Development of small molecule PET-tracers targeting PD-L1

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Introduction and Objective

Immune checkpoints are receptor-ligand systems and regulate immune responses by stimulation or inhibition. Programmed cell death protein 1 (PD-1) and its ligand Programmed cell death ligand 1 (PD-L1) function as an inhibitory checkpoint and upon binding negatively regulate immune responses, obstruct T cell signaling and proliferation, induce apoptosis and prevent excessive immune reactions and autoimmunity for self-tolerance.

Cancer cells may abuse this mechanism for immune evasion by overexpression of PD-L1. PD-L1 expression is used as a predictive biomarker for anti-PD-1/PD-L1 immunotherapy. The current method for quantifying PD-L1 expression by immunohistochemistry is confronted with intrinsic problems of invasive sampling (biopsy) as well as heterogeneous expression of PD-L1.

For more accurate patient stratification, positron emission tomography with PD-L1 selective tracers may be an alternative to immunohistochemistry allowing for non-invasive and concurrent expression measurements in primary and metastatic tumors.

Methods

- Select commercially available small-molecule precursor candidates with known affinity towards PD-L1 by literature research
- Synthesize new potential PET-tracer candidates by modification of precursors applicable for PET-tracer synthesis (e.g. methylation)
- Determine the lipophilicity (log*P*) according to an HPLC method[1] as well as in-solution stability (pH 7.4, 0°C or room temperature) and cytotoxicity using PD-L1 expressing CHO-K1 cells and MTT assay
- Evaluate binding affinities (IC_{50}) using a cell-free homogeneous time-resolved fluorescence (HTRF) assay from Cisbio[2], which is based on competitive binding of the ligands with PD-L1 (5 nM) and PD-1 (50 nM)
- Establish radiolabeling and optimize reaction conditions utilizing an automatable GE TRACERlab FX C Pro synthesis module
- Identify advantageous substructures using extensive literature research, LigandScout software and a manually curated chemical database of bioactive molecules with drug-like properties (ChEMBL)

Results

- ✓ Identification of essential features of the ligands pharmacophores using LigandScout software and published PD-L1/small-molecule co-crystal structures
- ✓ Successful synthesis of four potential small-molecule ligands based on two commercially available precursors with known affinity[3] by O- and N-methylation (Figure 1)
- ✓ The measured log*P* values were in line with the general perception that methylation increases lipophilicity (Table 1); It should be noted that higher log*P* values may lead to additional non-specific binding
- ✓ Precursors and methylated products show reasonable stability at both 0°C (Table 1) and room temperature (data not shown)
- ✓ IC_{50} values were successfully determined with a HTRF assay and GraphPad Prism 7 software using the non-linear regression, variable slope (four parameters) curve fitting. In general, methylation led to a decrease of binding affinity (Table 1)
- ✓ The radiolabeling of two products was successfully established and optimizations regarding reaction temperature, reaction time and precursor amount resulted in decay corrected radiochemical yields of up to 48.6 and 53.5%, respectively, with regard to the activity of the [^{11}C]methylating agent (Table 1)
- ✓ Substructures were identified using the LigandScout software pointing the way towards improved compounds

Conclusion

The synthesized ligands show affinity towards PD-L1. However, methylation of functional groups reduced the binding affinity. With the knowledge of the binding mechanism and the underlying structural framework, the future synthesis of high-affinity PD-L1 ligands can be directed towards substances with lower lipophilicity and higher binding affinity.

Acknowledgments

Many thanks to Univ.-Prof. Mag. Dr. Thierry Langer for the access to the LigandScout software, to Dr. Katharina Pallitsch and her research group for the NMR and mass spectrometric measurements, and to the MIC research cluster. This project is supported by the Austrian Federal Government within the framework of the COMET K1 Centre Programme of the province of Styria and the province of Vienna.

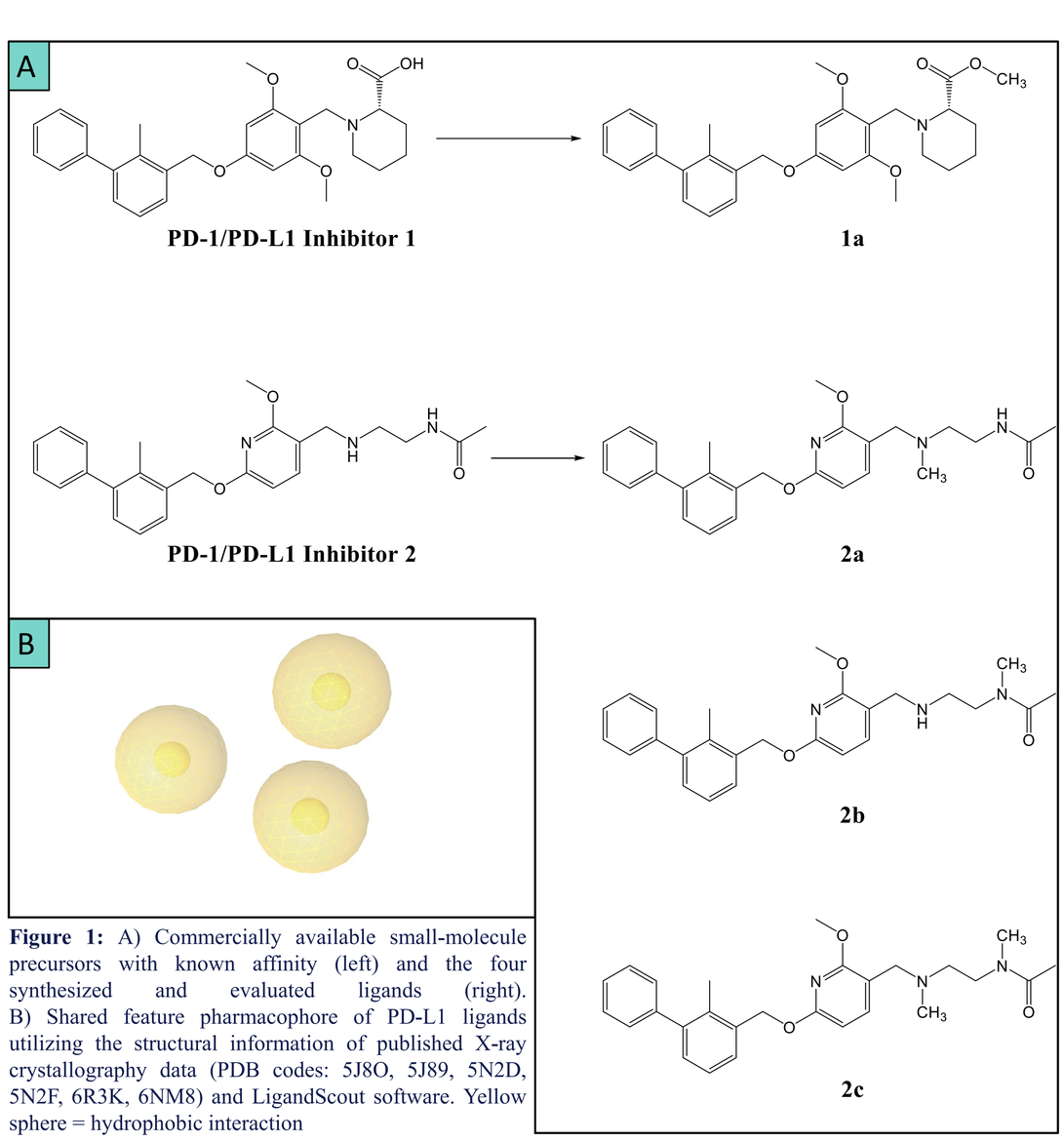
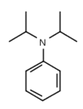
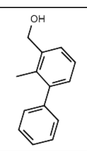
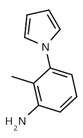
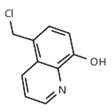


Figure 1: A) Commercially available small-molecule precursors with known affinity (left) and the four synthesized and evaluated ligands (right). B) Shared feature pharmacophore of PD-L1 ligands utilizing the structural information of published X-ray crystallography data (PDB codes: 5J8O, 5J89, 5N2D, 5N2F, 6R3K, 6NM8) and LigandScout software. Yellow sphere = hydrophobic interaction

Table 1: Measured HPLC log*P* values, evaluated binding affinities (IC_{50} values), optimized radiochemical yields (RCY), toxicological EC_{50} values and in-solution stability followed for 20 days.

Substance	μHPLC log$P_{OW}^{pH\ 7.4}$	IC_{50} [nM]	RCY [%]	Cytotoxicity EC_{50} [μM]	In-solution stability [%]
PD-1/PD-L1 Inhibitor 1	3.16 ± 0.16	154 ± 37	-	52.3 ± 11.7	96
1a	4.90 ± 0.27	3746 ± 399	48.6	-	86
PD-1/PD-L1 Inhibitor 2	3.88 ± 0.12	69 ± 17	-	24.9 ± 4.1	99
2a	4.13 ± 0.16	232 ± 16	53.5	-	99
2b	-	398	-	-	95
2c	4.28 ± 0.18	677 ± 84	-	-	99

Table 2: Top 4 substructures of the essential pharmacophore identified with LigandScout and a molecule database. The shared feature pharmacophore obtained from available and valid X-ray crystallography data (Figure 1: B) was screened against a ChEMBL database containing 34207 molecules and the Pharmacophore-Fit Scores and Binding Affinity Scores of 2695 hits were calculated. All hits were aligned to the (hydrophobic) pharmacophore of PDB 5J89 (Ligand: PD-1/PD-L1 Inhibitor 2) to include exclusion volumes of the protein – leaving 1295 hits. Substances were ranked according to their normalized scores (i.e. 10 points per category; max score = 40 points). Note: Good Binding Affinity Scores are given by negatives values.

Structure	Pharmacophore-Fit Score	Binding Affinity Score	Aligned Pharmacophore-Fit Score	Aligned Binding Affinity Score	Points
	38.86	-27.29	38.77	-26.91	**37.30**
	38.81	-24.51	38.85	-23.22	**36.26**
	38.78	-24.21	38.76	-24.89	**36.21**
	38.84	-23.32	38.61	-24.21	**35.70**

References

[1] Vraka C, et al. Log*P*, a yesterday's value? Nucl Med Biol. 2017; 50:1-10.
[2] PD1/PD-L1 Binding Assay Kit (Cisbio Bioassays, part no. 64ICP01PEG)
[3] Bristol-Myers Squibb Company. Compounds useful as immunomodulators. WO 2015/034820 A1.