

Online Research @ Cardiff

This is an Open Access document downloaded from ORCA, Cardiff University's institutional repository: <http://orca.cf.ac.uk/98328/>

This is the author's version of a work that was submitted to / accepted for publication.

Citation for final published version:

Hamdy, Rania, Ziedan, Noha, Ali, Samina, Bordoni, Cinzia, El-Sadek, Mohamed, Lashin, Elsaid, Brancale, Andrea, Jones, Arwyn Tomos and Westwell, Andrew D 2017. Synthesis and evaluation of 5-(1H-indol-3-yl)-N-aryl-1,3,4-oxadiazol-2-amines as Bcl-2 inhibitory anticancer agents. *Bioorganic & Medicinal Chemistry Letters* 27 (4) , pp. 1037-1040. 10.1016/j.bmcl.2016.12.061 file

Publishers page: <http://dx.doi.org/10.1016/j.bmcl.2016.12.061>
<<http://dx.doi.org/10.1016/j.bmcl.2016.12.061>>

Please note:

Changes made as a result of publishing processes such as copy-editing, formatting and page numbers may not be reflected in this version. For the definitive version of this publication, please refer to the published source. You are advised to consult the publisher's version if you wish to cite this paper.

This version is being made available in accordance with publisher policies. See <http://orca.cf.ac.uk/policies.html> for usage policies. Copyright and moral rights for publications made available in ORCA are retained by the copyright holders.



Graphical Abstract

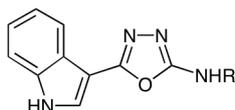
To create your abstract, type over the instructions in the template box below.
Fonts or abstract dimensions should not be changed or altered.

Synthesis and evaluation of 5-(indol-3-yl)-*N*-aryl-

Leave this area blank for abstract info.

1,3,4-oxadiazol-2-amines as Bcl-2 inhibitory anticancer agents

Rania Hamdy, Noha Ziedan, Samia Ali, Cinzia Bordoni, Mohamed El-Sadek, Elsaid Lashin, Andrea Brancale, Arwyn T. Jones and Andrew D. Westwell



8a: R=2-NO₂Ph; Bcl-2 IC₅₀ = 1.24 μM (ELISA)
8e: R=3-Cl-Ph; Bcl-2 IC₅₀ = 0.66 μM (ELISA)

Synthesis and evaluation of 5-(1*H*-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines as Bcl-2 inhibitory anticancer agents

Rania Hamdy^{a,b}, Noha Ziedan^{a,b}, Samia Ali^{a,c}, Cinzia Bordoni^a, Mohamed El-Sadek^b, Elsaid Lashin^b, Andrea Brancale^a, Arwyn T. Jones^a and Andrew D. Westwell^a

^aSchool of Pharmacy and Pharmaceutical Sciences, Cardiff University, Redwood Building, King Edward VII Avenue, Cardiff, CF10 3NB, Wales, U.K.

^bFaculty of Pharmacy, Zagazig University, Egypt

^cNational Research Centre, Cairo, Egypt

ARTICLE INFO

Article history:

Received

Revised

Accepted

Available online

Keywords:

Anti-apoptotic Bcl-2

Indoles

Oxadiazoles

Anticancer

Drug discovery

ABSTRACT

A series of 5-(1*H*-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines **8a-j** has been designed, synthesized and tested *in vitro* as potential pro-apoptotic Bcl-2-inhibitory anticancer agents based on our previous lead compound **8a**. Synthesis of the target compounds was readily accomplished through a cyclisation reaction between indole-3-carboxylic acid hydrazide (**5**) and substituted isothiocyanates **6a-j**, followed by oxidative cyclodesulfurization of the corresponding thiosemicarbazide **7a-j** using 1,3-dibromo-5,5-dimethylhydantoin. Active compounds of the series **8a-j** were found to have sub-micromolar IC₅₀ values selectively in Bcl-2 expressing human cancer cell lines; notably the 2-nitrophenyl analogue **8a** was found to exhibit potent activity, and compounds **8a** and **8e** possessed comparable Bcl-2 binding affinity (ELISA assay) to the established natural product-based Bcl-2 inhibitor, gossypol. Molecular modeling studies helped to further rationalise anti-apoptotic Bcl-2 binding, and identified compounds **8a** and **8e** as candidates for further development as Bcl-2 inhibitory anticancer agents.

Resistance to apoptosis is well documented as one of the hallmarks of cancer,¹ and the Bcl-2 family of proteins play a critical role in regulating the intrinsic apoptosis process in response to apoptotic stimuli. The structurally related Bcl-2 protein family is structurally and functionally classified as either anti-apoptotic (e.g. Bcl-2, Bcl-x_L, Bcl-w, Mcl-1); pro-apoptotic (e.g. Bak, Bax, Bcl-rambo); or as pro-apoptotic BH3 domain only proteins (e.g. Bad, BID, Bim, Noxa) that bind and regulate anti-apoptotic Bcl-2 proteins. The carefully regulated balance between pro- and anti-apoptotic protein family members is crucial in dictating cellular survival or commitment to apoptosis.²

In terms of development of new cancer therapeutics, small molecule anti-apoptotic Bcl-2 inhibitors offer exciting prospects.³ Following Bcl-2 inhibition, the resulting cellular mitochondrial membrane permeabilisation and release of pro-apoptotic factors commits the cell to apoptosis and subsequent removal. Resistance to apoptotic stimuli is commonly induced by over-expression of anti-apoptotic Bcl-2 proteins that are also known to contribute to therapy failure and subsequent generation of a more resistant cancer phenotype.²

The exciting potential of small molecule anti-apoptotic Bcl-2 inhibitors in cancer therapy has led to a number of advances in this area,^{3,4} focusing on the design of new cell permeable small molecule inhibitors that block sites of protein-protein interaction⁵

between anti-apoptotic Bcl-2 family proteins and pro-apoptotic partners. Amongst the most advanced and well-documented examples of Bcl-2 inhibitors that have progressed to clinical evaluation are the orally bioavailable navitoclax (ABT-263, **1**), obatoclax (**2**) and the polyphenolic natural product-based experimental drug gossypol (**3**), shown in Figure 1. Perhaps most significantly, the Bcl-2 inhibitor venetoclax (ABT-199, **4**) has been granted breakthrough designation by the FDA for treatment of patients with relapsed or refractory chronic lymphocytic leukaemia (CLL) who have the 17p chromosomal deletion.⁶

One notable feature of the first generation of anti-apoptotic Bcl-2 family inhibitors is their large size (M_r = 975 in the case of navitoclax) and chemical complexity, in part arising from the perceived requirement to make multiple drug binding interactions across a substantial protein-protein interaction interface. The development of smaller, less complex molecules that retain selective Bcl-2/Bcl-x_L inhibition and antitumour activity is an ongoing challenge. The pro-apoptotic Bcl-2 inhibitory compound obatoclax mesylate⁷ suggests that structurally simple heterocyclic compounds may possess the necessary characteristics for selective Bcl-2 inhibitory antitumour properties. Previous work within our group has identified pro-apoptotic Bcl-2 inhibitory compounds of the indolyl-oxadiazole,⁸ isoxazole⁹ and indolyl-triazole¹⁰ classes; in this paper we report the discovery and early

evaluation of a new series of Bcl-2 inhibitory 5-(1*H*-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines.

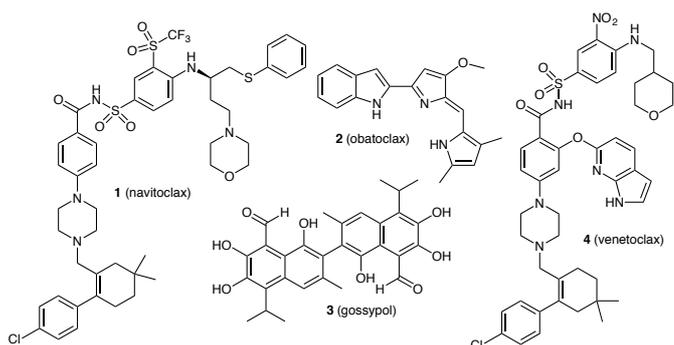
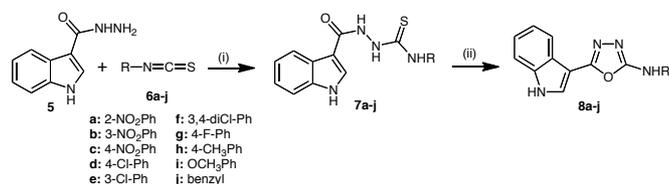


Figure 1. Examples of small molecule Bcl-2 inhibitors.

The lead compound for this study (**8a**) was obtained as a virtual screening hit of the ZINC database against a Bcl-2 pharmacophore model, using methodology previously described.¹¹ Structure activity relationships were studied by variation of the phenyl/benzyl group substituents that bind within a hydrophobic groove of our model, as shown in Scheme 1.



Reagents and conditions: (i) EtOH, reflux; (ii) 1,3-dibromo-5,5-dimethylhydantoin, KI, *i*-PrOH/CH₃CN (aq), 50°C

Scheme 1: Synthesis of indolyl-*N*-aryl-oxadiazolamines.

Synthesis of the target compounds was readily achieved in two synthetic steps, through adaptation of a literature route.^{12,13} The first step involved heating indole-3-carbonylhydrazide (**5**) with substituted phenyl/benzyl isothiocyanates (**6a-j**) in refluxing ethanol to produce the intermediate thiosemicarbazide (**7a-j**) that was used in the next step without further purification.¹⁴ Treatment of the indolyl-3-carbonyl-*N*-phenyl/benzyl thiosemicarbazide (**7a-j**) with KI and 1,3-dibromo-5,5-dimethylhydantoin in basic solution afforded the product 5-(1*H*-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines (**8a-j**) in high yield (58-86%) following recrystallisation from ethanol.^{15,16}

Evaluation of the newly synthesized indolyl-oxadiazolamines was carried out across four human cancer cell lines. Viability in the established triple-negative breast cancer cell line MDA-MB-231 and cervical cancer (HeLa) cells (both Bcl-2 expressing) was assessed using the MTT endpoint assay. To test the effect of differing Bcl-2 status, further evaluation of new compounds was carried out in the human cancer cell lines KG1a (acute myelogenous leukaemia) and Jurkat (T cell leukaemia). The Jurkat cell line has previously been characterized as Bcl-2 negative by our group¹⁷ and others,¹⁸ unlike the Bcl-2 expressing KG1a cell line.¹⁹ For studies on the leukaemic cells we used the CellTiter-Blue® viability assay, appropriate for endpoint determinations in these non-adherent cells. The results, expressed as mean values following testing on at least three separate occasions, are presented in Table 1.

The results shown in Table 1 indicated that in general the HeLa cell line was the most sensitive to the effects of test compounds with IC₅₀ values in the sub- to low-micromolar range

of 0.3 – 19 μM (with the exception of inactive compound **8g**). The MDA-MB-231 breast cancer cell line also gave sub- to low-micromolar IC₅₀ values for the majority of new compounds. The observation of sub- to low-micromolar inhibitory activity for the majority of compounds in the Bcl-2 expressing KG1a leukaemic cell line, but not in the Bcl-2 negative T-cell leukaemia Jurkat cells, suggests that Bcl-2 may play a role in mediating the observed anticancer activity.

The most active compound overall was the 2-nitrophenyl derivative **8a** that shows potent (sub-micromolar) IC₅₀ values across the three Bcl2-expressing human cancer cell lines (MDA-MB-231, HeLa and KG1a) with less potent inhibitory activity in the Bcl-2 negative Jurkat cells. The most active and selective compounds from the cell line assay (**8a**, **8c** and **8e**), along with moderately active compounds **8h** and **8i** were chosen for more detailed mechanistic study using the enzyme-linked immunosorbent (ELISA) sandwich binding assay along with gossypol as positive control.

The ELISA assay was used to determine the ability of the test compounds to compete with immobilized Bim peptide for binding to His-tagged Bcl-2 protein, according to our previously described protocol^{10,19} (see Supplementary Information). The ELISA binding results (Table 2) displayed some consistency with the predicted activity of the killing pattern, in that the potently active compound **8e** showed similar binding affinity to Bcl-2 compared with reference standard gossypol. Compound **8e** was found to be two-fold more active than our initial lead compound **8a**.

Compound number	ELISA IC ₅₀ , μM
8a	1.24 ± 0.18
8c	3.83 ± 0.05
8e	0.66 ± 0.06
8h	21.12 ± 0.18
8i	29.05 ± 0.17
Gossypol (3)	0.60 ± 0.09

Table 2. ELISA binding activity values (IC₅₀) for selected compounds. Results are expressed as triplicate testing mean values.

Further evidence that Bcl-2 might be responsible for the observed anticancer activity of the new indolyl-*N*-aryl-oxadiazolamines was obtained through molecular modeling studies. Interactions between the most active compounds **8a** and **8e**, and a published Bcl-2 crystal structure (PDB code 4AQ3²⁰), were studied within MOE²¹ making use of the LeadIt software²² for analyzing molecular interactions. Docking of compound **8a** at the Bim peptide-binding site revealed that **8a** mimics residues Leu95 and Ile97 of Bim, making important interactions between Leu96 and the extracellular NH group, and between Arg105 and the oxadiazole ring nitrogen (3-position), within the Bcl-2 hydrophobic groove. Compound **8e** occupies a similar Bcl-2 binding site mimicking the Phe101, Leu95, and Ile97 residues of Bim peptide, similarly making interactions between Leu96 and the extracellular NH group, and between Arg105 and the oxadiazole ring nitrogen (3-position). For compound **8e**, an additional interaction between key Bcl-2 hydrophobic groove residue Tyr67 and the indole ring of **8a** is apparent. Representations of the binding models of **8a** (A) and **8e** (B) within the Bcl-2 hydrophobic pocket are shown in Figure 2.

Table 1. Growth inhibitory activity (IC_{50} , μM) values for 5-(1*H*-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines **8a-j** in human cancer cell lines MDA-MB-231 (breast), HeLa (cervical), KG1a (acute myelogenous leukaemia) and Jurkat (T cell leukaemia). Results are expressed as triplicate testing mean values.

Compound	MDA-MB-231	HeLa	KG1a	Jurkat
8a	0.90 ± 0.02	0.30 ± 0.04	0.85 ± 0.08	30.2 ± 1.5
8b	34.6 ± 0.64	15.12 ± 0.32	25.5 ± 0.65	>100
8c	3.54 ± 0.03	1.76 ± 0.02	0.89 ± 0.04	45.0 ± 1.3
8d	9.77 ± 0.82	3.80 ± 0.20	3.16 ± 0.19	30.2 ± 1.5
8e	8.89 ± 0.05	5.60 ± 0.09	32.7 ± 0.68	>100
8f	31.08 ± 0.6	19.29 ± 1.09	45.3 ± 0.34	>100
8g	>100	>100	21.1 ± 1.6	>100
8h	27.25 ± 0.98	15.79 ± 0.96	4.00 ± 0.13	>100
8i	10.08 ± 0.83	5.82 ± 0.07	0.82 ± 0.05	>100
8j	6.47 ± 0.52	7.81 ± 0.87	>100 ± 0.03	>100

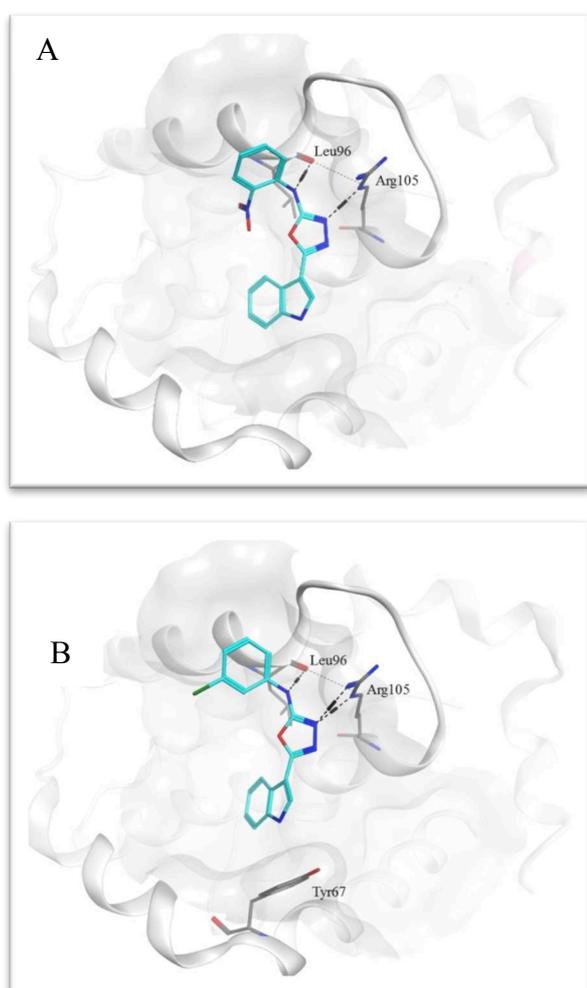


Figure 2. Binding of indolyl-*N*-aryl-oxadiazolamine hit compounds **8a** (A) and **8e** (B) within the Bcl-2 hydrophobic binding pocket. Only the Bcl-2 residues involved in hydrogen bonding (Leu-96, Arg-105) or in π -H interaction (Tyr-67) with compounds **8a** and **8e** are represented.

In conclusion, we have identified the 5-(1*H*-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines **8e** and **8a** as new Bcl-2 inhibitory small molecules with moderately potent anti-proliferative activity against Bcl-2 expressing human cancer cell lines and Bcl-2 protein. These new compounds are useful lead compounds for

further development against this well-established anti-apoptotic target.

Acknowledgments

We thank the Egyptian Government for the award of a PhD studentship (to NIZ) and the award of a Channel Scholarship (to RH and SA). We acknowledge the support of Cancer Research Wales for a PhD studentship (to CB). We are also grateful to the EPSRC UK National Mass Spectrometry Facility at Swansea University (U.K.) for provision of compound accurate mass determinations.

References and notes

- Hanahan, D.; Weinberg, R. A. *Cell* **2011**, *144*, 646.
- Chipuk, J. E.; Moldoveanu, T.; Llambi, F.; Parsons, M. J.; Green, D. R. *Mol. Cell* **2010**, *37*, 299.
- Vogler, M.; Dinsdale, D.; Dyer, M. J.; Cohen, G. M. *Cell Death Differ.* **2009**, *16*, 360.
- Ziedan, N. I.; Kadri, H.; Westwell, A. D. *Mini Rev. Med. Chem.* **2008**, *8*, 711.
- White, A. W.; Westwell, A. D.; Brahehi, G. *Expert Rev. Mol. Med.* **2008**, *10*, 1.
- Cang, S.; Iragavarapu, C.; Savooji, J.; Song, Y.; Liu, D. *J. Hematol. Oncol.* **2015**, *8*, 129.
- Nguyen, M.; Marcellus, R. C.; Roulston, A.; Watson, M.; Serfass, L.; Madiraju, S. R. M.; Goulet, D.; Viallet, J.; Bélec, L.; Billot, X.; Acoca, S.; Purisima, E.; Wiegmanns, A.; Cluse, L.; Johnstone, R. W.; Beuparlant, P.; Shore, G. C. *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 19512.
- Ziedan N. I.; Stefanelli, F.; Fogli, S.; Westwell, A. D. *Eur. J. Med. Chem.* **2010**, *45*, 4523.
- Tohid, S. F. M.; Ziedan, N. I.; Stefanelli, F.; Fogli, S.; Westwell, A. D. *Eur. J. Med. Chem.* **2012**, *56*, 263.
- Hamdy, R.; Ziedan, N. I.; Ali, S.; El-Sadek, M.; Lashin, E.; Brancale, A.; Jones, A. T.; Westwell, A. D. *Bioorg. Med. Chem. Lett.* **2013**, *23*, 2391.
- Ziedan, N. I. *Ph.D. Thesis*, Cardiff University, 2010.
- Begum, A.; Aparna, A. V.; Sireesha, B.; Devi, C. S.; Raghavaiah, P. *Ind. J. Chem. B* **2009**, *48*, 1565.
- Rivera, N. R.; Balshells, J.; Hansen, K. B. *Tetrahedron Lett.* **2006**, *47*, 4889.
- General method for synthesis of indolyl-3-carbonyl-N-phenyl/benzyl thiosemicarbazide (7a-j)*. To a solution of indole-3-carbonylhydrazide (**5**, 1.0 mmol) in absolute ethanol (100 mL) was added a solution of substituted phenyl/benzyl isothiocyanate (**6a-j**) in ethanol (50 mL) with continuous stirring. The reaction mixture was heated under reflux for 1h. After cooling to room temperature, the precipitate formed was collected by filtration, and washed with ice-cold ethanol (30 mL) to give the corresponding

thiosemicarbazide (**7a-j**), which was used in the next step without further purification.

15. *General method for synthesis of 5-(1H-indol-3-yl)-N-aryl-1,3,4-oxadiazol-2-amines (8a-j)*. To a suspension of the indolyl-3-carbonyl-*N*-phenyl/benzyl thiosemicarbazide (**7a-j**, 1.5 mmol) in isopropanol (10 mL) was added a solution of potassium iodide (1.5 mmol) in water (2 mL) and 5N NaOH (1.5 mmol) at 5 °C, and the mixture was stirred until a clear solution was formed. A solution of 1,3-dibromo-5,5-dimethyl hydantoin (3.8 mmol) in acetonitrile (10 mL) was added over 1h whilst maintaining the temperature below 10 °C. The mixture was stirred for a further 1h at 10 °C and then the reaction mixture was quenched with aqueous NaHSO₃ and the crude product collected by filtration. Recrystallisation from ethanol gave the corresponding pure 5-(1H-indol-3-yl)-*N*-aryl-1,3,4-oxadiazol-2-amines (**8a-j**).
16. Representative characterization data: 5-(1H-Indol-3-yl)-*N*-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine (**8c**): (85% yield). ¹H NMR (DMSO-*d*₆) δ 7.26 (2H, m, ArH), 7.55 (1H, d, *J* 7.4 Hz, ArH), 7.84 (2H, d, *J* 9.1 Hz, H-3', H-5'), 8.03 (1H, d, *J* 2.7 Hz, H-2), 8.11 (1H, d, *J* 7.0 Hz, ArH), 8.30 (2H, d, *J* 9.1 Hz, H-2', H-6'), 11.40 (1H, bs, NH), 11.91 (1H, bs, NH). ¹³C NMR (DMSO-*d*₆) δ 99.48 (C-3), 112.38 (ArCH), 116.65 (ArCH), 120.18 (ArCH), 120.96 (ArCH), 122.78 (ArCH), 123.99 (ArC), 125.48 (ArCH), 126.93 (ArCH), 136.38 (ArC), 140.80 (ArC), 145.31 (ArC), 156.54 (ArC), 157.49 (ArC). MS (ESI⁺) 322.1 (M⁺+1). Analysis

calcd. for C₁₆H₁₁N₅O₃: C, 59.81; H, 3.45; N, 21.80. Found: C, 59.46; H, 3.35; N, 21.47.

17. Watkins, C. L.; Sayers, E. J.; Allender, C. J.; Barrow, D.; Fegan, C.; Brennan, P.; Jones, A. T. *Mol. Therap.* **2011**, *19*, 2124.
18. Kolluri, S. K.; Zhu, X. W.; Zhou, X.; Lin, B. Z.; Chen, Y.; Sun, K.; Tian, X. F.; Town, J.; Cao, X. H.; Lin, F.; Zhai, D. Y.; Kitada, S.; Luciano, F.; O'Donnell, E.; Cao, Y.; He, F.; Lin, J. L.; Reed, J. C.; Satterthwait, A. C.; Zhang, X. K. *Cancer Cell* **2008**, *14*, 285.
19. Rao, J.; Xu, D.-R.; Zheng, F.-M.; Long, Z.-J.; Huang, S.-S.; Wu, X.; Zhou, W.-H.; Huang, R.-W.; Liu, Q. *J. Trans. Med.* **2011**, *9*, 71.
20. Research Collaboration for Structural Bioinformatics (RCSB) Protein Data Bank (PDB); www.rcsb.org/pdb
21. Molecular Operating Environment (MOE), Chemical Computing Group Inc., Montreal, Canada; www.chemcomp.com
22. BioSolveIT GmbH; www.biosolveit.de/LeadIT

Supplementary Material

Chemical analytical and spectroscopic data (NMR, mass spectrometry and % C,H,N elemental analysis) are detailed in Supplementary Material, along with detailed protocols for cell culture and endpoint assays, plus the Bcl-2 ELISA assay and molecular modelling studies.