



HHS Public Access

Author manuscript

Expert Opin Ther Pat. Author manuscript; available in PMC 2017 September 01.

Published in final edited form as:

Expert Opin Ther Pat. 2016 September ; 26(9): 973–977. doi:10.1080/13543776.2016.1206527.

Inhibitors of programmed cell death 1 (PD-1): a patent review (2010–2015)

Tryfon Zarganes-Tzitzikas¹, Markella Konstantinidou¹, Yongzhi Gao¹, Dobrosława Krzemien², Krzysztof Zak², Grzegorz Dubin², Tad A. Holak^{2,3}, and Alexander Dömling^{1,*}

¹University of Groningen, Department of Drug Design, A. Deusinglaan 1, Groningen, The Netherlands ²Jagiellonian University, Malopolska Centre of Biotechnology, Gronostajowa 7a, Krakow ³Faculty of Chemistry, Jagiellonian University, Ingardena 3, 30-060 Krakow

Keywords

Programmed death-1; PD-1; PD-L1; immune checkpoint; T-cell exhaustion; immune-oncology

1. INTRODUCTION

The search for the magic bullet in cancer immunotherapies is ongoing since more than 100 years, but only recent clinical success of immune checkpoint directed antibodies significantly revived the field of immune-oncology.¹ A key protein target in this area is the protein-protein interaction between PD-1 and its ligand PD-L1. Functionally, PD-1 also called programmed death-1 protein comprises an immune checkpoint located on T-cells. The PD-1/PD-L1 axis is hijacked by viruses and tumor/cancer cells to suppress the immune surveillance. For example, PD-L1 is expressed on tumor cells and also on immune cells (e.g. myeloid tumor-infiltrating cells). Binding of PD-1 to PD-L1 determines a downregulation of T-cell effector functions in cancer patients, inhibiting the antitumor immune response and leading to T-cell exhaustion.² In viral diseases a similar mechanism is used by viruses to undermine the effective immune recognitions and answer.³ Current medication directed towards the PD-1/PD-L1 axis includes monoclonal antibodies. These have shown impressive clinical results in the treatment of several types of tumours, including melanoma and lung cancer.⁴ Currently two humanized monoclonal antibodies targeting PD-1 are approved by the regulatory bodies, pembrolizumab and nivolumab.⁵ Multiple additional clinical trials either as single agents or in combination with other agents are ongoing to extend their

*Corresponding author: Alexander Dömling, University of Groningen, Department of Drug Design, A. Deusinglaan 1, Groningen, The Netherlands, a.s.domling@rug.nl.

Declaration of interest

The authors have received research support from the National Institute of Health (NIH) (1R01GM097082-01), the European Lead Factory (IMI; under grant agreement number 115489), the Qatar National Research Foundation (NPRP6-065-3-012), and the ITN AEGIS. Additional research support was received from the National Science Centre, Poland, (under grant UMO-2012/06/A/ST5/00224) by the Project operated within the Foundation for Polish Science TEAM Program, co-financed by the EU European Regional Development Fund and the Marie Curie FP7-Reintegration-Grant within the 7th European Community Framework Programme. 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 apart from those disclosed.

indication areas. Therapeutic antibodies, however exhibit several disadvantages such as limited tissue and tumour penetration, very long half-life time, lacking oral bioavailability, immunogenicity, and difficult and expensive production. Moreover current PD-1/PD-L1 axis directed monoclonal antibodies lead to a tumour response only in a fraction of cases and tumour types. Therefore, a search for non-mAbs, including small molecules, peptides, cyclo-peptides and macrocycles is ongoing and will be reviewed here.

2. BODY

Recently the co-crystal structure between the human PD-1 and PD-L1 has been determined for the first time.⁶ The Å-resolution crystal structure provides a possible starting point for the design of molecules against the protein-protein interaction (PPI). The interface between the two proteins is extended (~1.700 Å²), hydrophobic and flat, without deep binding pockets, which makes the interface likely a difficult target for small molecules. The hydrophobic interface also increases the chances to discover false positive hits considerably. Moreover direct competitor PD-1/PD-L1 antagonists are potentially very hydrophobic molecules, which can lead to downstream development issues, including toxicity, selectivity and poor water solubility, just to name a few. Nonetheless, currently several series of small molecules, peptides and cyclo-peptides have been disclosed targeting the PD-1/PD-L1 PPI. In the following, we focus on discussing first small molecules, followed by peptide and cyclic peptide derivatives.

A group from Harvard University has discovered sulphonamide derivatives (**1**) and (**2**) to work in a similar fashion as reference mAbs.⁷ (Fig.1). The two compounds are active antagonists in an IFN γ -release assay in transgenic mouse T cells that express PD-1.

Workers from the company Bristol-Myers Squibb (BMS) have disclosed scaffold (**3**) (Fig.2) binding to PD-L1.⁸ It consists of a tri-aromatic structure, including a mono-ortho substituted biphenyl substructure. Moreover, another phenyl ring is connected to the biphenyl and contains also a methylene amine moiety. The biological activity of the claimed compounds was established by a homogenous time-resolved fluorescence (HTRF) binding assay in which Europium cryptate-labeled anti-Ig was used. To assess selectivity the PPIs PD-1/PD-L2 and PD-L1/CD80 were tested as well. Typical examples are BMS-8, BMS-37, BMS-202, BMS-230 and BMS-242. No further in vitro or in vivo assays have been described supporting the biological activity of compounds based on scaffold (**3**) (Fig.2). The structural basis of BMS-202 and BMS-8 as PD-1/PD-L1 antagonists was recently rigorously proven by a co-crystal structure and other biophysical methods.⁹

Certain compound classes have been recently described to interfere with the time-resolved fluorescence resonance energy transfer (TR-FRET) for the PD-1/PD-L1 assay during a HTS campaign, producing false positive hits.¹⁰ Examples include the salicylates NCI 211717 and NCI 211845. Mechanistically, the interaction of the chelator moiety salicylate with the cryptand-ligated europium FRET donor leading to a change in the assay signal is suggested (Fig.3).

Several PD-1/PD-L1 antagonists based on peptide structures including bioisosteres such as oxadiazole, and urea have been disclosed. Workers from Aurigene Ltd. described cyclic peptidomimetic compounds as immunomodulators able to interfere with the programmed death (PD-1) signalling pathway. The general formula (**4**) is exemplified in 20 explicit examples. It consists of a central 1-oxa-, or 1-thia-3,4-diazole fragment with a serine or threonine side chain in position 2 and another amino acid or dipeptide linked in position 5. Dipeptides are bound by a urea unit. In the majority of examples the first amino acid in position 5 is asparagine, glutamine, aspartic acid or glutamic acid. The biological activity of the compounds was tested by using a rescue assay of mouse splenocytes in the presence of recombinant mouse PD-1/PD-L1. Compound (**5**) for example was able to rescue the mouse immune cells to 92% at 100 nM. (Fig.4).

Positional isomeric 1-oxa-2,4-diazoles (**6**) with otherwise similar sidechains were also disclosed by the same company. Compound (**7**) exhibited 91% rescue in the above mouse splenocyte assay (Fig.4).

Aurigene Ltd. described also small peptidomimetics comprising of 3–4 amino acid moieties comprising a hydrazine and a urea linker with general structure (**8**). A total of 14 compounds were explicitly exemplified.¹¹ Compound (**9**) for example was able to rescue the mouse immune cells to 84% at 100 nM (Fig.4).

A team from Zhengzhou and Tsinghua Universities described the discovery of the first hydrolysis-resistant D-peptide antagonists to target the PD-1/PD-L1 interaction.¹² The optimized compound DPPA-1 could bind PD-L1 at an affinity of 0.51 μ M in vitro. A blockade assay at the cellular level and tumor-bearing mice experiments indicated that DPPA-1 could also effectively disrupt the PD-1/PD-L1 interaction in vivo (Fig.5).

Researchers at Aurigene Ltd. developed compound (**10**) for the treatment of cancer. Sequences of the extracellular domain of PD-1 critical for the PD-L1/PD-L2 binding interaction, overlapping or in close proximity to the known ligand binding regions, were identified and used as starting points for the design and evaluation of 7- to 30-mer peptides derived from human and murine PD-1 sequences. Compound (**10**) is highly effective in antagonizing PD-1 signalling, with in vivo exposure upon subcutaneous dosing. It is claimed to inhibit tumour growth and metastasis in preclinical models of cancer and to be well tolerated with no obvious toxicity at any of the tested doses. The structure of compound (**10**) is shown in (Fig.5).¹³

BMS chemists disclosed macrocyclic peptides with general structure (**11**) that inhibit the PD-1/PD-L1 with nanomolar potency in HTRF assays, as well as cellular binding assays (Fig.5).⁸ In addition, the peptides also demonstrate biological activity in CMV recall and HIV Elispot assays demonstrating their utility in ameliorating hyperproliferative disorders such as cancer. The peptides are competing with the binding of PD-L1 with anti-PD-1 monoclonal antibody nivolumab (BMS-936558, MDX-1106) that are known to block the interaction with PD-1, enhancing CMV-specific T-cell IFN γ secretion and enhancement of HIV-specific T-cell IFN γ secretion.

3. EXPERT OPINION

Small molecules and peptide antagonists of the PD-1/PD-L1 interaction are highly sought after, since they could have considerable therapeutic advantages over the current clinically used mAbs. The PD-1/PD-L1 target consists of a very hydrophobic, large and flat protein-protein interaction and would be classically described as ‘undruggable’ by small molecules. Nonetheless, several compound classes have been described as PD-1/PD-L1 antagonists. Currently the majority of compound classes seem to competitively antagonize the PD-1/PD-L1 interaction and no allosteric mechanism was established. It should be mentioned that care has to be taken during the screening process to avoid false positives by carefully triaging compound hits. The sequence and structural differences between mouse and human PD-1/PD-L1 should be taken into account when recombinant proteins or mouse models are used to screen compounds. The hydrophobicity and large molecular weight of direct PD-1/PD-L1 antagonists might also result in development issues such as low target selectivity, poor oral bioavailability, low solubility, fast metabolism and toxicity. For example, many compounds based on scaffold (3) have rather high cLogP. The nature of the PD-1/PD-L1 interaction has also resulted into several distinct peptides, including linear and cyclic peptides derived from interfacial epitopes or discovered by other techniques. Small molecule PD-1/PD-L1 antagonists might be advantageous over current mAbs due to their easy diffusion across physiologic barriers such as the blood–brain barrier and plasma membranes resulting in an overall better tumor tissue uptake. In contrast, large biomolecules such as mAbs largely rely on tumor endothelium permeability to penetrate solid tumors, and endocytic consumption can strongly affect their biodistribution. A recently described PD-1-targeting high affinity small protein, 100 × smaller than a mAb and lacking its Fc moiety, penetrates deeper into tumors as seen by fluorescence microscopy and, unlike antibodies, does not cause unwanted depletion of PD-L1–positive T-cells that mediate antitumor immunity.¹⁴ PD-1/PD-L1 antagonists are proven useful in the indication areas cancer and potentially useful in viral or bacterial infections and most recently therapeutic application in Alzheimers disease (AD) are also claimed. In the first area mAbs are well established as therapeutic agents, not so in the field of viral and bacterial infections. Small molecules and peptides antagonizing PD-1/PD-L1 might therefore easier penetrate the later indications. Moreover, in AD small molecules might be advantageous due to their blood-brain-penetration. The area of non-mAb based PD-1/PD-L1 targeting is at the very beginning and currently none of the discussed compound classes is reported to have progressed further to clinical trials yet. Thus it remains to be seen if the protein- (and cell-) based activities translate in vivo and lead to an advancement in cancer treatment and/or in the anti-infective or AD areas in clinical trials. Advanced techniques such as radiotracers for immunoPET imaging of PD-1 checkpoint expression on tumor-infiltrating lymphocytes and PD-L1 in general might therefore become very valuable to assess the prognostic value of PD-1/PD-L1 axis targeting molecules in preclinical models of immunotherapy and may ultimately aid in predicting response to therapies targeting immune checkpoints.¹⁵

References

Reference annotations

* Of interest

** Of considerable interest

1. Dömling A, Holak TA. Programmed Death-1: Therapeutic Success after More than 100 Years of Cancer Immunotherapy. *Angewandte Chemie International Edition*. 2014; 53:2286–88. [PubMed: 24474145]
2. Keir ME, Butte MJ, Freeman GJ, Sharpe AH. PD-1 and its ligands in tolerance and immunity. *Annu Rev Immunol*. 2008; 26:677–04. [PubMed: 18173375]
3. Day CL, Kaufmann DE, Kiepiela P, et al. PD-1 expression on HIV-specific T cells is associated with T-cell exhaustion and disease progression. *Nature*. 2006; 443:350–54. An excellent paper about PD-1 expression on human immunodeficiency virus (HIV)-specific CD8 T-cells. [PubMed: 16921384]
4. Carvahlo S, Levi-Schaffer F, Sela M, Yarden Y. Immunotherapy of cancer: from monoclonal to oligoclonal cocktails of anti-cancer antibodies: IUPHAR Review X. *Br J Pharmacol*. 2016; 173:1407–24. [PubMed: 26833433]
5. Wolchok JD. PD-1 Blockers. *Cell*. 2015; 162:937. [PubMed: 26317459]
6. Zak KM, Radoslaw K, Sara P, et al. Structure of the Complex of Human Programmed Death 1, PD-1, and Its Ligand PD-L1. *Structure*. 2015; 23:2341–48. An excellent paper describing the interactions of Human Programmed Death 1, PD-1, and Its Ligand PD-L1. [PubMed: 26602187]
7. President and Fellows of Harvard College. Modulators of immunoinhibitory receptor PD-1, and methods of use thereof. WO2011/082400 A3. 2011.
8. Bristol-Myers Squibb Company. Compounds useful as immunomodulators. WO2015/034820 A1. 2015.
9. Zak KM, Grudnik P, Guzik K, et al. Structural basis for small molecule targeting of the programmed death ligand 1 (PD-L1). *Oncotarget*. 2016; An excellent paper about small molecules targeting The programmed death ligand 1 (PD-L1) along with the first x-ray structure. doi: 10.18632/oncotarget.8730
10. Hanley RP, Horvath S, An J, et al. Salicylates are interference compounds in TR-FRET assays. *Bioorg Med Chem Lett*. 2016; 26:973–77. [PubMed: 26733476]
11. Aurigene Discovery Technologies Limited. 1,3,4-oxadiazole and 1,3,4-thiadiazole derivatives as immunomodulators. WO2015/033301 A1. 2015.
12. Chang HN, Liu BY, Qi YK, et al. Blocking of the PD-1/PD-L1 Interaction by a D-Peptide Antagonist for Cancer Immunotherapy. *Angewandte Chemie International Edition*. 2015; 54:11760–64. An excellent paper describing the use of hydrolysis-resistant D-peptide antagonists targeting the PD-1/PD-L1 interaction. [PubMed: 26259671]
13. Aurigene Discovery Technologies Limited. Immunosuppression modulating compounds. US2011/0318373 A3. 2011.
14. Mautea RL, Gordona SR, Mayere AT, et al. Engineering high-affinity PD-1 variants for optimized immunotherapy and immuno-PET imaging. *PNAS*. 2015; 112:6506–14.
15. Baruch K, Deczkowska A, Rosenzweig N, et al. PD-1 immune checkpoint blockade reduces pathology and improves memory in mouse models of Alzheimer's disease. *Nature Medicine*. 2016; 22:135–7. An excellent paper suggesting that immune checkpoints may be targeted therapeutically in AD.

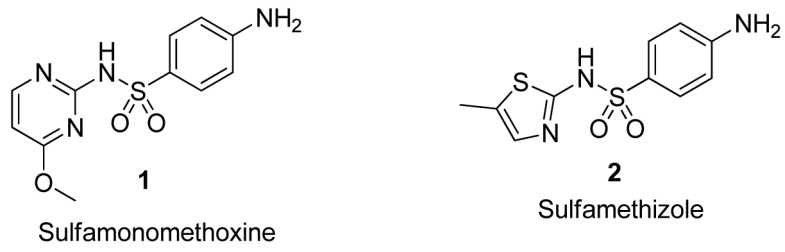


Fig. 1.
Small-molecule antagonists of the PD-1 pathway.

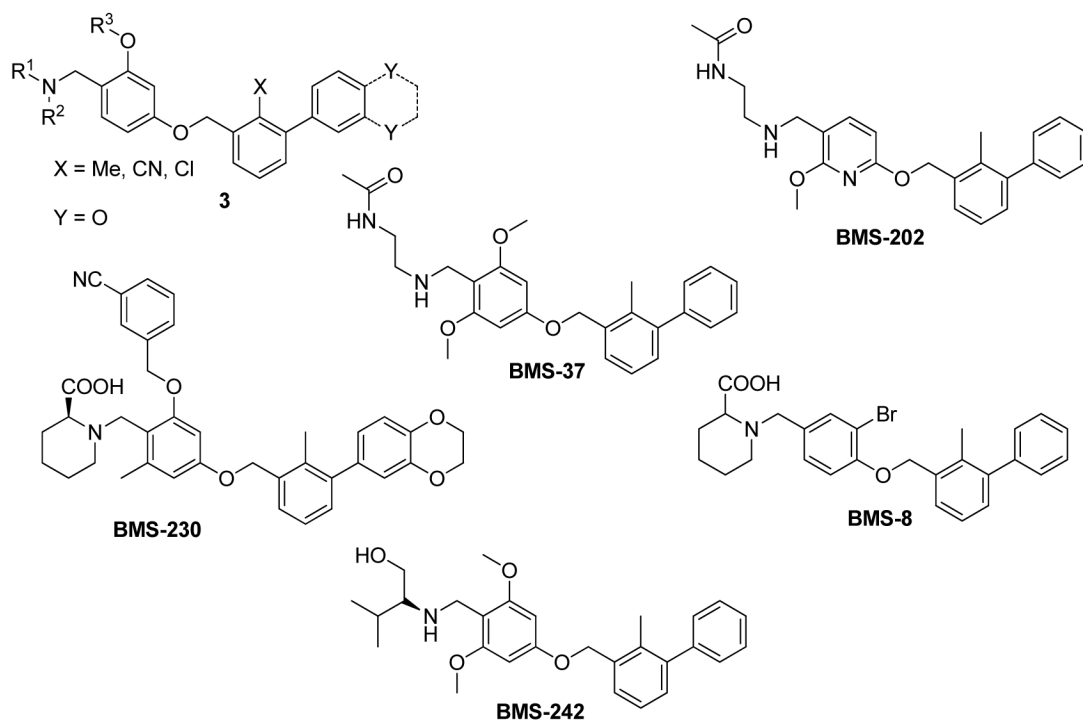


Fig. 2.
PD-1/PD-L1 inhibitors synthesized by BMS.

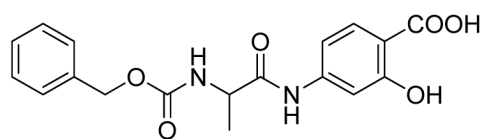
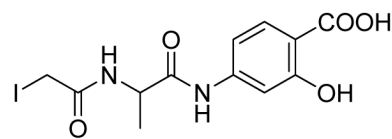
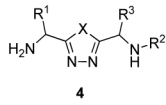
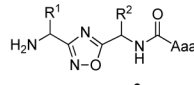
**NCI 211717****NCI 211845**

Fig. 3.
False positive PD-1/PD-L1 inhibitors



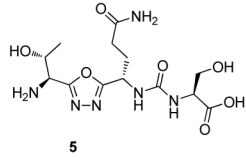
4

X= O or S
 R¹= Ser, Thr, Phe, Ala or Asn
 R²= H or -CO-Aaa
 R³= Ser, Ala, Glu, Gln, Asn or Asp

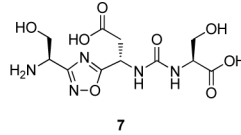


6

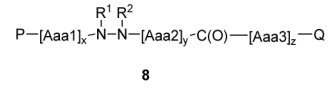
R¹= Ser, Thr
 R²= Glu, Gln, Asn, Asp
 Aaa= Thr or Ser



5

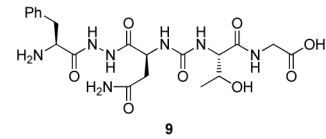


7



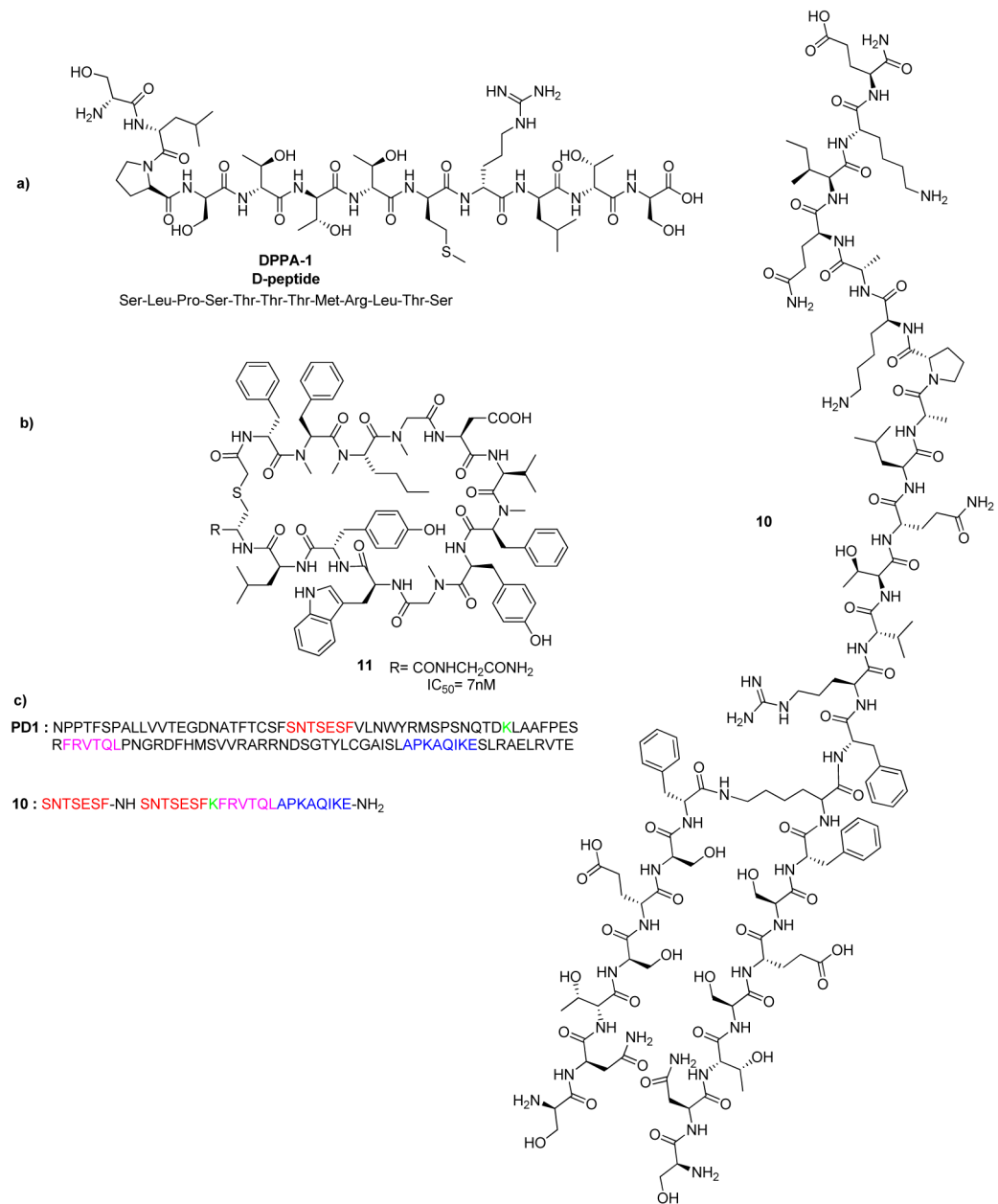
8

x, y, z are integers from 1 to 2
 Aaa1, Aaa2, Aaa3 are aminoacids
 P = N-terminus of Aaa1
 Q = C-terminus of Aaa3
 R¹ and R² are hydrogen or alkyl



9

Fig. 4. Peptidomimetic inhibitors of the PD-1/PD-L1 interaction with oxa- and thiazole core moieties

**Fig. 5.**

a) Hydrolysis-resistant D-peptide antagonist to target the PD-1/PD-L1 b) Macrocyclic peptidic inhibitor c) Peptide antagonist of PD-L1 (**10**) along with its sequence similarities to PD-1