiapoptotic proteins, Bak is displaced and induces cell death.[32] The previously reported small molecules that compete with BH3 domains in these protein-protein interactions induce apoptosis in cell lines[33] although probably they do not directly initiate the apoptotic process in solid tumours, but enhance the effects of death signals.[34] As mentioned above, quinacrine can activate different death signals[19,30] and in addition have a self-amplification effect by also inhibiting Bcl-xL.

Acknowledgments

This work was partially supported by the Spanish Ministry of Science and Innovation (MICINN) BIO2004-998, BIO2007-60666 and CSD2008-00005 to EPP and BFU2005-09141 to APL, the Generalitat Valenciana GVPRE/2008 to MO and the Centro de Investigación Príncipe Felipe. Laura Mondragón is supported by a FPI fellowship from MICINN. We are grateful to Leticia Ortí for technical assistance. The p11 plasmid was a generous gift from Professor Dr. Walid Houry (Department of Biochemistry, University of Toronto).

Supplementary data

Supplementary data (Materials and methods) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.02.020.

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