



HAL
open science

Drimane derivatives as the first examples of covalent BH₃-mimetics that target MCL-1

Florian Daressy, Florian Malard, Line Seguy, Vincent Guérineau, Cécile Apel, Vincent Dumontet, Aude Robert, Anne-claire Groo, Marc Litaudon, Jérôme Bignon, et al.

► To cite this version:

Florian Daressy, Florian Malard, Line Seguy, Vincent Guérineau, Cécile Apel, et al.. Drimane derivatives as the first examples of covalent BH₃-mimetics that target MCL-1. *ChemMedChem*, 2021, 16 (11), pp.1789-1798. 10.1002/cmdc.202100011 . hal-03279424

HAL Id: hal-03279424

<https://hal.science/hal-03279424>

Submitted on 6 Jul 2021

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Drimane derivatives as the first examples of covalent BH3-mimetics that target MCL-1

Florian Daessy,^{[a,b]§} Florian Malard,^[a] Line Seguy,^[c] Vincent Guérineau,^[a] Cécile Apel,^[a] Vincent Dumontet,^[a] Aude Robert,^{[b]¶} Anne-Claire Groo,^[c] Marc Litaudon,^[a] Jérôme Bignon,^[a] Sandy Desrat,^[a] Aurélie Malzert-Fréon,^[c] Joëlle Wiels,^{[b]§} Ewen Lescop,^[a] and Fanny Roussi^{*[a]}

[a] Dr. F. Daessy, Dr. F. Malard, V. Guérineau, C. Apel, Dr. V. Dumontet, Dr. M. Litaudon, Dr. J. Bignon, Dr. S. Desrat, Dr. E. Lescop, Dr. F. Roussi, Institut de Chimie des Substances Naturelles, CNRS UPR 2301, Université Paris-Saclay, Avenue de la terrasse, 91198 Gif-sur-Yvette Cedex, France, E-mail: fanny.roussi@cnrs.fr

[b] Dr. F. Daessy, Dr. A. Robert, Dr. J. Wiels, Institut Gustave Roussy, CNRS UMR8126, Université Paris-Saclay, 114 rue Edouard-Vaillant, 94805 Villejuif Cedex, France

[c] L. Seguy, Dr. A. C. Groo, Dr. A. Malzert-Fréon, Centre d'Etudes et de Recherche sur le Médicament de Normandie, Normandie Université, UNICAEN, Boulevard Becquerel, 14032 Caen Cedex, France

§ Present address: UMR9018 CNRS Institut Gustave Roussy, Université Paris-Saclay, 94805, Villejuif, France.

¶ Present address: Université Paris-Saclay, Inserm, Institut Gustave Roussy, UMR1279, 94805, Villejuif, France

Abstract: Drimane sesquiterpenoid dialdehydes are natural compounds with antiproliferative properties. Nevertheless, their mode of action has not yet been discovered. In this publication, we have demonstrated that various drimanes are potent inhibitors of MCL-1 and BCL-xL, two proteins of the BCL-2 family that are overexpressed in various cancers, including lymphoid malignancies. Subtle changes in their structure significantly modified their activity on the target proteins. The two most active compounds are MCL-1 selective and bind in the BH3 binding groove of the protein. Complementary studies, by NMR and mass spectrometry analyses but also synthesis showed that they are covalent inhibitors of MCL-1, via the formation of a pyrrole adduct. In addition, cytotoxic assays revealed that these two compounds show a cytotoxic selectivity for BL2, a MCL-1/BCL-xL dependent cell line and induce apoptosis.

Introduction

Apoptosis is a well-known form of programmed cell death that is essential for tissue homeostasis through the elimination of useless or potentially harmful cells.^[1] One of the most studied protein families implicated in the intrinsic (mitochondrial pathway) of apoptosis is the BCL-2 family.^[2] This protein family is divided into anti- and pro-apoptotic members. The anti-apoptotic proteins such as MCL-1, BCL-xL and BCL-2 are multidomain proteins and they share short conserved sequenced regions (BH1 to BH4 motifs). Pro-apoptotic proteins are of two types and play a distinct role: either multidomain like Bax and Bak with three to four domains of homology (BH1 to BH4) or monodomain (BH3-only) like Bad, Bid or Bim. In normal conditions, the anti-apoptotic proteins sequester the BH3 domain of the pro-apoptotic proteins at a hydrophobic groove formed by their BH1, BH2 and BH3 domains via protein-protein interactions (PPIs), thus preventing apoptosis.^[3] Cellular stress signals such as DNA damage lead to the up-regulation of BH3-only proteins that trigger apoptosis. They bind to anti-apoptotic proteins, unleashing pro-apoptotic multidomain proteins that oligomerize in the mitochondrial outer membrane leading to its permeabilization, release of cytochrome c into the cytoplasm, activation of caspases and cell death.^[4]

Numerous studies have shown that overexpression of the anti-apoptotic BCL-2 proteins is involved in the development of many kinds of cancers or confers resistance to apoptosis induced by standard anticancer therapies.^[5] For example, proteins of the BCL-2 family play a central role in lymphocytes biology and are very often deregulated in lymphoid malignancies. Indeed, anti-apoptotic members of this family of proteins are overexpressed through translocation, amplification of the gene or upon action of transforming agents (such as oncogenic viruses for example) in haematological malignancies.^[6] On the other hand, a recent survey of various solid tumours revealed that, most of the time, MCL-1 is more abundant than BCL-2 or BCL-xL, thereby

suggesting that there might be some benefit in specifically targeting MCL-1 in these tumours.^[7] Similarly, in haematological cancers, patients with poor prognosis are often associated with an elevated level of MCL-1.^[8] These findings are not surprising because MCL-1 is one of the most amplified genes,^[9,10] and its overexpression is directly correlated with tumorigenicity.^[11] Consequently, targeting this protein family, and particularly MCL-1, is a highly promising strategy for anticancer therapy that has emerged over the last decades.^[12] Particularly, the development of small molecules, called BH3-mimetics, able to bind the hydrophobic cleft of the anti-apoptotic proteins, liberating the pro-apoptotic ones and restoring apoptosis has proven its great efficiency.^[13] Currently, such BH3-mimetics that target MCL-1 are evaluated in clinical trials as S63845,^[14] S64315,^[15] AMG-176,^[16] and AZD-5991.^[17]

Over the past years, our team has been involved in the identification of natural compounds that target the anti-apoptotic proteins of the BCL-2 family thanks to a large screening of ICSN extract library by polarization fluorescence assay (FPA).^[18] Various original natural molecules active on BCL-xL, BCL-2, and/or MCL-1 have been isolated,^[19,20,21] and chemically modified or synthesized.^[22,23,24,25,26,27]

A few years ago, we reported the isolation of various drimane sesquiterpenoids **1** from barks and leaves of three *Zygodium* (Winteraceae) of New Caledonia and their chemical modification.^[28,29] Drimane sesquiterpenoids^[30] are widespread secondary metabolites, produced by various higher plants (of the Canellaceae, Taxaceae, Polygonaceae and Winteraceae families), but also fungi, sponges, ferns and nudibranch. They are characterized by a common trans-decalin structure of type **2**, decorated by a wide range of substituents (Figure 1). This structural diversity goes along with a vast array of biological activities, including cytotoxic, antibacterial, antifungal and antifeedant properties.

A cinnamate ester at C1 confers originality to compounds of type **1** isolated in our group. We have disclosed that most of these molecules are cytotoxic on HCT-116, HL60 and/or KB cell lines if aldehyde functions are present at C8 and C9. We have also shown that their reduction, oxidation or cyclisation is detrimental.^[28] These results were confirmed by Fratoni *et al.*,^[31] who have pointed out that drimane derivatives with similar structures as **1** isolated from *Drimys brasiliensis* (Winteraceae) are cytotoxic against various myeloid and lymphoid cell lines. Contrarily, drimanes like polygodial **2** that do not possess a cinnamoyl group at C1 are found much less cytotoxic.^[32, 33]

Albeit their promising biological activity, little is known about the mode of action and biological target of drimanes of type **1**. In this paper, we want to disclose that their cytotoxic effect is due to their interaction with anti-apoptotic proteins from BCL-2 family, and more specifically with MCL-1.

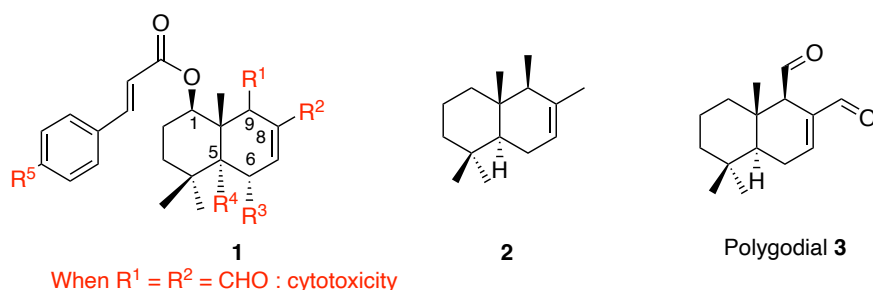


Figure 1. General structure of drimane sesquiterpenoids.

Results and Discussion

As part of our continuing search for novel plant-derived anti-cancer agents, the ethyl acetate bark and leaf extracts of various *Zygodium* (Winteraceae) from New Caledonia were found to significantly disrupt

interactions between the anti-apoptotic proteins BCL-xL with pro-apoptotic proteins Bak. This urged us to investigate the pro-apoptotic potency of various pure drimane sesquiterpenoids **4-9** previously isolated in our team from these plants (Figure 2).^[28, 29]

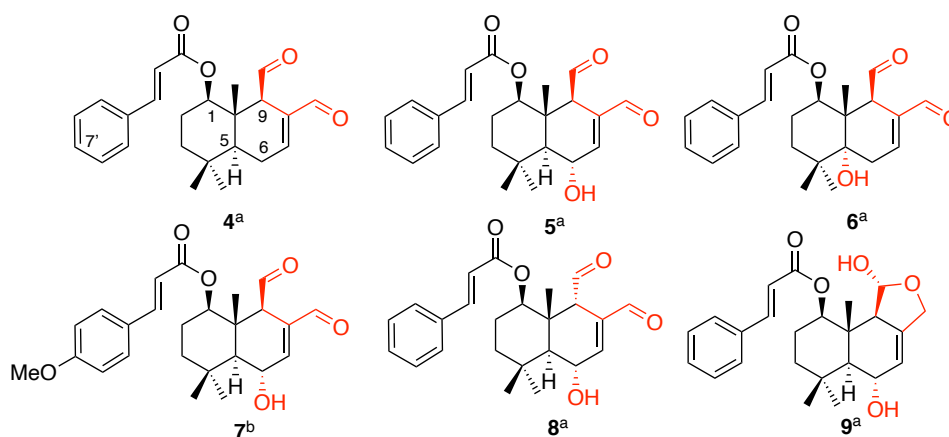


Figure 2. Structure of some drimane sesquiterpenoids isolated in our team from a- *Zygogynum pancheri*; b- *Zygogynum baillonii* and evaluated for their activity on BCL-2 family proteins.

The binding affinities of these natural compounds (**4-9**) for human isoforms of BCL-2, BCL-xL and MCL-1 has been evaluated by competition against fluorescently Labeled BH3 pro-apoptotic proteins, Bim, Bak and Bid, respectively, as described by Qian *et al.*^[34] They were compared to three known compounds used as references: meiogynin A a natural pan-BCL-2 inhibitor,^[19] ABT 737 a dual BCL-xL and BCL-2 inhibitor,^[35] and S63845^[14] that is MCL-1 selective (Table 1). We have found that all tested drimanes are MCL-1 and BCL-xL inhibitors with no affinity for BCL-2 (Table 1). In addition, the apparent affinity of all these compounds toward MCL-1 and BCL-xL is increased by a factor of four when they are incubated in the presence of the proteins for a period of 6 h instead of 2. This is a noticeable result as it is not the case for the control compounds.

Table 1. Binding affinities for compounds **4-9** compared to control molecules (meiogynin A, ABT-737, and S63845) on BCL-xL/Bak, MCL-1/Bid and BCL-2/Bim^[a] measured by fluorescence polarization.

Compd	K _i ^[b] (μM)			
	MCL-1/Bid 2h ^[c]	MCL-1/Bid 6h ^[d]	BCL-xL/Bak 2h ^[c]	BCL-xL/Bak 6h ^[d]
4	5.4 ± 1.8	1.5 ± 0.06	>23	7.0 ± 1.8
5	1.5 ± 0.1	0.3 ± 0.03	9.6 ± 0.9	3.0 ± 0.4
6	7.5 ± 0.8	2.0 ± 0.2	>23	11.7 ± 0.8
7	2.9 ± 0.5	0.6 ± 0.03	3.9 ± 0.3	1.3 ± 0.05
8	14.8 ± 4.1	5.7 ± 0.2	>23	>23
9	17.5 ± 0.5	5.3 ± 0.06	> 23	>23
Meiogynin A ^[e]	5.4 ± 0.4	4.7 ± 0.5	4.7 ± 1.4	4.1 ± 1.1
ABT-737 ^[e]	ND	ND	0.1 ± 0.01	0.03 ± 0.01
S63845 ^[e]	0.2 ± 0.02	0.2 ± 0.02	ND	ND

[a] Compounds **4-9** show no binding affinity for Bcl-2, [b] K_i is the concentration corresponding to 50% of inhibition of the binding of the labelled reference compound Bak to Bcl-xL and Bid to Mcl-1, and corrected for experimental conditions according to Cheng and Prusoff.^[36] The values presented (mean \pm S.D.) are from two independent experiments performed in triplicate, [c] Binding affinities were determined after 2 h of incubation, [d] Binding affinities were determined after 6 h of incubation, [e] Displacement assays for control molecule are also described in following references: meioyinin A see [25]; ABT-737 see [35]; S63845 see [14]

The structure-activity relationship studies that can be deduced from these experiments match perfectly to those published by us^[28] and Madeiros's group^[31] following the cytotoxicity studies on similar compounds. Aldehyde functions in position 8 and 9 are required as demonstrated with compound **9** that presents a low affinity for MCL-1 with $K_i = 5.26 \mu\text{M}$ and is not active on BCL-xL. In addition, position of the hydroxyl group greatly influences the affinity when comparing compounds **4**, **5** and **6**: an OH at position 5 is detrimental for activity on MCL-1. On the other hand, absolute configuration of aldehyde at C9 is decisive as compounds **9** and **5** is 16 times more potent than **9** and **8**. On the contrary, functionalization of the cinnamoyl moiety has a low impact (see compounds **5** and **7**).

Finally, the most potent and interesting compounds are **5** and **7** that only differ by the presence of a methoxy group on the cinnamoyl moiety. Functionalization in position 7' does not affect the affinity for MCL-1 as both present sub-micromolar activities (with K_i of 0.35 and 0.57 μM respectively) in the same range of order than S63845. However, the presence of a methoxy group in position 7' increases the affinity of compound **7** for BCL-XL (1.27 μM) compared to compound **5** (3.03 μM). Compound **5** is more selective towards MCL-1 than compound **7** (*i.e.*, it is 9 times more active on Mcl-1 than on BCL-xL compared to twice for compound **7**). As selectivity for MCL-1 with regard to BCL-xL is an important criteria in lymphoid malignancies, where MCL-1 is overexpressed but where BCL-xL is constitutively present and should not be totally inhibited to prevent thrombocytopenia,^[37] compound **5** seems more promising than **7**.

NMR experiments were carried out to confirm the interaction between compounds **5** or **7** and the proteins MCL-1 and BCL-xL in order to determine more precisely their binding mode. Preliminary solubility experiments (Figure S1) indicated that both molecules are soluble enough ($\geq 200\text{-}400 \mu\text{M}$) in presence of organic co-solvent (3.5 % DMSO) for interaction studies with MCL-1 and BCL-xL proteins.

We first titrated a uniformly ^{15}N -labeled Mcl-1 solution with increasing concentration of compound **7** (Figure 3). The titration was followed by 2D ^1H - ^{15}N SOFAST-HMQC NMR experiments that permit the observation of ^1H - ^{15}N correlation peaks for backbone amide groups but also for the long living NH/NH₂ side chains from tryptophane, arginine, asparagine and glutamine residues. During the titration, ligand **7** induced two types of successive spectral changes of the protein, in a concentration dependent manner (Figures 3A, 3B). The first one was characterized upon addition of compound **7** at low concentration (up to 2 eq.), by the progressive decrease in peak intensity, without chemical shift variation, for a consequent (~ 15) set of ^1H - ^{15}N resonances. Notably, no new cross peak appeared in the spectra, suggesting extensive line broadening for the 15 above-mentioned residues in the formed complex. Disappearing residues were mapped on MCL-1 structure (Figure 4C). These were located within the BH3-binding groove of the protein composed by α -helices α_2 , α_3 , α_4 and α_5 . We observed a similar behaviour in α -helix α_6 that does not directly interact with the ligand in most crystal structures, as already reported for MCL-1 complex with BH3-derived peptides.^[38] The second type of spectral changes was identified at higher ligand concentration (> 2 eq.), where all signals from MCL-1 decreased uniformly and almost no signal could be seen anymore at maximum ligand concentration (300 μM , 6 eq). In conclusion, compound **7** binds to MCL-1 with a strong affinity in the BH3-binding pocket and likely induces the oligomerization of MCL-1 at high concentration ($> 200 \mu\text{M}$). These experiments also showed that several NMR signals are missing for residues in the ligand binding sites of the formed complex. This is usually indicative of

conformational sampling at the intermediate timescale (~ms) and suggests potential conformational dynamics within the MCL-1/compound **7** complex.

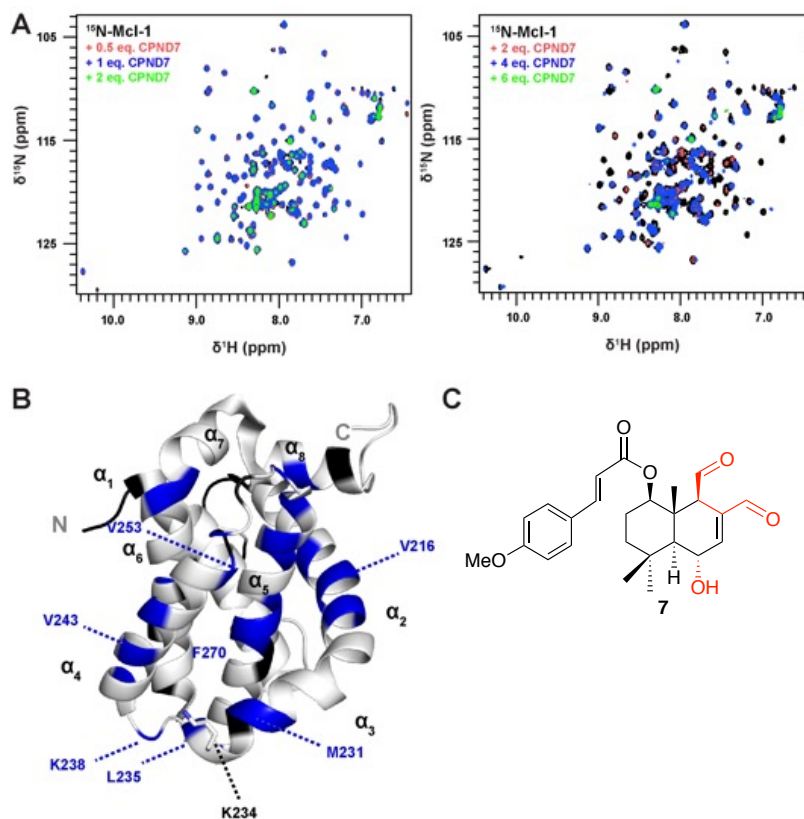


Figure 3. Compound **7** binds to human MCL-1 with strong affinity in the BH3 binding-groove. (A) Overlay of SOFAST ^1H - ^{15}N HMQC spectra from isolated MCL-1 (50 μM) (black) and in presence of increasing amount of compound **7** with 1 hour incubation at r. t. after each increment and prior to measurement. (B) Disappearance of ^1H - ^{15}N resonances in presence of compound **7** (2 eq.) and corresponding residues (blue) mapped on MCL-1 structure (pdb code: 2MHS): L213, V216, G217, G219, V220, G230, M231, L235, K238, E240, S243, R246, V251, S253, T259, W261, R263, V265, S269, F270, G271, A272, E292, T301, K302 and F315. Residues for which no data are available are highlighted (black). Those include prolines and others that could not be assigned to a ^1H - ^{15}N resonances in the spectrum of free MCL-1. Side-chain of K234 residue is oriented toward the binding pocket (black dash and label); (C) Compound **7**.

In order to locate the binding pocket of compound **5** on human MCL-1, ^1H - ^{15}N SOFAST HMQC spectra of ^{15}N -labelled MCL-1 were recorded in presence of increasing amounts of ligands **5** up to its solubility limit (Figure 4). For the highest ligand concentration, we incubated the sample overnight at r. t. to reach apparent system equilibrium. Throughout the titration, spectral perturbations were clearly visible by progressive shifts of several resonances, mainly in rapid exchange regime at the chemical shift timescale, although some peaks also underwent some line broadening (Figures 4A and 4B). At the ligand maximum concentration (225 μM), the protein (50 μM) was not fully saturated. Residues located in helical regions (α_2 , α_3 , α_4 and α_5) forming the BH3-binding groove of the protein were predominantly affected upon ligand addition as judged from large chemical shift perturbation upon ligand binding (Figure 4C). This indicates that compound **5** and BH3 motifs do share a common binding pocket in the protein. Consistently, helical regions that do not form the BH3-binding groove (α_1 , α_6 , α_7 , α_8) did not show perturbation above threshold upon binding of compound **5**. Surprisingly, we observed a new set of cross-peaks in the spectra when increasing ligand concentration (Figure 4B), close to cross-peaks that underwent rapid exchange shifts at lower ligand concentration. For the

appearing crosspeaks, higher ligand concentration strengthened signal intensity but did not impact on ^1H - ^{15}N chemical shifts. This suggests a second type of events for MCL-1/compound **5**. We excluded the possibility of a second binding site since it would be of stronger affinity as indicated from the slow (or nul) exchange between the new peaks and the first binding mode. Since we previously observed a time-dependent inhibition mode for compound **5** in the FPA experiments, we next hypothesized that compound **5** could chemically cross-react with MCL-1 to yield a covalent complex at longer time and large ligand excess.

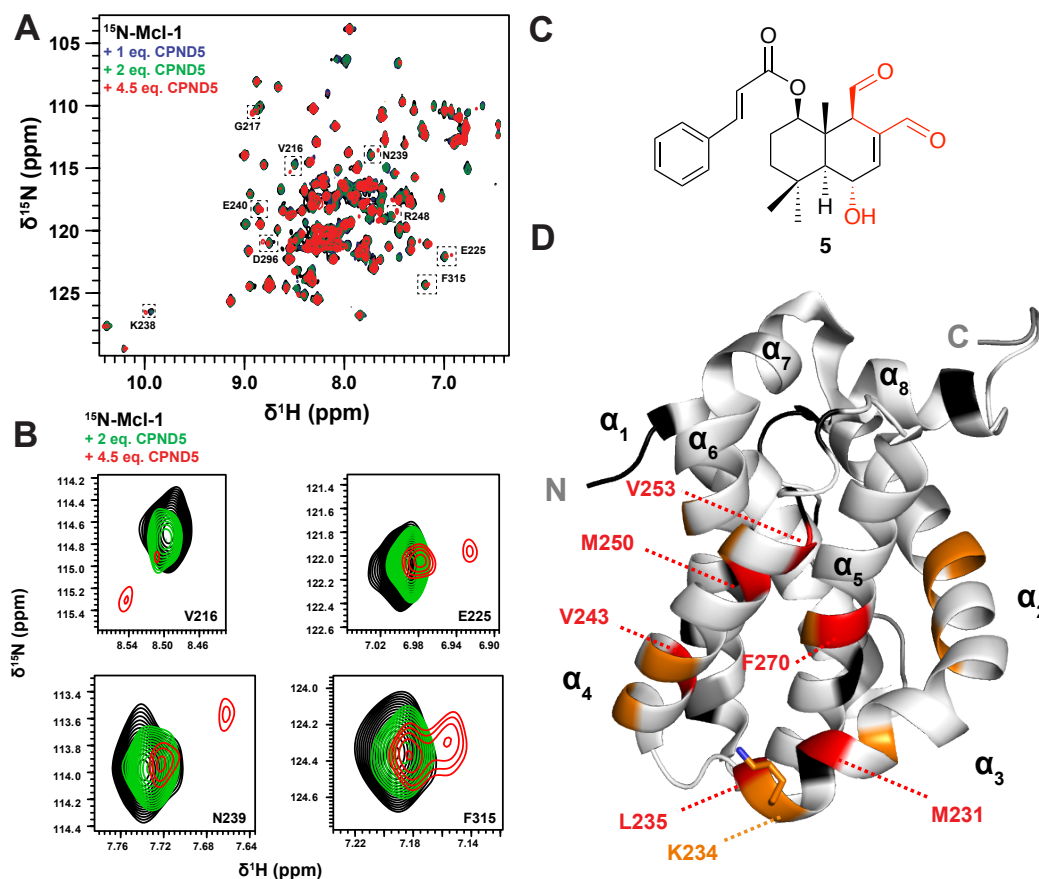


Figure 4. Compound **5** binds to human MCL-1 with micromolar affinity in the BH3 binding-groove. (A) Overlay of ^1H - ^{15}N SOFAST HMQC spectra of MCL-1 alone (50 μM) (black) and in presence of compound **5** at 1 eq. (red), 2 eq. (blue) and 4.5 eq. (green). We incubated samples during 1 hour (1eq., 2eq.) or overnight (4.5eq.) prior to measurement. The spectral regions where new cross-peaks were observed at the largest ligand concentration are highlighted (dashed black frames) and the closest neighboring residue ^1H - ^{15}N resonance is labelled for each frame; (B) Close-up view of several spectral regions for which new cross-peaks (black arrow) were observed upon increase of ligand concentration; (C) Mapping of combined ^1H - ^{15}N chemical shift perturbations on MCL-1 structure (pdb code: 2MHS): red > 0.1 ppm (M231, L235, V243, M250, V253, F270), 0.1 > orange > 0.05 ppm (V216, G217, K234, S245, I251, G271), grey < 0.005 ppm. Residues for which no data are available are highlighted (black). Those include prolines and others that could not be assigned to a ^1H - ^{15}N resonances in the spectrum of free MCL-1; (D) Compound **5**.

NMR experiments demonstrated that compound **5** and **7** bind human MCL-1 BH3 domain with good affinity. The same experiments performed with BCL-xL showed that compound **5** binds to BCL-xL in the BH3-binding groove of the protein with rather low affinity (Figure S2). Although several residues from the BH3-binding groove of the protein (α_2 , α_3 , α_4 and α_5) were affected upon ligand addition, the analysis of the bound BCL-xL spectrum reveals modest changes, with most spectral perturbations in rapid exchange regime. However, for a very limited number of residues (< 5), intermediate and slow exchange regime shifts could be

observed. BCL-xL was far (< 50%) from being saturated at the maximum used concentration of ligand (150 μ M) since the rapid exchange shifts are linear regarding the concentration of ligand along the whole titration. This indicated the apparent lower affinity of compound **5** for BCL-xL when compared to MCL-1.

A last experience was carried out to compare the relative affinity of compound **7** for MCL-1 and BCL-xL. The ^1H - ^{15}N SOFAST HMQC spectrum of free ^{15}N -labelled MCL-1 was compared with the ^1H - ^{15}N spectra of MCL-1 in presence of compound **7** (1 eq.) with or without BCL-xL (1 eq.). About 75 % of MCL-1 was bound to compound **7** in absence of BCL-xL as seen from the partial disappearing of resonances from residues in the BH3 binding groove of the protein (Figure S3). Upon addition of BCL-xL, no significant change was observed in the spectrum of ^{15}N -labelled MCL-1 in term of both ^1H - ^{15}N chemical shifts and intensity (Figure S3). This confirmed the selectivity of compound **7** for human MCL-1 compared to BCL-xL. Considering the relative concentration of the two proteins, this suggests that the relative affinity of compound **7** for BCL-xL could be at least 20 times lower compared to MCL-1 in this kind of experiments.

This last NMR experiment as well as the ^1H - ^{15}N HMQC spectrum of MCL-1 in presence of 4.5 eq. of compound **5** and intriguing FPA results, may also account for the ability of compounds **5** and **7** to form a covalent bond with MCL-1 or BCL-xL via a Paal-Knorr condensation (Figure 5).

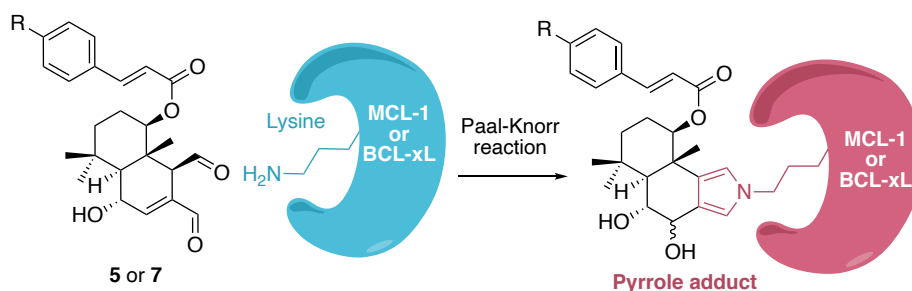
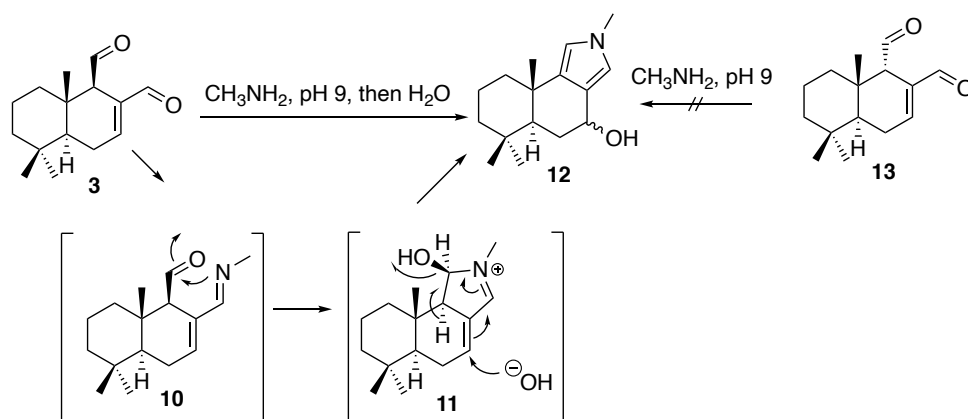


Figure 5. Suggested covalent interaction between compound **5** and MCL-1 protein via a Paal-Knorr rearrangement.

There are, indeed, few examples in the literature, of Paal-Knorr reactions between natural products and their target.^[39,40,41,42] Moreover, the activity of 9β -polygodial **3** on the taste sense has been linked, in the literature, to its ability to interact covalently with a lysine of the taste receptors by a modified Paal-Knorr condensation.^[43,44] The inactive 9α -isomer **13** was supposed to be unable of such reaction. So far, only ^1H NMR spectroscopic monitoring of **3** and **13** with methylamine and, later on, chemical reaction between **3** and 4-nitroaniline^[32,33] were able to support this hypothesis (Scheme 1). Indeed, newly formed pyrroles are most of the time unstable and not isolable.



Scheme 1. NMR spectroscopic monitoring between 9β- or 9α-polygodial **3** and **13** and methylamine to mimic the covalent interaction with a lysine of the protein of the taste sense.^[43,44] Sodano *et al.* have shown that only 9β-polygodial is able to react via a Paal-Knorr rearrangement, whereas 9α isomer is unreactive.

In our case, this assumption of a covalent interaction between 9□-5 and 9□-7 and MCL-1 and BCL-xL by a Paal-Knorr reaction is substantiated by the fact that both are much more potent on MCL-1 and BCL-xL than 9□-8. The short residue section (231-238) encompassing lysine 234 and lysine 238 is affected upon addition of compound **5** or compound **7** on MCL-1. Thus, these lysine residues probably contribute in ligand binding, which consequently suggests their side-chain may orient toward the ligand. Note that due to fast solvent exchange at neutral pH and at room temperature, the sidechain amino group of these lysines were not observable in the NMR 2D 1H -15N SOFAST-HMQC maps. Upon the formation of the adduct, the pyrrole nitrogen is 15N labelled. However, the poor sensitivity of 15N direct detection precluded further information regarding the binding mode of these compounds. To support this hypothesis, MALDI experiments were carried out on MCL-1 incubated or not with 0.5, 1 and 2 equivalents of compound **5** (Figures 6A and S4). After 2 h of incubation with 2 equivalents of **5**, more than 95% of the peak corresponding to native MCL-1 has disappeared in favour of a peak with a higher mass of about 378 Da. This difference in mass corresponds perfectly to the formation of a covalent adduct between compound **5** and a lysine *via* a Paal-Knorr rearrangement. A similar result was obtained with 2 equivalents of compound **7** (Figure S5A). On the contrary, the same experiment carried out with 2 equivalents of compound **8** did not show the formation of such covalent adduct (Figure 6B). This observation corroborates that of Sodano^[44] who has proposed that the ability to perform this rearrangement depends on the configuration of C9, due to the difference in distance between the two carbonyl functions. In our case, this information is crucial as **5** and **7** are 16 times more active on MCL-1 than compound **8**. We assume that this difference in affinity is due to the formation of a covalent bond between the two molecules and the protein. Likewise, MALDI experiments carried out on BCL-xL incubated with either **5** or **7** (2 eq.) showed the formation of covalent adducts but in less extent than with MCL-1 (*i.e.*, of the order of 50%), perfectly in accordance with FPA results (Figures 6C and S5B).

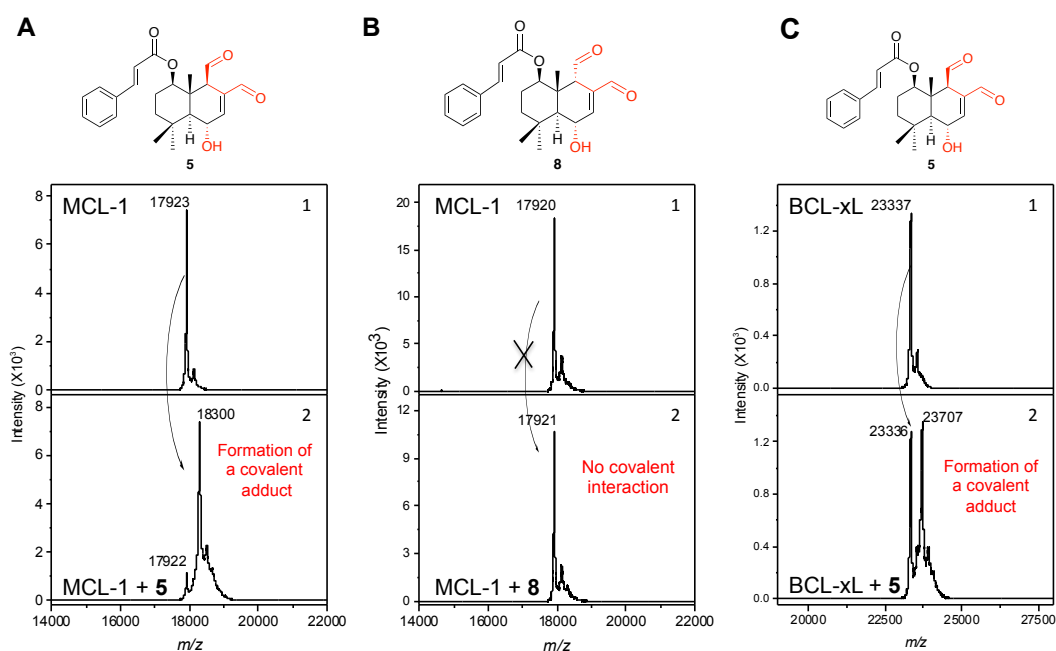
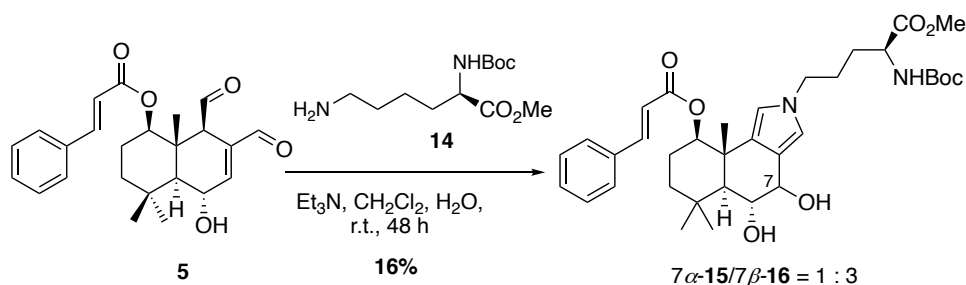


Figure 6. MALDI experiments of MCL-1 and BCL-xL incubated at 22 °C for 2 h. (A) 1- MCL-1 alone; 2- MCL-1 with 2 eq. of **5**; (B) 1- MCL-1 alone; 2- MCL-1 with 2 equivalents of compound **8**; (C) 1- BCL-xL alone; 2- BCL-xL with 2 eq. of **5**

To go further and characterize unambiguously the adduct thus formed, compound **5** was engaged in a reaction with protected lysine derivative **14** in wet dichloromethane at room temperature for 48 h (Scheme 2). ^1H NMR analysis of the crude mixture revealed the disappearance of protons at 9.35 and 9.75 ppm corresponding to the aldehyde functions, as well as the apparition of two singlets around 6.4 ppm, characteristic of the protons of a pyrrole ring. In the flask, reactions between **5** and lysine took much more time than within MCL-1. This suggests the protein plays a key role in orientating the aldehydes of **5** so that they interact correctly with the lysine in the BH3 groove. Gratifyingly, the Paal-Knorr products 7α -**15** and 7β -**16** could be isolated. They resulted from a rearrangement between **5** and lysine **14** followed by a subsequent regioselective addition of water on C7. The 1:3 selectivity in favor of compound 7β -**16** can be explained by the preferential addition of water on the less hindered side of the molecule *i.e.*, anti to the adjacent alcohol. Both were sensitive to acidic conditions and prone to degradation.



Scheme 2. Formation of Paal-Knorr pyrrole adducts 7α -**15** and 7β -**16** by reaction between compound **5** and lysine derivative **14**.

As it has been demonstrated that drimane derivatives are cytotoxic against myeloid and lymphoid cell lines,^[31] we intended to associate the cytotoxicity of compounds **5** and **7** to their affinity to anti-apoptotic members of BCL-2, and more particularly to MCL-1 as these proteins are known to play a central role in haematological malignancies tumorigenicity. *In vitro* tests were realized on 2 haematological cells lines whose dependences to anti-apoptotic members of BCL-2 family have been previously characterized.^[25] Thus, BL2, a Burkitt lymphoma cell line, is dependent on BCL-xL and MCL-1 for their survival whereas Remb1, a lymphoblastoid cell line (EBV-transformed normal B lymphocytes), is dependent on BCL-xL. First, cells were treated for 24 h with increasing concentrations of compounds **4** to **9** (0-50 μM) to determine the LC_{50} (lethal concentration 50%). Cell viability was evaluated by MTT assays (Table 2). Results obtained on both cell lines indicated that, apart **9**, which is non cytotoxic, only compounds **5** and **7** showed a real cytotoxic selectivity for BL2, the MCL-1/BCL-xL dependent cell line (4.0 and 3.8 μM respectively) over Remb1 ($\text{LC}_{50} > 15 \mu\text{M}$), the BCL-xL dependent cell line. The same selectivity was observed for S63845, an MCL-1 inhibitor. On the contrary, ABT 737 a BCL-2 and BCL-xL inhibitor was only cytotoxic for Remb1. As expected, the cytotoxicity associated to compounds **5** and **7** seems correlated to MCL-1 dependency.

Table 2. Cytotoxic activities of compounds **4-9** on haematological cancer cell lines.

Compd.	BL2 ^[a] IC ₅₀ (μM)	Remb1 ^[a] IC ₅₀ (μM)
4	5.5 \pm 1.1	5.6 \pm 1.5
5	4.0 \pm 0.7	>15
6	5.7 \pm 0.3	8.9 \pm 1.0
7	3.8 \pm 0.3	>15
8	2.3 \pm 0.1	5.3 \pm 0.9
9	>15	>15
S63845	0.13 \pm 0.01	>15
ABT-737	>15	0.9 \pm 0.03

[a] Dependencies: MCL-1/BCL-xL (BL2), BCL-xL (Remb1), [b] IC₅₀ measures the drug concentrations required for 50% cell death after 24 h. The values presented (mean \pm S.D.) are from three independent experiments.

For a better characterisation of the nature of the cytotoxicity observed, cells were then treated (or not) with 5 μM of each compound for 24 h. Apoptosis was measured by flow cytometry after labelling the cells with Annexin V-FITC and PI (Figure 7). Compounds **5** and **7** were shown to promote a strong induction of apoptosis on BL2 cell line (respectively 86% and 78% of AV⁺ cells), whereas they had a much weaker effect in Remb1 cell line (respectively 35% and 40% of AV⁺ cells).

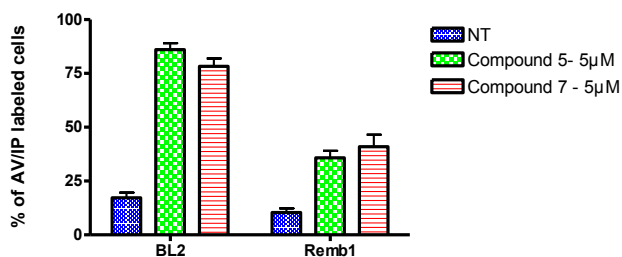


Figure 7. Apoptosis induction by compounds **5** and **7** on haematological cell lines. Cells were treated (or not) with 5 μ M of each compound for 24 h and apoptosis was determined by flow cytometry after labelling with annexin V-FITC and PI the proportion of cells in apoptosis was determined by flow cytometry after labelling with annexin V-FITC and PI. Dot plots representative of at least three independent experiments.

Conclusion

In summary, we have shown that drimane-type natural compounds **4-8** are inhibitors of proteins of the BCL-2 family. Among them, compounds **5** and **7** are the most potent molecules, with a sub-micromolar affinity for MCL-1. At cellular level, these two compounds are selective of BL2, a MCL-1/BCL-xL dependent cell line and induce apoptosis. A more detailed study regarding their mode of action, based on NMR and MALDI experiments revealed that compounds **5** and **7** are covalent inhibitors of MCL-1 and in a less extent of BCL-xL. This was confirmed by the synthesis of the corresponding Paal-Knorr adducts **15** and **16**. Although for many years, there were reluctances to develop covalent inhibitors of a target due to safety concerns, there has been recent renewed interest for such compounds.^[45,46] In fact, these inhibitors often present a strong affinity for their target, a prolonged effect in patients since they are not eliminated by the bloodstream and limit the risk of acquired resistance. The covalent bond between compounds **5** and **7** and MCL-1 is created thanks to a selective Paal-Knorr rearrangement. If Cimino *et al.* have hypothesised this rearrangement for the first time in 1984; we were able to confirm it indirectly thanks to the synthesis of compounds **15** and **16**. These drimane derivatives **5** and **7** are thus the first examples of covalent BH3-mimetics that target MCL-1.^[47] These two compounds can be isolated at multi-gram scale and could constitute good candidates for a new therapeutic strategy.

Experimental Section

General experimental details: All reagents and solvents were used as purchased from commercial suppliers. Purification by column chromatography on silica gel was performed using Merck Silica Gel 60 (70–230 mesh). ^1H and ^{13}C NMR spectra were recorded on Bruker ARX500 or Bruker AVANCE III 700 instruments using CDCl_3 or CD_3CN as internal reference. Chemical shifts (δ values) are given in parts per million (ppm), and multiplicity of signals are reported as follows: s, singlet; bs, broad singlet; d, doublet; t, triplet; q, quartet; dd, doublet of doublets; m, multiplet. HRMS analyses were performed using a Waters LCT Premier instrument by ElectroSpray Ionization (ESI). Protein-ligand NMR experiments were recorded on a Bruker 600 or 800 (Avance III) and 950 MHz (Avance III HD) spectrometers equipped with cryoprobes. A MALDI-TOF/TOF UltrafleXtreme mass spectrometer (Bruker Daltonics, Bremen) was used for all MALDI-TOF experiments. Mass spectra were

obtained in linear positive ion mode. The laser intensity was set just above the ion generation threshold to obtain peaks with the highest possible signal-to-noise (S/N) ratio without significant peak broadening. All data were processed using the FlexAnalysis software package (Bruker Daltonics). Sinapic acid (SA, used as the matrix for MALDI-TOF experiments, was of the highest grade available and used without further purification) was purchased from Sigma Aldrich.

Plant material: For compounds **4**, **5**, **6**, **8** and **9**: Trunk barks and leaves of *Z. pancheri* subsp. *pancheri* were collected in the rainforest of Nodela (South Province of New Caledonia) by one of us (V. D.). The corresponding voucher specimen (DUM-578) is kept at the Herbarium of New Caledonia (NOU). See ref [29]. For compound **7**: Trunk barks of *Zygodium baillonii* were collected in the high-altitude scrubland of "Monts Dzumac", South Province of New Caledonia, by one of us (M.L.). A voucher specimen (LIT-1275) is deposited in the Herbarium of New Caledonia (NOU).

Extraction and isolation: Extraction, isolation and characterisation of these compounds have already been described: see [29] for compounds **4**, **5**, **6**, **8** and **9**; see [28] and [48] for compound **7**.

Human MCL-1 production and purification: Plasmid pET15b (AmpR) encoding for the human MCL-1 deleted for the PEST domain and C-terminal transmembrane segment (MCL-1 sequence from 172 to 329) in fusion with an N-terminal histidine tag followed by a Maltose Binding Protein (MBP) segment was generously shared by L. Carlier (LBM, CNRS/Sorbonne-Universités) and incorporated into BL21 Rosetta pLysS (ChloR) using electroporation method. This biomass was made in a 1 L culture at 37 °C, to an optical density of 0.6-0.9 prior to induction. Induction of protein expression was carried out by adding isopropyl β-D-1-thiogalactopyranoside (IPTG) and lasted 18 h at 15 °C, and then the culture was finally harvested and the bacterial pellet containing the protein of interest was collected. After the bacterial lysis, a histidine affinity chromatography was performed (5 mL HisTrap FF crude, GE). The elution product from histidine-affinity chromatography was digested by prescission enzyme. The completion of protein digestion was checked through SDS-PAGE experiments. Once completed, the digestion product was concentrated with an adapted centrifugal filter (Amicon) and the His-TAG was removed by a histidine affinity chromatography column (5 mL HisTrap FF crude, GE). Finally, MCL-1 was purified by size-exclusion chromatography (SEC) with S200 column (buffer: 20 mM Tris pH 7, 300 mM NaCl).

BCL-xL, MCL-1 and BCL-2 binding affinity assays: The binding affinities of compounds for BCL-2, BCL-xL and MCL-1 were evaluated by competition against fluorescently Labelled reference compounds, Bim, Bak and Bid, respectively, as described by Qian *et al.*^[34] Human isoform BCL-2 (isoform 2), human 45-84/C37 BCL-xL and human 172-327 MCL-1 proteins were recombinantly produced at ICSN. Bim, FITC-Ahx-Bim-OH, Bak, FITC-Ahx-Bak-OH, Bid and FITC-Ahx-Bid-OH peptides were synthesized by GenePep Laboratories (Montpellier, France). Labelled and unlabelled peptides were diluted in assay buffer, which contained 20 μM Na₂HPO₄ (pH 7.4), 50 μM NaCl, 2 μM EDTA, 0.05% Pluronic F-68 (without pluronic acid for storage at -20 °C). Labelled BH3 peptide (15 nM of Bak and Bid, 3 nM of Bim), 100 nM protein, and 100 μM of unlabelled BH3 peptide or compound (first diluted in 10 mM DMSO and then buffer for final concentration from 10⁻⁹ to 10⁻⁴ M) into a final volume of 20 μL were distributed in a 384-well black flat-bottomed microplate (PS, Hibase, Small Volume, Med. Binding, VWR Garnier Bio-One 784076). The microplate was then incubated at room temperature for 2 and 6 h (except for BCL-2 not stable at RT for 6 h) and shaken before fluorescent polarization

was measured. Fluorescence polarization in millipolarization units was measured with a Beckman Coulter Paradigm® using FP cartridge (λ_{ex} 485 nm, λ_{em} 535 nm). The exposure time was 300 ms per channel. All experimental data were collected using the Biomek Software® (Beckman Coulter, Inc, Brea, CA, USA) and analyzed using Microsoft Excel 2010 (Microsoft, Redmond, WA, USA). Results are expressed as binding activity, *i.e.* percentage of inhibition of the binding of labelled reference compound, converted into K_i , the concentration corresponding to 50% of such inhibition, and corrected for experimental conditions according to Kenakin rearranged equation,^[49] which is adapted from Cheng and Prusoff equation.^[36] Unlabelled peptides Bim, Bak and Bid were used as positive controls. The performance of the assays was monitored by use of Z' factors as described by Zhang *et al.*^[50] The Z' factors for these assays were 0.85 and 0.8 for BCL-xL/Bak (at 2h and 6h respectively); 0.8 for MCL-1/Bid (at 2 and 6 h); 0.7 for BCL-2/Bim indicating the robustness of such assays.

NMR experiments: Spectroscopic measurements were performed in the NMR spectroscopy buffer (NaPO₄ 20 mM pH 7.4, 50 mM NaCl, 2 μ M EDTA, 2 mM TCEP, 3.5% DMSO, 95% / 5% H₂O / D₂O, 0.05% pluronic acid 10% + Roche anti-protease EDTA free) at 298 K. Reference experiments with both isolated, ¹⁵N-labelled MCL-1 (human) and BCL-xL (human) were carried out by using 50 μ M of protein in a 3 mm (200 μ L) NMR tube. For each titration point, the content of the NMR tube was added into a lyophilized aliquot of the desired compound. After vigorous resuspension of the ligand, the solution was transferred back in the NMR tube for measurement. Experiments for the assessment of ligand solubility were performed in the same manner, except that the reference experiment was protein-free and buffer-only. Combined ¹H-¹⁵N chemical shift perturbations were calculated as follows:

$$\Delta\delta_{\text{H,N}} = \sqrt{(\Delta\delta_{\text{H}})^2 + 0,14 * (\Delta\delta_{\text{N}})^2}$$

With:

$$\Delta\delta_{\text{X}} = \Delta\delta_{\text{X,bound}} - \Delta\delta_{\text{X,free}}$$

MALDI-TOF mass Spectrometry: Sample for MALDI analysis was prepared at a concentration of 50 μ M in a buffer (NaPO₄ 20 mM pH 7.4, 50 mM NaCl, 2 μ M EDTA, 3.5% DMSO, 0.05% pluronic acid 10% + Roche anti-protease EDTA free). The matrix solution was prepared at a concentration of 45 mM in H₂O/CH₃CN/TFA 1:1:0.1. The sample was prepared by mixing the sample solution with matrix solution at a volume ratio of 1:9.

Synthetic procedure and description of compounds 15 and 16: Dialdehyde compound **5** (61 mg, 0.15 mmol), lysP*HCl **14** (92 mg, 0.31 mmol, 2 eq.) and triethylamine (43 μ L, 0.31 mmol) were dissolved in wet dichloromethane. The reaction was stirred at room temperature for 48 h then concentrated *in vacuo*. The crude residue was purified by flash chromatography on silica gel using a gradient of heptane and ethyl acetate to give compound **7 α -15** (4 mg, 4%) and **7 β -16** (11 mg, 12%). **Compound 7 α -15:** ¹H NMR (700 MHz, CDCl₃): δ 7.74 (d, J = 16.0 Hz, 1H), 7.68 (m, 2H), 7.43 (m, 3H), 6.65 (d, J = 16.0 Hz, 1H), 6.54 (s, 1H), 6.26 (s, 1H), 5.46 (s, NH), 4.76 (dd, J = 4.1, 11.3 Hz, 1H), 4.53 (t, J = 4.4 Hz, 1H), 4.19 (td, J = 4.4, 9.9 Hz, 1H), 3.98 (m, 1H), 3.68 (t, J = 6.9 Hz, 2H), 3.61 (s, 3H), 2.99 (brs, 1H, OH), 2.75 (d, J = 10.7 Hz, 1H, OH), 1.82 (m, 3H), 1.77 (m, 1H), 1.58 (m, 3H), 1.49 (m, 1H), 1.46 (m, 1H), 1.38 (s, 9H), 1.37 (s, 3H), 1.19 (s, 3H), 1.19 (m, 2H), 1.17 (s, 3H), ppm; ¹³C NMR (175 MHz, CDCl₃): δ 174.2, 167.4, 145.5, 135.7, 131.5, 131.0, 130.0 (2C), 129.3 (2C),

120.7, 120.0, 119.1, 115.0, 82.1, 80.2, 71.1, 67.0, 54.6, 52.6, 50.9, 50.0, 42.3, 41.8, 36.1, 33.9, 32.0, 31.6, 28.5 (3C), 25.8, 23.8, 22.5, 20.9 ppm; HRMS (ESI)*: *m/z* calcd. for C₃₆H₅₀N₂NaO₈ [M-OH+OMe+Na]⁺ 661.3465; found 661.3451. **Compound 7β–16**: ¹H NMR (700 MHz, CDCl₃): δ 7.74 (d, J = 16.0 Hz, 1H), 7.68 (m, 2H), 7.44 (m, 3H), 6.66 (d, J = 16.0 Hz, 1H), 6.46 (s, 1H), 6.26 (s, 1H), 5.49 (s, NH), 4.71 (dd, J = 4.1, 11.4 Hz, 1H), 4.35 (d, J = 7.8 Hz, 1H), 4.00 (m, 1H), 3.97 (m, 1H), 3.66 (brs, 2H), 3.62 (s, 3H), 3.14 (OH), 2.93 (OH), 1.83 (m, 1H), 1.76 (m, 1H), 1.58 (m, 4H), 1.48 (m, 1H), 1.44 (s, 3H), 1.41 (m, 1H), 1.38 (s, 9H), 1.21 (s, 3H), 1.19 (m, 2H), 1.16 (s, 3H) ppm; ¹³C NMR (175 MHz, CDCl₃): δ 173.8, 167.0, 145.6, 135.7, 131.5, 131.2, 130.0 (2C), 129.3 (2C), 122.0, 120.3, 117.0, 115.5, 82.2, 80.4, 75.6, 75.5, 55.4, 54.7, 52.7, 49.8, 42.3, 40.7, 36.1, 34.2, 32.1, 31.6, 28.7 (3C), 25.7, 23.7, 22.5, 21.7 ppm; HRMS (ESI)*: *m/z* calcd. for C₃₆H₅₀N₂NaO₈ [M-OH+OMe+Na]⁺ 661.3465; found 661.3488. * Mass experiments were performed in MeOH

Cell lines: BL2 and Remb1 cell lines were kindly provided by the International Agency for Research on Cancer (IARC, Lyon). All cell lines were cultured in RPMI 1640 medium (Invitrogen) containing 2 mM L-glutamine, 1 mM sodium pyruvate, 20 mM glucose, 100 U/mL penicillin and 100 µg/mL streptomycin and supplemented with 10% heat-inactivated fetal calf serum (complete RPMI medium). Cell lines were maintained at 37 °C in a humidified atmosphere containing 5% CO₂.

MTT assay: Cell viability was measured by the MTT (3-(4, 5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide) assay. 7 x 10⁴ cells (in triplicate) were incubated for 24 h at 37 °C with various concentrations (0/0.625/1.25/2.5/10/25/50 µM) of both compounds in 100 µL of complete RPMI medium. Then, 20 µL of MTT reagent (final concentration 1 mg/mL) was added and plates were incubated for 2h. Finally, 100 µL of lysis buffer (30% SDS, 33% dimethylformamide, 2% glacial acetic acid, 0.025 N HCl, pH = 4.7) was added and absorbance was recorded at 570 nm after an overnight incubation. The LC₅₀ values were calculated after plotting the dose-response curves using the GraphPad Prism software.

Quantification of apoptosis: Apoptosis was assessed by labelling cells with annexin-V-FITC and propidium iodide (PI). 3 x 10⁵ cells were incubated for 24 h at 37 °C with 5 µM of each compound in complete RPMI medium. Cells were then washed in PBS, resuspended in annexin buffer (10 mM HEPES/NaOH, pH = 7.4, 150 mM NaCl, 5 mM KCl, 1 mM MgCl₂, 1.8 mM CaCl₂) containing 2.5 µg/mL annexin V-FITC (eBioscience) and incubated for 5–10 min. Then, 100 µL of an annexin buffer supplemented with PI (10 µg/mL; Sigma-Aldrich) was added. Analysis (n = 10 000) was performed by flow cytometry (Accuri C6 cytometer, Becton-Dickinson). Annexin V-positive cells (PI negative and positive) were considered as apoptotic cells.

Acknowledgements

The authors are very grateful to North and South Provinces of New Caledonia, which facilitated our field investigation. The authors warmly thank L. Carlier (LBM, Jussieu) for the gift of MCL-1 plasmid and J.- F. Gallard (ICSN) who performed NMR experiments on the 700 MHz apparatus. This work has been supported by University Paris-Saclay through a PhD fellowship to F. D. and the “IDI 2016” project ANR-11-IDEX-0003-02 (PhD fellowship to F.M.). This work has also benefited from an “Investissement d’Avenir” grant managed by Agence Nationale de la Recherche (CEBA, ref ANR-10-LABX-25-01) and from the IR-RMN-THC FR 3050 CNRS.

Keywords: Covalent inhibitors • BCL-2 proteins • Protein-protein interaction • Cancer • Natural compounds

- [1] A. Strasser, S. Cory, J. M. Adams, *EMBO J.* **2011**, *30*, 3667-3683.
- [2] P. E. Czabotar, G. Lessene, A. Strasser, J. M. Adams, *Nat. Rev. Mol. Cell Biol.* **2014**, *15*, 49-63.
- [3] S. N. Willis, J. M. Adams, *Curr. Opin. Cell Biol.* **2005**, *17*, 617-625.
- [4] J. Montero, A. Letai, *Cell Death Differ.* **2018**, *25*, 56-64.
- [5] P. Gimenez-Bonafe, A. Tortosa, R. Perez-Tomas, *Curr. Cancer Drug Targets* **2009**, *9*, 320-340.
- [6] J.C. Reed, *Blood* **2008**, *111*, 3322-3330.
- [7] W.J. Placzek, J. Wei, S. Kitada, D. Zhai, J.C. Reed, M. Pellecchia, *Cell Death Dis.* **2010**, *1*, e40.
- [8] A. H. Wei, A. W. Roberts, A. Spencer, A. S. Rosenberg, D. Siegel, R. B. Walter, S. Caenepeel, P. Hughes, Z. McIver, K. Mezzi, P. K. Morrow, A. Stein, *Blood Rev.* **2020**, 100672, 1-12.
- [9] a) R. Beroukhim, C. H. Mermel, D. Porter, G. Wei, S. Raychaudhuri, J. Donovan, J. Barretina, J. S. Boehm, J. Dobson, M. Urashima, K. T. Mc Henry, R. M. Pinchback, A. H. Ligon, Y.-J. Cho, L. Haery, H. Greulich, M. Reich, W. Winckler, M. S. Lawrence, B. A. Weir, K. E. Tanaka, D. Y. Chiang, A. J. Bass, A. Loo, C. Hoffman, J. Prensner, T. Liefeld, Q. Gao, D. Yecies, S. Signoretti, E. Maher, F. J. Kaye, H. Sasaki, J. E. Tepper, J. A. Fletcher, J. Taberner, J. Baselga, M.-S. Tsao, F. Demichelis, M. A. Rubin, P. A. Janne, M. J. Daly, C. Nucera, R. L. Levine, B. L. Ebert, S. Gabriel, A. K. Rustgi, C. R. Antonescu, M. Ladanyi, A. Letai, L. A. Garraway, M. Loda, D. G. Beer, L. D. True, A., Okamoto, S. L. Pomeroy, S. Singer, T. R. Golub, E. S. Lander, G. Getz, W. R. Sellers, M. Meyerson, *Nature* **2010**, *463*, 899-906.
- [10] T. I. Zack, S. E. Schumacher, S. L. Carter, A. D. Cherniack, G. Saksena, B. Tabak, M. S. Lawrence, C.-Z. Zhang, J. Wala, C. H. Mermel, C. Sougnez, S. B. Gabriel, B. Hernandez, H. Shen, P. W. Laird, G. Getz, M. Meyerson, R. Beroukhim, *Nat. Genet.* **2013**, *45*, 1134-11140.
- [11] K. J. Campbell, M. L. Bath, M. L. Turner, C. J. Vandenberg, P. Bouillet, D. Metcalf, C. L. Scott, S. Cory, *Blood* **2010**, *116*, 3197-3207.
- [12] M. S. Davids, A. Letai, *J. Clin. Oncol.* **2012**, *30*, 3127-3135.
- [13] S. D'Aguanno, D. Del Bufalo, *Cells* **2020**, *9*, 1287, 1-25.
- [14] A. Kotschy, Z. Szlavik, J. Murray, J. Davidson, A. L. Maragno, G. Le Toumelin-Braizat, M. Chanrion, G. L. Kelly, J.-N. Gong, D. M. Moujalled, A. Bruno, M. Csekei, A. Paczal, Z. B. Szabo, S. Sipos, G. Radics, A. Proszenyak, B. Balint, L. Ondi, G. Blasko, A. Robertson, A. Surgenor, P. Dokurno, I. Chen, N. Matassova, J. Smith, C. Pedder, C. Graham, A. Studeny, G. Lysiak-Auvity, A.-M. Girard, F. Gravé, D. Segal, C. D. Riffkin, G. Pomilio, L. C. A. Galbraith, B. J. Aubrey, M. S. Brennan, M. J. Herold, C. Chang, G. Guasconi, N. Cauquil, F. Melchiorre, N. Guigal-Stephan, B. Lockhart, F. Colland, J. A. Hickman, A. W. Roberts, D. C. S. Huang, A. H. Wei, A. Strasser, G. Lessene, O. Geneste, *Nature* **2016**, *538*, 477-482.
- [15] Z. Szlavik, M. Csekei, A. Paczal, Z. B. Szabo, S. Sipos, G. Radics, A. Proszenyak, B. Balint, J. Murray, J. Davidson, I. Chen, P. Dokurno, A. E. Surgenor, Z. M. Daniels, R. E. Hubbard, G. Le Toumelin-Braizat, A. Claperon, G. Lysiak- Auvity, A.-M. Girard, A. Bruno, M. Chanrion, F. Colland, A.-L. Maragno, D. Demarles, O. Geneste, A. Kotschy *J. Med. Chem.* **2020**, *63*, 13762-13795.

- [16] S. Caenepeel, S. P. Brown, B. Belmontes, G. Moody, K. S. Keegan, D. Chui, D. A. Whittington, X. Huang, L. Poppe, A. C. Cheng, M. Cardozo, J. Houze, Y. Li, B. Lucas, N. A. Paras, X. Wang, J. P. Taygerly, M. Vimolratana, M. Zancanella, L. Zhu, E. Cajulis, T. Osgood, J. Sun, L. Damon, R. K. Egan, P. Greninger, J. D. McClanaghan, J. Gong, D. Moujalled, G. Pomilio, P. Beltran, C. H. Benes, A.W. Roberts, D. C. S. Huang, A. Wei, J. Canon, A. Coxon, P. E. Hughes, *Cancer Discov.* **2018** 1582-1597.
- [17] A. E. Tron, M. A. Belmonte, A. Adam, B. M. Aquila, L. H. Boise, E. Chiarparin, J. Cidado, K. J. Embrey, E. Gangl, F. D Gibbons, G. P. Gregory, D. Hargreaves, J. A. Hendricks, J. W. Johannes, R. W. Johnstone, S. L. Kazmirski, J. G. Kettle, M. L. Lamb, S. M. Matulis, A. K. Nooka, M. J. Packer, B. Peng, P. B. Rawlins, D. W. Robbins, A. G. Schuller, N. Su, W. Yang, Q. Ye, X. Zheng, J.P. Secrist, E.A. Clark, D.M. Wilson, S.E. Fawell, A.W. Hird, *Nat. Commun.* **2018**, 9, 5341, 1-14.
- [18] C. Kieffer, J. -P. Jourdan, M. Jouanne, A.-S. Voisin-Chiret, *Drug Discov. Today* **2020**, 25, 1592-1603.
- [19] M. Litaudon, H. Bousserouel, K. Awang, O. Nosjean, M.-T. Martin, M. E. Tran Huu Dau, H. A. Hadi, J. A. Boutin, T. Sévenet, F. Guéritte, *J. Nat. Prod.* **2009**, 72, 480-483.
- [20] C. Apel, C. Geny, V. Dumontet, N. Birlirakis, F. Roussi, V. C. Pham, D. T. M. Huong, V. H. Nguyen, V. M. Chau, M. Litaudon. *J. Nat. Prod.* **2014**, 77, 1430-1437.
- [21] C. Gény, G. Rivière, J. Bignon, N. Birlirakis, E. Guittet, K. Awang, M. Litaudon, F. Roussi, V. Dumontet, *J. Nat. Prod.* **2016**, 79, 838-844.
- [22] D. Fomekong Fotsop, F. Roussi, A. Leverrier, A. Bretéché, F. Guéritte, *J. Org. Chem.* **2010**, 75, 7412-7415.
- [23] S. Desrat, A. Pujals, C. Colas, J. Dardenne, C. Geny, L. Favre, V. Dumontet, B. Iorga, M. Litaudon, M. Raphaël, J. Wiels, F. Roussi, *Bioorg. Med. Chem. Lett.* **2014**, 24, 5086-5088.
- [24] S. Desrat, C. Remeur, C. Geny, G. Rivière, C. Colas, V. Dumontet, N. Birlirakis, B. Iorga, F. Roussi, *Chem Comm.* **2014**, 50, 8593-8596.
- [25] A. Abou Samra, A. Robert, C. Gov, L. Favre, L. Eloy, E. Jacquet, J. Bignon, J. Wiels, S. Desrat, F. Roussi, *Eur. J. Med. Chem.* **2018**, 148, 26-38.
- [26] S. Gapil Tiamas, F. Audet, A. Abou Samra, J. Bignon, M. Litaudon, C. Fourneau, A. Ariffin, K. Awang, S. Desrat, F. Roussi, *Eur. J. Org. Chem.* **2018**, 42, 5830-5835.
- [27] S. Gapil Tiamas, F. Daressy, A. Abou Samra, J. Bignon, V. Steinmetz, M. Litaudon, C. Fourneau, K. Hoong Leong, A. Ariffin, K. Awang, S. Desrat, F. Roussi, *Bioorg Med Chem Lett.* **2020**, 30, 127003-127006.
- [28] D. Fomekong-Fotsop, F. Roussi, C. Le Callonec, Hadjira Bousserouel, M. Litaudon, F. Guéritte, *Tetrahedron* **2008**, 64, 2192-2197 .
- [29] N. Allouche, C. Apel, M.-T. Martin, V. Dumontet, F. Guéritte, M. Litaudon, *Phytochem.* **2009**, 70, 546-553.
- [30] B. M. J. Jansen, A. de Groot, *Nat. Prod. Rep.* **2004**, 21, 449-477.

- [31] E. Fratoni, A. E. de Athayde, M. da Silva Machado, T. Zermiani, I. Venturi, M. Corêa dos Santos, F. Lobato, V. C. Filho, G. C. Franchi Jr., A. E. Nowill, J. R. Santin, A. Malheiros, *Biomed. Pharmacother.* **2018**, *103*, 1498-1506.
- [32] R. Dasari, A. De Carvalho, D. C. Medellin, K. N. Middleton, F. Hague, M. N. M. Volmar, L.V. Frolova, M. F. Rossato, J. J. De La Chapa, N. F. Dybdal-Hargreaves, A. Pillai, R. E. Kälin, V. Mathieu, S. Rogelj, C. B. Gonzales, J. B. Calixto, A. Evidente, M. Gautier, G. Munirathinam, R. Glass, P. Burth, S. C. Pelly, W. A. L. van Otterlo, R. Kiss, A. Kornienko, *Eur. J. Med. Chem.* **2015**, *103*, 226-237.
- [33] R. Dasari, A. De Carvalho, D. C. Medellin, K. N. Middleton, F. Hague, M. N. M. Volmar, L.V. Frolova, M. F. Rossato, J. J. De La Chapa, N. F. Dybdal-Hargreaves, A. Pillai, V. Mathieu, S. Rogelj, C. B. Gonzales, J. B. Calixto, A. Evidente, M. Gautier, G. Munirathinam, R. Glass, P. Burth, S. C. Pelly, W. A. L. van Otterlo, R. Kiss, A. Kornienko, *ChemMedChem.* **2015**, *10*, 2014-2026.
- [34] J. Qian, M. J. Voorbach, J. R. Huth, M. L. Coen, H. Zhang, S.-C. Ng, K. M. Comess, A. M. Petros, S. H. Rosenberg, U. Warrior, D. J. Burns, *Anal. Biochem.* **2004**, *328*, 131-138.
- [35] T. Oltersdorf, S. W. Elmore, A. R. Shoemaker, R. C. Armstrong, D. J. Augeri, B. A. Belli, M. Bruncko, T. L. Deckwerth, J. Dinges, P. J. Hajduk, M. K. Joseph, S. Kitada, S. J. Korsmeyer, A. R. Kunzer, A. Letai, C. Li, M. J. Mitten, D. G. Nettesheim, S. Ng, P. M. Nimmer, J. M. O'Connor, A. Oleksijew, A. M. Petros, J. C. Reed, W. Shen, S. K. Tahir, C. B. Thompson, K. J. Tomaselli, B. Wang, M. D. Wendt, H. Zhang, S. W. Fesik, S. H. Rosenberg, *Nature* **2005**, *435*, 677-681.
- [36] C. Yung-Chi, W.H. Prusoff, *Biochem. Pharmacol.* **1973**, *22*, 3099-3108.
- [37] C. Tse, A. R. Shoemaker, J. Adickes, M. G. Anderson, J. Chen, S. Jin, E. F. Johnson, K. C. Marsh, M. J. Mitten, P. Nimmer, L. Roberts, S. K. Tahir, Y. Xiao, X. Yang, H. Zhang, S. Fesik, S. H. Rosenberg, S. W. Elmore, *Cancer Res.* **2008**, *68*, 3421-3428.
- [38] Q. Liu, T. Moldoveanu, T. Sprules, E. Matta-Camacho, N. Mansur-Azzam, K. Gehring, *J. Biol. Chem.* **2010**, *285*, 19615-19624.
- [39] A. Kornienko, J. J. La Clair, *Nat. Prod. Rep.* **2017**, *34*, 1051-1060.
- [40] M. Bury, A. Girault, V. Mégalizzi, S. Spiegl-Kreinecker, V. Mathieu, W. Berger, A. Evidente, A. Kornienko, P. Gailly, C. Vandier, R. Kiss, *Cell Death Dis.* **2013**, *4*, e561
- [41] C. C. Rodrigues, M. d. L. A. Vieira, C. A. Rosa, L. H. Rosa, C. L. Cantrell, D. E. Wedge, T. M. A. Alves, C. L. Zani, R. S. Pimenta, J. P. A. Sales, S. M. F. Murta, A. J. Romanha, *Nat. Prod. Res.* **2016**, *30*, 478-481.
- [42] R. Dasari, M. Masi, R. Lisy, M. Ferdérin, L. R. English, A. Cimmino, V. Mathieu, A. J. Brenner, J. G. Kuhn, S. T. Whitten, A. Evidente, R. Kiss, A. Kornienko, *Bioorg. Med. Chem. Lett.* **2015**, *25*, 4544-4548.
- [43] G. Cimino, A. Spinella, G. Sodano, *Tetrahedron Lett.* **1984**, *25*, 4151-4152.
- [44] G. Cimino, G. Sodano, A. Spinella, *Tetrahedron* **1987**, *43*, 5401-5410.
- [45] J. Singh, R.C. Petter, T.A. Baillie, A. Whitty, *Nat. Rev. Drug Discov.* **2011**, *10*, 307-317.
- [46] A.K. Ghosh, I. Samanta, A. Mondal, W.R. Liu, *ChemMedChem* **2019**, *14*, 889-906.

- [47] S. Lee, T. E. Wales, S. Escudero, D. T. Cohen, J. Luccarelli, C. G. Gallagher, N. A. Cohen, A. J. Huhn, G. H. Bird, J. R. Engen, L. D. Walensky, *Nat. Struct. Mol. Biol.* **2016**, 23, 600-607.
- [48] L. Larsen, S. D. Lorimer, N. B. Perry, *Biochem. Syst. Ecol.* **2007**, 35, 286-292.
- [49] Z. W. Nikolovska-Coleska, R. Wang, X. Fang, H. Pan, Y. Tomita, P. Li, P. P. Roller, K. Krajewski, N. G. Saito, J. A. Stuckey, S. Wang, *Anal Biochem.* **2004**, 332, 261-273.
- [50] J. H. Zhang, T. D. Y. Chung, K. R. Oldenburg, *J. Biomol. Screen* **1999**, 4, 67-73.