Proceedings of the 36th European Peptide Symposium
Michal Lebl (Editor)
European Peptide Society, 2022 *https://doi.org/10.17952/36EPS.2022.203*

Design, Synthesis, and Characterization of Aza-BODIPY-Peptide Conjugates Derived from LfcinB: Approximation to Photodynamic Therapy

A. Verónica Rodríguez-Mayor[1], Maricela Morales[1], Zuly J. Rivera[2], Javier E. García[2], Norberto Farfan[3], and Rosa Santillan[1]

[1]Departamento de Química- CINVESTAV. 07360, CDMX, México; [2]Facultad de Química, Departamento de Química Orgánica, UNAM. 04510, CDMX, México; [3]Facultad de Ciencias, Universidad Nacional de Colombia, 111321, Bogotá D.C. Colombia

Introduction

In 2020, the World Health Organization had reported 19 million cases of cancer. Among the available treatments for cancer, the most frequently used are surgery, radiotherapy and chemotherapy while immunotherapy and photodynamic therapy have also become available recently [1,2]. In spite of constant efforts to provide treatments that improve the survival and quality of life of people around the world; the high cost, low availability, and adverse side effects require the development of therapeutic alternatives more specific, selective and with less adverse effects [3].

Phototheranostics is a promising strategy for precise tumor treatment. It consists of optical diagnosis, photodynamic therapy, and photothermal therapy. Aza-boron-dipyrromethenes (aza-BODIPYs) are excellent photosensitizer candidates due to their properties such as absorption/emission close to the near-infrared region, potential to generate highly reactive oxygen species and photothermal conversion efficiency. Conjugation of aza-BODIPY photosensitizers with amphiphilic molecules can provide selectivity in target tumor sites, enhanced permeability and retention effect, improving diagnosis and therapeutic efficacy [4].

Peptides have been considered promising candidates for the design and development of anticancer therapeutic agents, in addition to presenting high selectivity for cancer cells and fewer adverse effects. Some promising peptides that have been studied are those derived from Bovine Lactoferricin (LfcinB). LfcinB is an Antimicrobial peptide (AMP) that has shown activity against Gram positive and Gram-negative bacteria, fungi, parasites, viruses, and tumor cells [5-7]. Further studies have shown that the minimum motif with activity is the hexapeptide RRWQWR [8,9] and that peptide derivatives [7,10] containing this sequence show significant activity against bacterial and cancer cell lines.

On the other hand, aza-BODIPY derivatives have shown cytotoxic activity against several cancer lines [11-13] and may be promising therapeutic molecules. The design of molecules with optical properties, such as Boron aza-dipyrromethes (aza BODIPY), is an area of interest currently. These compounds belong to the BODIPY (Boron dipyrromethene) family and are characterized by having a nitrogen atom in the *meso* position of the structure (Figure 1). These compounds are chromophores with characteristics which include: high photostability, structural versatility and the possibility of modifying the periphery of the core allowing to enhance the chemical and optical properties [14].

The possibility to achieve structural diversity and generate molecules with optical and therapeutic properties from a small number of units is attractive. BODIPY-peptide conjugates having low toxicity and high specificity towards target cells, with potential application in phototherapy and imaging [15-17] have been reported, however, to our knowledge, no reports on the conjugation of aza-BODIPYs with peptides have been described.

This work describes the design and synthesis of modified peptides derived from Bovine Lactoferricin (LfcinB) and their conjugation with aza-BODIPY type donor-acceptor-donor molecules with thiophene and methoxy groups. Conjugation of the Aza-BODIPY fragment at the *N*-terminal end of the peptide chain using click chemistry is expected to provide new molecules with favorable optical properties and antitumoral activity that will be evaluated as therapeutic agents in photodynamic therapy.

Results and Discussion

Aza-BODIPY molecules with Donor-Acceptor-Donor architecture were designed to give emission bands shifted towards the NIR by incorporation of electron-donor groups such as amino phenyl, p-methoxyphenyl, and thiophene in the periphery. For conjugation with peptides, we envisioned to functionalize the aza-BODIPYs with maleimide or alkyne groups to obtain monomeric conjugates (with a unit of peptide) or dimeric conjugates (with two units of peptide) (Figure 1).

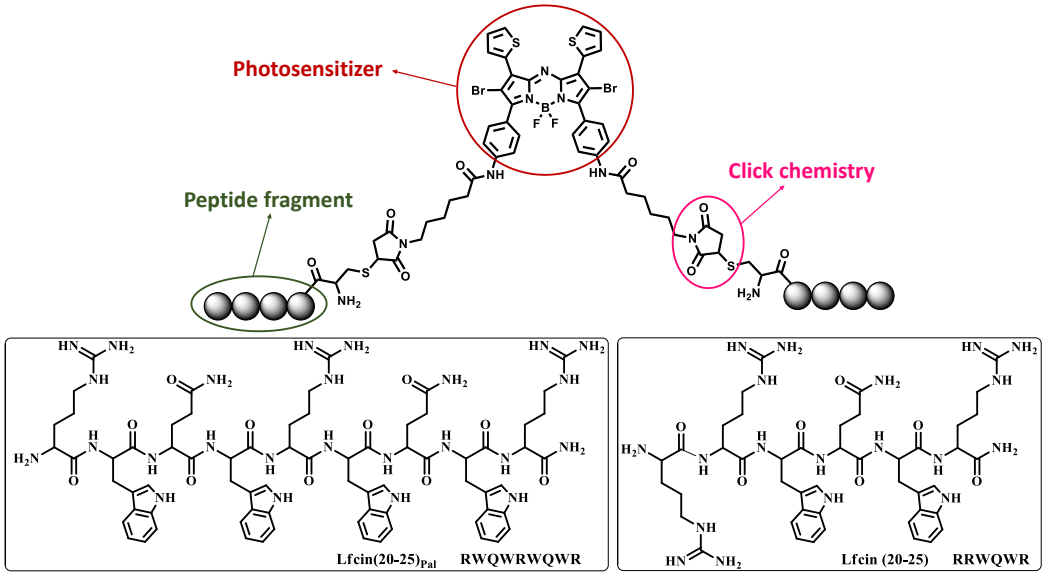

Fig. 1. Structural design of aza-BODIPY-peptide conjugates derived from LfcinB. Peptides Lfcin(20-25) and Lfcin(20-25)$_{pal}$ are represented by circles.

Peptides derived from LfcinB containing cysteine and Lys(N_3) residues in the *N*-terminal end, were also designed to be conjugated to aza-BODIPY by click chemistry [18,19]. The peptides were obtained by manual SPPS (Solid Phase Peptide Synthesis) using the Fmoc/tBu strategy and characterized by HPLC and mass spectrometry MALDI-TOF (Table 1).

Table 1. Characterization of peptides HPLC and MALDI-TOF data.fig.

Peptide	*Chromatography* [a]		*Mass Spectrometry*	
	$t_{(R)}$ *(min)*	*Area (%)*	$[M+H]^+$	*m/z*
RRWQWR	5.3	99	986,536	985,279
K(N_3)-RRWQWR*	5.4	83	1139,631	1139,781
C-RRWQWR*	4.9	97	1088,545	1088,224
RWQWRWQWR	6.9	97	1485,753	1484,589
K(N_3)-RWQWRWQWR*	6.8	65	1693,848	1640,678
C-RWQWRWQWR*	6.5	96	1588,762	1589,101

* *Crude peptide* [a]*Peptides were analyzed using a Linear gradient from 5% to 50% of B solvent (ACN-TFA 0.05%) in A solvent (H_2O-TFA 0.05%) on Chromolith@ Performance RP-18e column for 8 min*

The synthesis of the aza-BODIPY derivatives was carried out by the O'Shea method [20] starting by condensation of *p*-amino-acetophenone or *p*-methoxy-acetophenone to give the α, β-unsaturated ketone, followed obtention of the corresponding nitroketone by Michael addition which upon

cyclization provides the aza-dipyrromethene intermediate. Finally, coordination using boron trifluoride provides the desired aza-BODIPYs which can be conjugated to the peptide molecule using click chemistry.

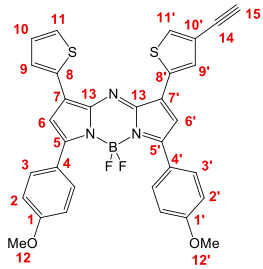

Fig. 2. Aza-BODIPY functionalized with alkyne group.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.06 and 8.01 (1H each, *J* = 8.9 Hz, H-3 and H3'), 7.91 (d, *J* = 3.8 Hz, 1H, H-11), 7.72 (d, *J* = 3.9 Hz, 1H, H-11'), 7.55 (d, *J* = 5.0 Hz, 1H, H-9), 7.31 (d, *J* = 3.9 Hz, 1H, H-9'), 7.19 (dd, *J* = 5.0, 3.8 Hz, 1H, H-10), 6.99 (1H each, *J* = 8.9, H-2 and H-2'), 6.93 and 6.87 (1H each, H-6 and H-6'), 3.88 (3H each, H-12 and H-12'), 3.54 (s, 1H, H-15).

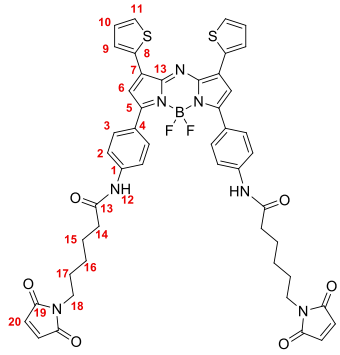

Fig. 3. Aza-BODIPY functionalized with maleimide group.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.14 (s, 2H, H-12), 7.96 (d, *J* = 8.6 Hz, 4H, H-3), 7.86 (d, *J* = 3.6 Hz, 2H, H-11), 7.58 (d, *J* = 8.6 Hz, 4H, H-2), 7.51 (d, *J* = 5.0 Hz, 2H, H-9), 7.14 (dd, *J* =5.0, 3.6 Hz, 2H, H-10), 6.88 (s, 2H, H-6), 6.63 (s, 4H, H-20), 3.47 (t, *J* = 7.0 Hz, 4H, H-18), 2.60-1.09 (m, 12H).

Two Aza-BODIPY compounds containing alkyne and maleimide groups with donor-acceptor architecture were synthesized and characterized by NMR and MS-ESI. Peptides were synthesized and characterized by HPLC and mass spectrometry MALDI-TOF, obtaining a high chromatographic purity of 95% except for the peptides with Lys(N3) residues which had 83% and 65% chromatographic purity, respectively. The compounds obtained will be halogenated and subsequently will be linked to peptide sequences with anticancer properties to evaluate their cytotoxic activity in photodynamic therapy.

Acknowledgments

The authors acknowledge support from CONACyT. A. Rodríguez thanks CINVESTAV for the financial support given and EPS for the registration grant to attend this meeting

References

1. Siegel, R.L., et al. *Cancer J. Clin.* **70,** 7-30 (2020), https://doi.org/10.3322/caac.21590
2. Agostinis, P., et. al. *Cancer J. Clin.* **61,** 250-281 (2011), https://doi.org/10.3322/caac.20114
3. Ferreiro, J.R., et al. *Gac Med Bilbao* **100**, 69-74 (2003),
4. Chen, D.Z. et al. *ACS Appl. Mater. Interfaces* **12,** 26914-26925 (2020), https://doi.org/10.1021/acsami.0c05021
5. Sinha, M., et al. *Int J Pept.* **2013,** 1-46 (2013), https://doi.org/10.1155/2013/390230
6. León-Calvijo, M.A., et al. *Biomed Res. Int.* **2015**, 1-8 (2015), https://doi.org/10.1155/2015/453826
7. Barragán-Cárdenas, A., et al. *RSC Adv.* **10**, 17593-17601 (2020), https://doi.org/10.1039/d0ra02688c
8. Richardson, A., et al. *Biochem. Biophys. Res. Commun.* **388**, 736-741 (2009), https://doi.org/10.1016/j.bbrc.2009.08.083
9. Hilchie, A.L., et al. *Exp. Mol. Pathol.* **95**, 192-198 (2013), https://doi.org/10.1016/j.yexmp.2013.07.006
10. Vargas Casanova, Y., et al. *Molecules,* **22**, 1641-1652 (2017), https://doi.org/10.3390/molecules22101641
11. Treekoon, J., et al. *Org. Biomol. Chem.* **19**, 5867-5875 (2021), https://doi.org/10.1039/d1ob00400j
12. Tian, J., et al. *Chem. Sci.* **6**, 5969-5977 (2015), https://doi.org/10.1039/c5sc01721a

13. Yu, Z.J., et al. *J. Med. Chem.* **63**, 9950-9964 (2020), https://doi.org/10.1021/acs.jmedchem.0c00882
14. Kubheka, G., et al. *Molecules.* **25**, 3689 (2020), https://doi.org/10.3390/molecules25163689
15. Zhao, N., et al. *Bioconjug. Chem.* **28**, 1566-1579 (2017), https://doi.org/10.1021/acs.bioconjchem.7b00211
16. Kaufman, N.E.M., et al. *J. Med. Chem.* **62**, 3323-3335 (2019), https://doi.org/10.1021/acs.jmedchem.8b01746
17. Williams, T.M., et al. *Photochem. Photobiol.* **96**, 581-595 (2020), https://doi.org/10.1111/php.13234
18. Rostovtsev, V.V., et al. *Angew. Chemie - Int. Ed.* **41**, 2596-2599 (2002), https://doi.org/10.1002/1521-3773(20020715)41:14<2596::AID-ANIE2596>3.0.CO;2-4
19. Nair, D.P., et al. *Chem. Mater.* **26**, 724-744 (2014), https://doi.org/10.1021/cm402180t
20. Killoran, J., et al. *Chem. Commun.* **317,** 1862-1863 (2002), https://doi.org/10.1039/b204317c