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Design, synthesis and biological evaluation of new inhibitors of Bax/Bcl-xL interaction in cancer cells

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ABSTRACT

We describe the synthesis of a series of new molecules containing phenol and triazoles moieties, compounds which have been evaluated for their ability to inhibit Bax/Bcl-xL interactions in cancer cells, by using BRET assays, and to induce cell death. Several derivatives exhibit a very promising activity, being more potent than the reference compounds acylpyrogallol ***A*** and ABT-737. These preliminary results demonstrate that derivatives of this family can be attractive to develop new molecules with potent anticancer activity.

Protein protein interactions (PPIs) play critical roles in numerous biological processes. Life death decisions are, in particular, regulated by a network of PPIs among Bcl-2 family members, with individual anti-apoptotic Bcl-2 homologues (such as Bcl-2, Bcl-xL, Mcl-1...) binding to, and inhibiting, pro-apoptotic counterparts (the effector multi-domain proteins Bax/Bak and their upstream regulators, BH3-only proteins such as Bim, Bid, Puma, Bad, Noxa...). This so called Bcl-2 network is often dysregulated in cancer cells, leading to aberrant survival. Developing small molecule inhibitors of PPIs engaged by Bcl-2 homologues is thus of major therapeutic interest, as their use may help override cancer cell outgrowth by promoting apoptosis. Numerous compounds have been developed on that basis and some have entered clinical trials.[1]

As part of our programme towards the research of new pro-apoptotic anti cancer agents,[2] we became interested in acylpyrogallol ***A***, which has been reported by the group of Wang as a potent inhibitor of Bcl-2.[3] Based on this core structure, we have first demonstrated that it is also an inhibitor of Bcl-xL and that the phenol group in position 3 played a key role for the biological activities in these series. Further, introduction of a *p*-fluorobenzyl group on the right part of the molecule resulted in a significant improvement of activity (triazole ***B***)[4] Then, we explored the possibility of replacing the aromatic group by 6-membered heteroaromatic structures (series ***C***) or 5-membered derivatives (triazoles series ***D***). In latter case in particular, some potent derivatives have been discovered.[5] An important point to notice is that most of the active compounds in these series of molecules (such as TW 37, TM 179,...) have, like first model ***A***, a carbonyl group vicinal to the phenolic core. Further, molecular docking studies have identified relevant interactions of latter carbonyl group inside the binding pockets of the proteins.[3,6] On the other hand, it is known that sp^2-hybridized nitrogen atoms are able to make stronger hydrogen bond (HB) interactions that oxygen.[7] Therefore, we tried to exploit this advantage by designing new target molecules (Fig. 1) where the benzophenone carbonyl group has been replaced by a nitrogen isostere. As first representative examples, we selected triazole derivatives (series ***E***) since this type of heterocycle has proved already to be very versatile in bioorganic and medicinal chemistry. The synthesis of these new molecules has been performed by using simple and efficient methods. We then used the BRET (Bioluminescence Resonance Energy Transfer) technique to monitor the

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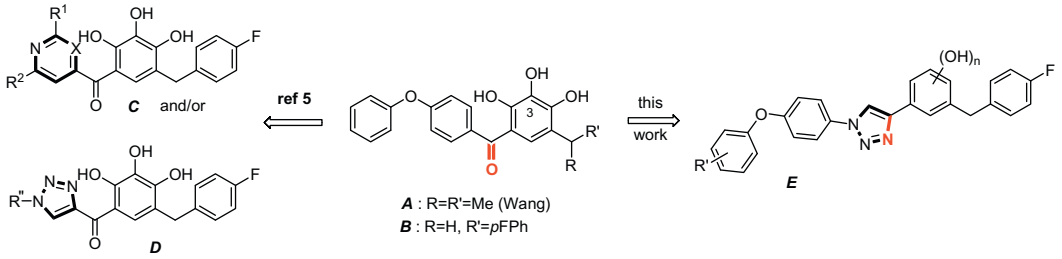


Figure 1. Previous studies and design of our target molecules.

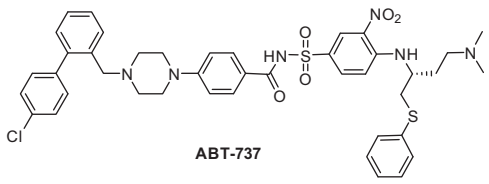


Figure 2. Structure of ABT-737.

incidence of these molecules on Bax/Bcl-xL interactions.[4] This technique allows detection and quantification of protein–protein interactions in whole living cells by measuring energy transfer between a donor fusion protein (Rluciferase-Bax in this work) and an acceptor fusion protein (eYFP-Bcl-xL in this work). Thus we demonstrated that some of these derivatives are significantly more potent inhibitors of the interaction between Bax and Bcl-xL than molecule ***A*** and than the classical reference ABT-737[8] (Fig. 2). The structure activity relationship has been analyzed in light of molecular docking experiments.

The synthesis of the triazoles is reported in Scheme 1. The reaction of known[4] aldehydes **1a**–**1c** with Ohira–Bestmann reagent[9] gave in 64–78% yield the alkynes **2a**–**2c**.

Then Huisgen's 1,3 dipolar cycloaddition[10] of appropriate azides, under click chemistry conditions,[11] afforded in good to excellent yields the desired triazoles **3a**–**5c**. A final deprotection step using BBr_3 in CH_2Cl_2 afforded, in fair to excellent yields after purification by chromatography on silica gel, the target molecules **6**–**13**. All these new molecules have been fully characterized by their spectral and analytical data.

The biological activity of these new type ***E*** analogues was evaluated using a BRET assay in whole living HeLa cancer cells, as previously described,[4] and the corresponding results are reported in Figure 3.

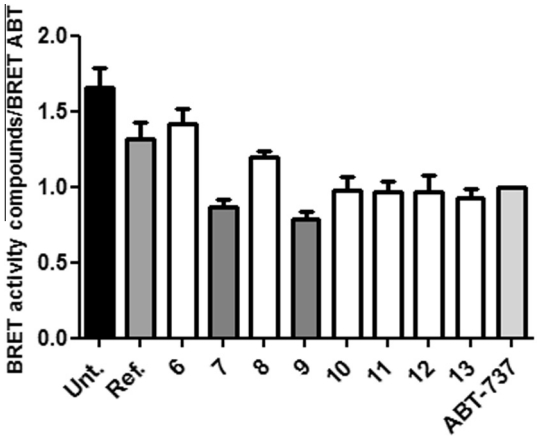
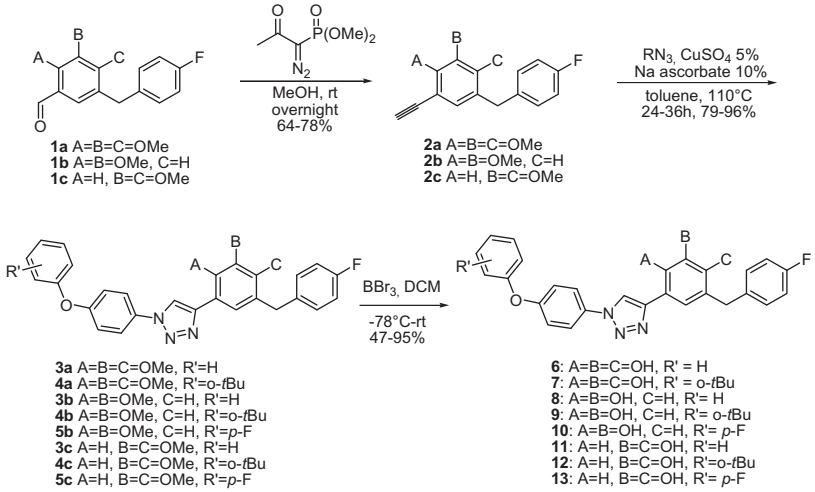


Figure 3. BRET activity regarding disruption of Bax/Bcl-xL interaction, for the new triazoles ***E*** series (10 μM each, 24 h treatment). **Unt**. is untreated and **Ref**. is model ***A***.

The first basic designed structure, triazole **6**, showed a moderate activity, slightly above reference model ***A***. Removal of one phenolic group led to some improvement in the case of **8** and better activity for **11** which is now as potent as ABT-737. However, most fruitful was the introduction of substituents on the diphenyl ether terminal aromatic ring. Addition of a *t*-butyl group in *ortho* position gave a triazole **7** which was now significantly more active than ABT 737 (p value: 0.0007). The analogues with only two OH group **9** and **12** gave also excellent results with the former being the most



Scheme 1. Synthesis of the triazoles **6**–**13**.

active of the series (p value: 0.0002). The introduction of a fluoro substituent in *para* position appeared to be also a good option since corresponding analogues **10** and **13** also demonstrated a good activity.

Cell death experiments have been then performed on HeLa cancer cells (Fig. 4). They establish that these compounds used as single agents for 48 h could induce significant cell death. Interestingly, **7**–**13** analogues were more efficient to trigger cell death than reference molecule ***A*** or analogue **6**, which were both poorly able to disrupt Bax/Bcl-xL interaction. In addition, analogues **7** and **9** induced cell death as efficiently as ABT-737 did, in good correlation with BRET assay. Noteworthy is the triazole **12** which exhibited a very high activity as compared to ABT-737, despite an equal disruptive activity in BRET assay, suggesting that this compound triggered cell death through additional signalling pathways.

All together, these preliminary data show that this novel scaffold bearing a triazole is very promising.

Molecular simulations confirmed that the binding pocket and ligand orientation of the novel triazole series are very close to those described for previous phenol-derived structures.[5,12] As compared with reference molecule ***B***, triazole **6** occupies the same crevice lined with Glu96, Tyr195, Trp137 and Glu92. The key interactions are conserved, with hydrogen bond (HB) between the polyphenol, Glu96 and Arg100 (Fig. 5). A weak HB (with suboptimal geometry) previously observed between Tyr101 and the ketone is however lost with this triazole. This could explain the moderate lowering of affinity. The position of diphenyl-ether is slightly shifted, but HB with Asn136 is maintained through a change in the rotameric state of the residue.

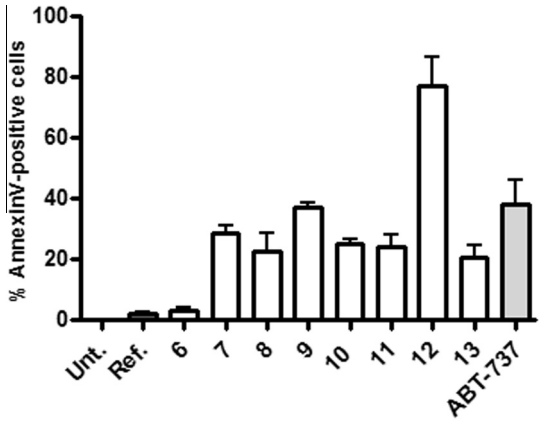


Figure 4. Cell death experiments. Hela cells were treated with the series of compounds as indicated (10 μM) or DMSO (Unt) for 48 h, then stained with AnnexinV, and analyzed by flow cytometry. Data are presented as% of AnnexinV-positive cells (corresponding to dead cells).

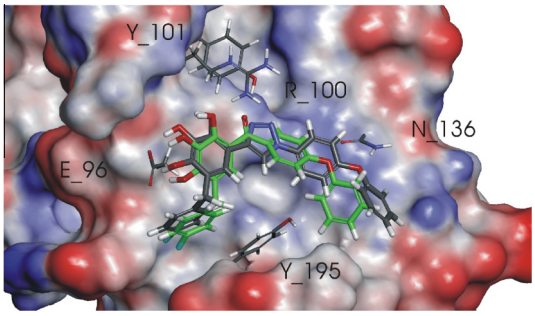


Figure 5. Superimposition of reference ***B*** (carbon atoms in green) and triazole **6** (carbon atoms in grey) docked in the Bcl-xL binding site. Key residues are labeled.

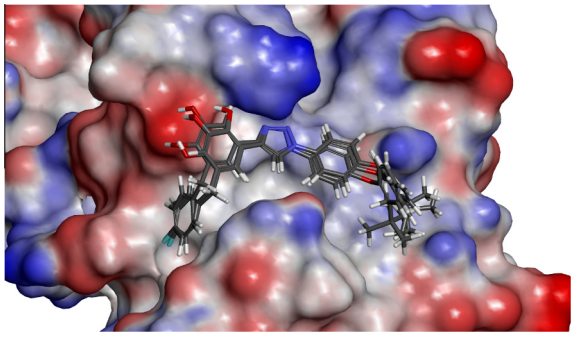


Figure 6. Triazoles **7**, **9** and **12** docked into the Bcl-xL binding site. The Connolly surface of the protein is colored according to electrostatic potential.

Substitutions of the terminal phenyl, either by *t*-Bu in *ortho*-position or fluorine in *para*-position, have a twofold advantage: they increase the van der Waals interactions between the ligands and the receptor, and they facilitate the desolvation of ligands. Further, since the triazoles have two and strong HB possibilities, they can easily compensate for the lack of one hydroxyl function for catechols (compounds **9**–**13**). Hence, a HB with Tyr101 is gained through a small displacement of the ligand (Fig. 6).

In conclusion, efficient strategies allowed the preparation of the mixed triazole–phenol target molecules[13] which were found as attractive new structures for Bax/Bcl-xL inhibition in cancer cells. The triazoles **7**, **9** and **12** especially, which are much more potent than ABT-737 and previous model derivatives ***A*** and ***B***, are promising hits for extended studies. Molecular docking gave a rationale for the interactions of these molecules with Bcl-xL and will be of much use for optimisation of their biological properties. Corresponding studies are under development in our groups and will be reported in due course.

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Supplementary data

Supplementary data (detailed experimental procedures and complete characterization data) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2014.02.035.

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13. *Representative synthetic procedures and spectral characterizations:*—General procedure for preparation of alkyne derivatives, example: preparation of terminal alkyne **2a**: To a stirred solution of aldehyde **1a** (526 mg, 1.73 mmol) in MeOH (10 ml) at 0 °C, was added K_2CO_3 (955 mg, 4 equiv), and Bestmann's reagent dimethyl-1-diazo-2-oxopropylphosphonate (443 mg, 1.3 equiv). The reaction mixture was allowed to warm up to rt and stirred under nitrogen for overnight. After evaporation of MeOH, the residue was purified on a silica gel column chromatography using the mixture pentane/Et_2O 8:2 as eluent to afford the desired product **2a** (332 mg, 64%) as a colorless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 7.14 (dd, J = 5.4, 8.8 Hz, 2H), 6.96 (t, J = 8.7 Hz, 2H), 6.95 (s, 1H), 3.96 (s, 3H), 3.88 (s, 3H), 3.85 (s, 2H), 3.75 (s, 3H), 3.21 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 161.3 (d, J = 243.9 Hz), 154.5, 152.9, 146.2, 136.2 (d, J = 3.2 Hz), 130.1 (d, J = 7.8 Hz), 129.1, 115.1 (d, J = 21.2 Hz), 111.5, 80.4, 79.6, 61.3, 60.9, 60.8, 35.0. ^{19}F NMR (282.4 MHz, $CDCl_3$) δ (ppm): −117.3. HRMS (ESI^+): $C_{18}H_{17}FO_3Na$ $[M+Na]^+$ m/z, calcd 323.1059 found. 323.1061.
General procedure for click chemistry, example: preparation of triazole **3a**: A solution of alkyne **2a** (105 mg, 0.35 mmol), *p*-phenoxyphenylazide (81.2 mg, 1.1 equiv), copper(II) sulfate (6.13 mg, 0.07 equiv), sodium ascorbate (49 µl, 0.14 equiv, 1 M in H_2O) in toluene (5 ml) was stirred under reflux for 24 h. After cooling down to rt, a NH_4OH solution was added to the mixture and then it was extracted with ethyl acetate. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified on a silica gel column using the mixture pentane/EA 8:2 as eluent to afford triazole **3a** (152 mg, 85%) as a colorless viscous oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 8.50 (s, 1H), 8.04 (s, 1H), 7.86 (d, J = 8.9 Hz, 2H), 7.52 (t, J = 7.9 Hz, 2H), 7.39–7.25 (m, 5H), 7.22–7.18 (m, 2H), 7.08 (t, J = 8.7 Hz, 2H), 4.10 (s, 2H), 4.07 (s, 3H), 4.04 (s, 3H), 3.88 (s, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 161.3 (d, J = 243.5 Hz), 157.7, 156.3, 152.0, 149.4, 146.6, 143.6, 136.8 (d, J = 3.2 Hz), 132.3, 130.7, 130.1 (d, J = 7.8 Hz), 130.0, 124.0, 123.2, 122.2, 120.2, 119.4, 119.3, 115.0 (d, J = 21.2 Hz), 60.7, 60.6, 60.3, 35.5. ^{19}F NMR (282.4 MHz, $CDCl_3$) δ (ppm): −117.2. HRMS (ESI^+): $C_{30}H_{26}FN_3O_4Na$ $[M+Na]^+$ m/z, calcd 534.1805 found. 534.1804.
General procedure for demethylation with BBr_3, example: preparation of triazole **6**: To a stirred solution of triazole **3a** (66.5 mg, 0.13 mmol) was added dropwise BBr_3 (1.17 ml, 9 equiv, 1 M in CH_2Cl_2) in CH_2Cl_2 (10 ml) at −78 °C under nitrogen. The reaction mixture was allowed to warm up to rt and stirred for overnight. The mixture was then cooled again to 0 °C and water was added, the solution diluted with CH_2Cl_2, stirred during 1 h before extraction with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. The crude product was purified on a silica gel column using the mixture pentane/EA 7:3 as eluent to afford mixed phenol-triazole compound **6** (34 mg, 55%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm): 10.93 (br s, 1H), 8.01 (s, 1H), 7.70 (d, J = 8.9 Hz, 2H), 7.40 (dd, J = 7.5, 8.5 Hz, 2H), 7.24–7.16 (m, 3H), 7.15 (d, J = 8.9 Hz, 2H), 7.09–7.06 (m, 2H), 6.97 (d, J = 8.7 Hz, 2H), 6.75 (s, 1H), 5.58 (br s, 2H), 3.95 (s, 2H). ^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 161.4 (d, J = 243.6 Hz), 158.5, 156.1, 148.3, 142.7, 142.4, 136.5 (d, J = 3.2 Hz), 132.0, 131.7, 130.1 (d, J = 7.8 Hz), 130.0, 124.4, 122.5, 119.7, 119.6, 119.2, 117.4, 116.2, 115.1 (d, J = 21.1 Hz), 106.1, 34.4. ^{19}F NMR (282.4 MHz, $CDCl_3$) δ (ppm): −117.2. HRMS (ESI^+): $C_{27}H_{20}FN_3O_4Na$ $[M+Na]^+$ m/z, calcd 492.1336 found. 492.1338.