**Antibody–Drug Conjugates**

**Diazaborines Are a Versatile Platform to Develop ROS-Responsive Antibody Drug Conjugates**


Abstract: Antibody–drug conjugates (ADCs) are a new class of therapeutics that combine the lethality of potent cytotoxic drugs with the targeting ability of antibodies to selectively deliver drugs to cancer cells. In this study we show for the first time the synthesis of a reactive-oxygen-species (ROS)-responsive ADC (VL-DAB31-SN-38) that is highly selective and cytotoxic to B-cell lymphoma (CLBL-1 cell line, \( IC_{50} \) value of 54.1 nM). The synthesis of this ADC was possible due to the discovery that diazaborines (DABs) are a very effective ROS-responsive unit that are also very stable in buffer and in plasma. DFT calculations performed on this system revealed a favorable energetic profile (Δ\( \Delta \)G = −74.3 kcal mol\(^{-1} \)) similar to the oxidation mechanism of aromatic boronic acids. DABs’ very fast formation rate and modularity enabled the construction of different ROS-responsive linkers featuring self-immolative modules, bioorthogonal functions, and bioconjugation handles. These structures were used in the site-selective functionalization of a VL antibody domain and in the construction of the homogeneous ADC.

**Introduction**

Antibody–drug conjugates (ADCs) have emerged as a promising therapeutic class for cancer treatment due to its enhanced toxicological profile and selectivity.[1] However, the construction of these functional bioconjugates is very demanding and recent studies show that their clinical success is intimately related with the linker technology. Because apart from connecting both functional components, this chemical spacer is responsible for enabling the biomolecule function-alization without altering its pharmacokinetic properties, for maintaining the conjugate integrity in circulation and for triggering the release of the active drug on the disease site.[2]

Therefore, most recent formats of ADCs, feature linkers with chemical functions that are sensitive to the disease chemical environment.[2,3] However, this responsiveness can also contribute to increase the instability in circulation and off-target toxicity of the ADC.[2,3] Therefore, the discovery of innovative linkages that are stable but responsive to specific molecular hallmarks of cancer, is instrumental for the construction of the next generations of ADCs.

In recent years, reactive oxygen species (ROS) gained considerable recognition because of their central role in cellular homeostasis through the regulation of numerous signaling pathways.[4] This family of signaling molecules is responsible for, among other mechanisms, controlling DNA transcription and regulating cell proliferation and differentiation. The vast majority of cellular ROS ([\( \approx \) 80%]) is generated during the oxidative phosphorylation in the electron transport chain of mitochondria, with the remaining part being produced in peroxisomes and endoplasmic reticulum.[5] In high levels, ROS are harmful to cells as they can damage proteins, lipids and DNA, ultimately leading to metabolic dysfunction and cell death.[6] Thus, cells have built-in mechanisms to counterbalance the increase of ROS levels which include a complex scavenging system based on glutathione (GSH) and redox enzymes such as superoxide dismutase, glutathione peroxidase, glutathione reductase, thioredoxin and catalase.[6-8] The preservation of this tenuous redox balance is essential for maintaining cellular homeostasis.

Contrary to healthy cells, cancer cells, due to a defective mitochondrial oxidative metabolism, have an inherently high level of ROS which is indispensable to sustain the biochemical alterations required for the initiation, promotion and progression of the disease.[9] The elevated oxidative stress in...
cancer cells, when compared with healthy cells, has been explored as a trigger to promote the selective delivery of cytotoxic and imaging payloads. These drug delivery systems are generally based on nanoparticles and polymeric supramolecular constructs bearing organochalcogen, thioether, thiovalerol or aryloxalate linkages that, when exposed to ROS, promote the payload release upon changing their physical properties.

Interestingly, although this strategy has been used to successfully promote the drug delivery of different nanosystems, to the best of our knowledge, these linkages have not been used in the construction of well-defined ROS-responsive targeting drug conjugates like ADCs. This lack of use is probably related with the fact that these functions endure the physiologic conditions more effectively when incorporated in a material’s supramolecular structure rather than when solvent exposed in the form of a bioconjugate linker. Therefore, the engineering of innovative architectures of well-defined ROS-responsive targeting drug conjugates relies on the discovery of chemical functions that sense disease-associated concentrations of ROS, while maintaining the structural integrity of the bioconjugate in circulation.

Aromatic boronic acids (BAs) are well-known for being readily oxidized to the corresponding phenols in the presence of various ROS, and this unique mechanism has been extensively explored in the design of multiple functional materials for drug delivery. However, aromatic BAs also exhibit poor stability under physiologic conditions and a promiscuous reactivity with endogenous molecules, such as vicinal diols or proteins, which often translates into poor pharmacokinetic and toxicity profiles. Therefore, despite very favorable oxidation kinetics in the presence of ROS, the instability and off-target reactivity of BAs constitute a major obstacle for the use of this function in the design of ROS-responsive linkers for targeted drug conjugates.

In the early 1980s, diazaborines (DABs) emerged as a promising class of boronated heterocycles with antibacterial properties. More recently, the very efficient formation of DABs under bioconjugation conditions established this reaction as a powerful click-type transformation in chemical biology. In DABs, the BA function is inserted in a B-N heterocycle where the boron vacant orbital is stabilized by the lone pair of electrons of the adjacent nitrogen. Despite this, as corroborated by recent studies, this architecture does not suppress the Lewis acidity of the boron center. Therefore, we envisioned that, if DAB’s boron center retains the BA’s oxidative sensitivity in the presence of ROS, the improved stability of this scaffold could contribute to bridge the technological gap that limits the construction of well-defined ROS-responsive linker for targeting drug conjugates. The demonstration of this hypothesis constitutes the main focus of this study.

Results and Discussion

Depending on the stereo-electronic properties of the hydrazine and 2-carbonylphenyl BA components, DABs with different stabilities under aqueous media can be obtained as a consequence of the equilibrium between the close and the open forms. In the open form, the BA function is exposed and the hydrazone linkage is expected to be more prone to hydrolysis. Therefore, we initiated this study by evaluating the influence of different substituents in the formation and stability of these heterocycles. Hence a panel of DABs was synthesized by mixing in water 2-formylphenyl BA (2-FPBA) or 2-acetyl phenyl BA (2-APBA) with hydrazines featuring different N-substituents (Figure 2a).
Once prepared, DABs 1–8 were tested for their stability in ammonium acetate pH 7.4 at 25°C. As shown in previous studies, also in this assay, DABs 2–4, prepared from 2-FPBA proved to be considerably more stable than those prepared from 2-APBA (5–8), as no degradation was observed for these compounds over 14 days (Figure 2c and Supplementary Figure S5). Despite being prepared from 2-FPBA, compound 1 displayed a poor stability which is possibly related with the low stability of the phenyl hydrazine in these conditions.

The reaction of hydrazines with 2-carbonylphenyl BA was shown to exhibit very fast kinetics and has been used recently as a click-type reaction to assemble bioconjugates. Therefore, we studied the formation kinetics of DABs 1–8 (7 was excluded from this study due to unreliable results possibly related to the already reported equilibrium of open and closed forms and the formation of dimers). As shown in Figure 2b, in ammonium acetate pH 7.4 (final concentration 60 μM), the reaction of benzyl hydrazine with 2-FPBA and 2-ABPA displayed the fastest kinetics, generating DABs 2 and 6 in less than 10 min, while hydrazines with electron withdrawing N-substituents were slightly slower, affording the respective DABs in up to 1 h. Interestingly, pairs bearing the same N-substituent appear to display similar reaction rates, implying that the stereo-electronic properties of the hydrazine substrate are essential to the reaction kinetics.

Considering these results, DABs featuring N-alkyl substituents like DAB 2 and 6 displayed the best performance in terms of stability in aqueous conditions and kinetics of formation. Therefore, we next tested if DABs retained the ability to be oxidized in the presence of ROS. Hence, a 50 μM solution of 2 was incubated with 100 equiv of H_2O_2 (ammonium acetate 20 mM pH 7.4). After 30 min, the mixture was analyzed by Electrospray Ionization-Mass Spectrometry (ESI-MS) and DAB 2 generated the corresponding salicylhydrazone 9 (Figure 3). Similar oxidations were observed for the remaining DABs 1–8 under these conditions (Supplementary Figure S8).

Encouraged by these results, the aqueous stability of DAB 2 was further tested in more demanding conditions, namely at different pHs and in human plasma. Hence, DAB 2 (100 μM) was incubated in pH 4.5 (acetate buffer 50 mM), 7.4 (KPi buffer 50 mM) and 9.0 (carbonate buffer 50 mM) at 25°C and the stability was monitored by High Performance Liquid Chromatography (HPLC) at different time points. As shown in Figure 4a, DABs concentration remained constant after 14 days in all three conditions. Similar results were obtained upon incubation of DAB 2 in human plasma at 37°C (120 μM), with no visible degradation after 5 days.

Pleased with its stability, we decided to study the oxidation process in detail. We incubated DAB 2 with 100 equiv of H_2O_2 at pH 7.4 (ammonium acetate 20 mM) and followed the disappearance of the starting material by HPLC. As observed in Figure 4b, at 100 μM the starting material was swiftly oxidized, with a calculated oxidation half-life around 15 min. Proportional results were obtained when repeating the experiment with 50 μM, 500 μM and 1 mM of starting material which enabled the determination of the reaction rate as 0.422 M⁻¹·s⁻¹ (Supplementary Figures S9 and S11).

Although ROS plays a fundamental role in cancer proliferation, the increased intracellular concentration of ROS can be noxious for cancer cells if not adequately counterbalanced. The major redox balancing mechanism in cancer cells encompasses an increase in GSH concentration and this delicate equilibrium is essential to maintain the redox homeostasis of cancer cells. Therefore, we questioned if H_2O_2 would still be able to oxidize DABs in the presence of high concentrations of GSH. To simulate cancer cell conditions, DAB 2 (100 μM) was incubated with 10 equiv of GSH (1 mM), followed by the addition of 10 equiv of H_2O_2 (1 mM). As expected, although the reaction in the presence of GSH is slightly slower (Figure 4d), importantly, the oxidation mechanism is still operative.

Next, we investigated the influence of the pH in the oxidation rate. (Figure 5). Accordingly, DAB 2 (100 μM) was incubated with 100 and 10 equiv of H_2O_2 in carbonate buffer 50 mM at pH 9.0. Under these conditions, the oxidation was
clearly accelerated, proceeding with half-lives of 5 and 14 min, respectively. Inversely, when the assay was performed in acetate buffer 50 mM at pH 4.5, the oxidation was completely inhibited, even in the presence of 100 equiv of oxidizing agent.

To rationalize some of the aforementioned observations, we studied the oxidation mechanism of DAB 2 in the presence of H₂O₂ by means of DFT calculations.[27] The result is depicted in a simplified way in Figure 6, showing the more relevant steps along the path. The detailed profile is presented as Supporting Information (Supplementary Figure S43).

The mechanism starts with nucleophilic attack of the peroxide O-atom to the boron atom of the DAB molecule, overcoming a barrier of 23.1 kcal mol⁻¹. The next relevant step is the aryl transposition from the boron to the O-atom with an associated barrier of ΔG° = 28.2 kcal mol⁻¹. This is the highest barrier of the entire mechanism (TS₉₈) and corresponds to the overall energy barrier of the reaction. The path proceeds with nucleophilic attack of a water molecule and B–N bond breaking and, finally, there is hydrolysis of the O–B bond by a second water molecule, producing the final phenol group and boric acid. The reaction is thermodynamically favorable with an overall free energy balance of ΔGᵣ = −74.3 kcal mol⁻¹. The mechanism calculated allows a rationalization of the observed pH influence on the reaction rate. Accordingly, under acidic conditions the H₂O₂ will be mostly protonated and, consequently, unable to attack the boron, whereas in basic medium its nucleophilicity is increased, facilitating the attack that is the starting point of the reaction.[28,29] The equivalent mechanism with phenyl BA as substrate was also calculated for comparison purposes (Supplementary Figure S44). These results indicate a slightly more facile reaction than the one involving DAB 2, with a lower overall barrier (ΔG° = 26.1 kcal mol⁻¹) as well as a more favorable free energy balance (ΔGᵣ = −91.9 kcal mol⁻¹). However, the difference is quite small and clearly support the observed oxidation of DABs.

Once established that alkyl-derived DABs are a valuable scaffold to design ROS-responsive linkers, we initiated a study to integrate this responsive moiety in the structure of a functional bioconjugate. Considering that, upon DAB oxidation a hydrolysis-susceptive hydrazone is generated, we first designed a linker in which the biomolecule is attached to the 2-FPBA component through a maleimide, while the functional payload is linked to the hydrazine. Therefore, the 2-FPBA was modified to install a maleimide function over 6 synthetic steps starting from 10 (Figure 7a and Supporting Information). Once prepared, cross-linker 11 was reacted with benzyl hydrazine to generate DAB 12, which was evaluated in the functionalization of a model peptide featuring a cysteine (Cys) residue. As shown in Figure 7, the bioconjugation with laminin fragment effectively afforded bioconjugate 13 which was successfully oxidized with H₂O₂ overnight (Figure 7b–d).

As shown in Figure 7, the oxidation process also promoted the hydrolysis of the hydrazone. Based on this observation, we
envisioned the possibility of exploiting this hydrolysis to trigger the release of a payload through a self-immolative mechanism. With this objective in mind, the strategies depicted in Figure 8 were delineated to study if either the hydrazone or the salicylaldehyde components could act as self-immolative modules. Considering the results previously obtained in Figure 7, we first evaluated route A (Figure 9). Hence, DABs 15 and 16 featuring a model benzyl alcohol were synthesized and evaluated in terms of stability (KPi 50 mM, pH 7.4) and oxidation (100 equiv H\textsubscript{2}O\textsubscript{2}, KPi 50 mM, pH 7.4). Interestingly, DAB 15 proved to be quite unstable even in the absence of hydrogen peroxide, favoring the elimination of benzyl acrylate 18. On the other hand, DAB 16 was tested in the same conditions and exhibited a half-life of 9.2 days in KPi buffer at pH 7.4. More importantly, in the presence of \textsubscript{H}2O\textsubscript{2}, DAB 16 was readily oxidized (80 min half-life) to generate salicylaldehyde 19, the hydrazone 20 and benzyl alcohol 21. Analyzing the evolution of the oxidation reaction (Figure 9), upon addition of the oxidant, the concentration of salicyl hydrazone 19 rapidly increases, followed by a second stage characterized by a slow decrease in concentration of 19 (Figure 9c). This kinetics profile suggests the oxidation step is faster than the hydrazone’s hydrolysis to release benzyl alcohol 21.

Based on these results, we next addressed the functionalization of the linker with the cytotoxic payload SN-38\textsuperscript{[30–32]}.

Recently, this topoisomerase I inhibitor was used in the construction of an antibody–drug conjugate (ADC–Sacituzumab govetecan: Trodelvy\textsuperscript{[33–36]}) that was approved by FDA for the treatment of triple-negative breast cancer.\textsuperscript{[33–36]}

Therefore, we explored the design used to construct Trodelvy\textsuperscript{[33–36]}, and installed the linker at the hydroxyl adjacent to the lactone ring in 22.

As shown in Figure 10, the once prepared DAB 23 was used in the functionalization of the laminin fragment peptide. The bioconjugation reaction produced mainly bioconjugate 24, though careful analysis of the ESI-MS spectra indicated that the hydrolyzed conjugate was also being produced in small amounts (Supplementary Figure S32).

Considering this observation, we studied the stability of DAB 23 in PBS at pH 7.4. In these conditions, the compound exhibited a half-life of 8 h, corroborating the observed hydrolysis of 24 (Supplementary Figure S19). This slight instability is likely related to the presence of the ester function, which is known to be labile in aqueous conditions.

Considering the hydrolysis profile observed for DAB 23, we evaluated if a self-immolative cascade directly promoted by the formation of the phenolic unit, would afford a more stable linker, as well as a more efficient release of the payload (Figure 11). Therefore, starting from salicylaldehyde, the 2-FPBA component was modified to include a benzyl alcohol para to the BA function, which then enabled the SN-38 phenol alkylation, as shown in Figure 11. The functionalization of the phenol motif in SN-38 has been used to generate produgs like irinotecan, that require activation to release the active SN-38 form.\textsuperscript{[30–32]}

Once prepared, compound 27 was reacted with hydrazine to afford DAB 29. This hydrazine 28, featuring a small polyethylene glycol, was selected to improve the aqueous

Figure 8. Two strategies to design a triggered self-immolative release of a payload. The self-immolative module is incorporated a) on the hydrazine moiety or b) in the boronic acid/phenol module.

Figure 9. DABs 15 and 16 featuring a model benzyl alcohol—stability and oxidation. a) DAB 15 was shown to be unstable at pH 7.4. b) DAB 16 is oxidized in the presence of 100 equiv \textsubscript{H}2O\textsubscript{2}, and the hydrazone formed promotes the release of benzyl alcohol 21. c) HPLC profiles (pH 7.4, 25°C) of DAB 16 and the intermediate salicyl hydrazone 19 after incubation with 100 equiv \textsubscript{H}2O\textsubscript{2}. d) HPLC profiles (pH 7.4, 25°C) of salicyl aldehyde 20 and benzyl alcohol 21 after incubation of DAB 16 with 100 equiv \textsubscript{H}2O\textsubscript{2}.

Figure 10. Synthetic route for the preparation of linker-payload 23 and its use in the modification of a laminin fragment. The bioconjugation reaction was analyzed by ESI-MS to confirm the presence of bioconjugate 24 and hydrolyzed conjugate 25 in small amounts. TFA: trifluoroacetic acid.
solubility of the linker and to enable its installation on the biomolecule surface by a strain-promoted azide-alkyne cycloaddition (SPAAC) reaction. Once prepared, DAB was tested for its stability in PBS pH 7.4 at 25°C where it displayed a half-life of over 100 h (Figure 12a). Moreover, a similar stability was observed for this compound in human plasma (Figure 12b). Nonetheless, by adding H₂O₂ to DAB in PBS at pH 7.4, the diazaborine was readily oxidized (half-life 8.1 h) and released the SN-38 drug (Figure 12c, d).

Then, we studied the conjugation of DAB to the laminin fragment. To this end, a commercially available maleimide-cyclooctyne was used to functionalize the peptide and to react with DAB by a SPAAC reaction to generate bioconjugate Laminin-DAB31-SN-38 (Figure 13). This reaction effectively generated the expected bioconjugate, thereby a similar methodology was employed to construct a functional ADC against cancer cells.

Recently, we initiated a program to develop an antibody fragment that selectively targets B-cell lymphoma cells and has the ability to internalize (manuscript in preparation). This antibody scaffold consists on a rabbit derived VL single-domain antibody that exhibits a free cysteine at position 82 and can be explored to develop antibody–drug conjugates (ADC). This antibody scaffold was therefore modified with the maleimide-cyclooctyne cross linker and converted to the homogenous targeting drug conjugate VL-DAB31-SN-38, using the DAB linker (Figure 14).

Once prepared, this ADC was tested in the canine B-cell lymphoma CLBL-1 cell line and Jurkat cell line. The VL antibody was developed to target canine B-cell lymphomas and does not recognize the human T lymphocyte Jurkat cell line. Therefore, this cell line was used as a control. The conjugate VL-DAB31-SN-38 revealed to be highly cytotoxic toward the CLBL-1 cancer cell line with an IC₅₀ value of 54.1 nM (Figure 15a). In contrast, the unconjugated VL did not show cytotoxicity under the same conditions. Additionally, both the conjugate VL-DAB31-SN-38 and the unconjugated VL, did not present cytotoxic activity against the Jurkat control cell line (Figure 15b). The free SN-38 drug presented a potent and similar cytotoxicity activity in both the targeted cancer and control cell line (IC₅₀ value of 4.67 nM and 10.6 nM, respectively). To further characterize the mechanism of action of VL-DAB31-SN-38, we have conducted control experiments for linker DAB-PEG-N₃ and DAB (SN-38-DAB-PEG-N₃) in both cell lines (Figure S41). Moreover, a cellular ROS quantification assay was conducted on the CLBL-1 and Jurkat cell lines. This assay demonstrated that the CLBL-1 and Jurkat cell lines presented similar cellular ROS levels (Figure S42). Altogether, these results indicate that the VL-DAB31-SN-38 exerts its cytotoxic activity via a novel mechanism involving the release of SN-38 and the activation of ROS.

Figure 11. Synthetic route for the preparation of linker-payload 29. Conditions for 27: Compound 26 (1.5 equiv), SN-38 (1 equiv), K₂CO₃ (1.5 equiv), DMF, 0°C–50°C, 4 h, 69%. Conditions for 29: HCl in H₂O/CH₃CN (4:3), 25°C, 24 h; hydrazine (6 equiv) in H₂O/CH₃CN (4:3), 25°C, 16 h, 39%. DMF: N,N-dimethylformamide.

Figure 12. DAB stability at different conditions analyzed by HPLC. a) DAB stability in PBS/DMSO pH 7.4 at 25°C. b) DAB stability in plasma at 25°C (compared with PBS pH 7.4 using the same analytical procedure). c) Oxidation profile of DAB oxidation in the presence of 100 equiv H₂O₂ (disappearance of the starting material) in PBS/DMSO pH 7.4 at 25°C. d) SN-38 release profile during DAB oxidation in the presence of 100 equiv H₂O₂ in PBS/DMSO pH 7.4 at 25°C. HPLC-MS traces of DAB oxidation are presented in the Supporting Information (Figures S26–S28).

Figure 13. Reaction of DAB in DMSO (2 mM) with laminin in ammonium acetate solution (10 mM, 20 mM, pH 8) at room temperature, after addition of commercially available maleimide-PEG-cyclooctyne. a) ESI-MS spectrum after the reaction of laminin with maleimide-PEG-cyclooctyne; b) ESI-MS spectrum after the addition of compound 29.
Figure 14. a) Conjugate VL-DAB31-SN-38 obtained from VL’s cysteine modification with commercially available cyclooctyne 30, followed by a SPAAc with DAB 29 (more details in the Supporting Information). b) HRMS spectrum of conjugate VL-DAB31-SN-38. c) Deconvoluted HRMS spectrum of VL (mass: 12879.2 Da). d) Deconvoluted HRMS spectrum of conjugate VL-DAB31-SN-38 (14361.9 Da). Mass spectral deconvolution was performed using Zscore algorithm in MagTran1.03 software.[27]

Figure 15. Cytotoxicity of conjugate VL-DAB31-SN-38. a) Cytotoxic effect of conjugate VL-DAB31-SN-38 on the CLBL-1 cell line. CLBL-1 cells (6x10⁶) were subjected to the indicated concentrations of VL-DAB31-SN-38, SN-38, and VL. After 48 h treatment, cell viability and proliferation were evaluated with WST-1 reagent. Two replicate wells were utilized to determine each data point and three independent experiments were carried out on different days. Best-fit IC₅₀ values of each compound were calculated using the log (inhibitor) vs. response (variable slope) function. b) Viability assay for conjugate VL-DAB31-SN-38 on the Jurkat cell line (incubation time of 48 h).

activity due to the ADC internalization and SN-38 release under ROS-responsive cleavage.

Conclusion

In summary, here we presented an innovative technology to synthesize ROS-responsive ADCs based on DABs. The high stability of DAB 2 in buffer (over 14 days at pH 4.5, 7.4 and 9); plasma) and in plasma (over 5 days), together with the ability to be oxidized in the presence of H₂O₂ (0.422 and 0.103 M⁻¹·s⁻¹ with 100 and 10 equiv of H₂O₂, respectively) made this scaffold a very useful tool to prepare well-defined ROS-responsive ADCs. A detailed DFT study was performed to elucidate the mechanism of DAB 2 oxidation in the presence of H₂O₂, which revealed a similar pathway to the known oxidation of aromatic boronic acids with H₂O₂. Once the high stability and ROS responsiveness of DABs were established, these scaffolds were applied in the synthesis of self-immolative linkers to release the cytotoxic payload SN-38. The linker was equipped with a maleimide that was used in the site-selective functionalization of a VL antibody. The obtained ADC VL-DAB31-SN-38 presented a high selectivity and potency (IC₅₀ = 54.1 nM) against B-cell lymphoma CLBL-1 cell line.

ROS is essential to sustain the biochemical alterations required for the initiation, promotion and progression of cancer, but is also a distinctive feature of many other important diseases (e.g neurodegeneration and inflammation), and until now, the development of ROS-responsive ADCs for these conditions has been limited by the existence of stable linkers that can effectively respond to ROS. The developed DAB oxidation technology is expected to overcome these limitations, and support the discovery of the next generation of stimuli-responsive ADCs.

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Conflict of Interest

J. P. M. António, J. I. Carvalho, and Pedro M. P. Gois are inventors of Portuguese provisional patent PPP11728 (Application number: 2021100023853). Patent rights granted to Technophage SA and Faculdade de Farmácia da Universidade de Lisboa, Frederico Aires-da-Silva, Joana N. R. Dias, Ana S. André, Sandra I. Aguia, Soraia Oliveira, and Luís Tavares are inventors of Portuguese provisional patent filed on 13-09-2021 entitled “Rabbit derived single-domain antibodies as promising scaffolds for the development of highly specific and potent antibody drug conjugates”. The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

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