Chapter 3

Design, synthesis, and biological evaluation of imidazopyridines as PD-1/PD-L1 antagonists


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This chapter is published


DOI: 10.1021/acsmedchemlett.1c00033
ABSTRACT

The PD-1/PD-L1 axis has proven to be a highly efficacious target for cancer immune checkpoint therapy with several approved antibodies. Also, small molecules based on a biphenyl core can antagonize PD-1/PD-L1 leading to the in vitro formation of PD-L1 dimers. However, their development remains challenging as we do not understand their mode of action fully yet. Here, we designed a new scaffold based on our previously solved high-resolution structures of small molecular weight inhibitors bound to PD-L1. A small compound library was synthesized using the Groebke-Blackburn-Bienaymé multicomponent reaction (GBB-3CR) resulting in the SAR of imidazo[1,2-a]pyridine-based inhibitors. These inhibitors were tested for their biological activity using various biophysical assays and cellular PD-1/PD-L1 blockade assay giving potent candidates with low µM PD-L1 affinities. An obtained PD-L1 co-crystal structure reveals the binding to PD-L1. Our results open the door to an interesting, bioactive scaffold that could lead to a new class of PD-L1 antagonists.

KEYWORDS

PD-L1 inhibitor, multicomponent reaction, Groebke-Blackburn-Bienaymé, imidazo[1,2-a]pyridine
INTRODUCTION

In the recent decades oncology has been revolutionized by immunotherapy.\(^1\) In particular, immune checkpoint blockade (ICB) targeting the PD-1/PD-L1 axis has shown impressive clinical benefit, with durable regression and even cure in a subset of hard-to-treat cancers.\(^2,3\) In general, ICB-responsive cancers are characterized by high levels of mutations and corresponding neo-antigens (“hot” tumors). These neo-antigens can be recognized by immune effector T cells, that under homeostatic conditions would result in cancer cell elimination.\(^4\) In cancer, this elimination is restrained by the immune checkpoint PD-1 expressed on T-cells. PD-1 binds to PD-L1 expressed on cancer cells, resulting in an inhibitory intracellular signaling cascade preventing proper T-cell activation.\(^5\) Consequently, inhibition of the interaction of PD-L1 and PD-1 receptors promotes T-cell activation. Several PD-1 and PD-L1 directed antibodies are in clinical use and numerous experimental ones are under development. However, current PD-1/PD-L1 directed therapies are useful only for a small subset of patients, are expensive to produce, have a risk of adverse effects, and show development of resistance, which limit their utility.\(^6,7\) Therefore, novel therapeutic modalities such as small molecules or peptides exhibit a lot of promise.\(^8,9\) The only small molecular inhibitor targeted against PD-L1 currently undergoing clinical trial is CA-170 (Figure 1B).\(^10\) However, it was recently proved, using various functional cell assays, not to be a direct binder to PD-1 or PD-L1 and its mode-of-action remains unclear.\(^11\)

As part of our ongoing efforts to understand and develop small molecules antagonizing PD-1/PD-L1, we present here the design, synthesis, biological activity and structural basis of imidazopyridine as PD-1/PD-L1 antagonists.\(^8,11-14\).
RESULTS AND DISCUSSION

Our recently published co-crystal structures of several small molecules binding to PD-L1 have been used to propose a generalized pharmacophore model for small molecule PD-L1 binder (Figure 1A). These structures triggered a wave of small molecule designs and subsequent patent applications. Accordingly, a twisted biphenyl moiety is linked via a two atom linker with a planar (hetero)aromatic ring fragment which has a methanamine para to the linker moiety. Symmetrical central biphenyl moieties with two times the linker (hetero)aromatic fragment have been also described as highly potent PD-L1 binders. The diversity of PD-L1 small molecule scaffolds based on our proposed pharmacophore model and claimed in patents is great (Figure 1B). The biphenyl component allows for less variations, however the linker and (hetero)aromatic moieties can be executed in a variety of designs. Finally, the water exposed part of molecule allows for many variations useful to tune drug-like properties such as water solubility.

To circumvent the lengthy and linear sequential syntheses of many small molecule PD-1/PD-L1 antagonists, we decided to explore multicomponent reactions for the one-pot assembly of the central (hetero)aromatic part of the pharmacophore model. For this, we choose the Groebke-Blackburn-Bienaymé reaction (GBB-3CR) which is a versatile three component reaction of heterocyclic amidines, aldehydes, and isocyanides giving access to ‘drug-like’ molecules (Figure 1C). A key fragment of the scaffold is a bicyclic imidazo-ring.

Figure 1. Design of PD-1/PD-L1 antagonists. A) Generalized pharmacophore model of PD-L1 antagonist. Aromatic (purple), hydrophobic (green) and basic (positive charged, blue) pharmacophores. B) Examples of potent PD-L1 dimer taken from patent literature. The pharmacophore is indicated by red and blue colors. C) Design of imidazopyridines accessible by GBB-
In our design, the bicyclic heteroaromatic moiety (**Figure 1C**, grey) is the central element of the scaffold having attached three suitable substituents including the biphenyl (**Figure 1C**, blue), methanamine (**Figure 1C**, yellow) and amino (**Figure 1C**, green) moieties. To decide on the position of the substituents, we used molecular-modeling performed via Moloc and Scorpion software (**Figure 1D**). The best fit into the receptor was to introduce the methanamine moiety by the GBB-3CR aldehyde component and the biphenyl moiety through a C-O coupling into the aminopyridine linker. Following the design and docking studies (**Figure 1D**), we opted for the imidazopyridine containing scaffold (**Figure 1C**) starting with the development of a corresponding synthesis route that yields the established scaffold.

We envisioned a synthetic route in which the step with the highest introduction of variation should be carried out last. Therefore, the GBB reaction was chosen as one of the last stages. Consequently, the preparation of the biphenyl substituted amidine was approached first. We started synthesizing the twisted biphenyl moiety via the Suzuki cross coupling reaction implementing (3-bromo-2-methylphenyl)methanol (2) and unprotected boronic acid (1a-c). Using Pd(dppf)Cl₂ as a catalyst in a solvent system consisting of toluene, ethanol, and saturated aqueous sodium hydrogen carbonate solution (5:1:5) and heating this for 12 hours at 85 °C generated the compounds 3a-c in excellent yields of 90-97%, respectively.

![Scheme 2. Synthetic route of compounds 5a-f. Reagents and conditions: a) Pd(dppf)Cl₂, toluene:ethanol:NaHCO₃ (sat. aq.) (5:1:5; 0.3 M), 85 °C, 12h; b) 5-fluoro-2-nitropyridine (4a) or 5-fluoro-4-methyl-2-nitropyridine (4b), KOH, dry DMSO, 0 °C-RT, 5 min-1h; c) Fe, HCl; EtOH:H₂O (5:1; 0.1M), 2h, reflux; R¹ = H, p-F, [3,4]-O(CH₂)₄; R² = H, CH₃.](image-url)

Following the Suzuki reaction, we selected the nucleophilic aromatic substitution involving 3a-c and 5-fluoro-2-nitropyridines (4a) or 5-fluoro-4-methyl-2-nitropyridine (4b, SI) as the best option to prepare the precursor to the amidines (6a-d).

By adding finely ground potassium hydroxide in dry DMSO at 0 °C, generating a superbasic medium, in presence of 3a-c and 4a-b, we were able to synthesize the corresponding products 5a-d with quantitative conversion and
with brief reaction times of 5 min for 5a-c and 1 h for 5d. An extraction step was performed and the resulting crude oil was utilized in the subsequent step. Lastly, to generate the desired amidines 6a-d, reduction of 5a-d was performed applying hydrochloric acid and iron powder in ethanol/water (5:1) under reflux conditions, to avoid a possible benzyl ether cleavage of the biphenylic component, i.e., via a classical Pd/C H₂ reduction. Following two hours reaction time and chromatographic purification, the required amidines were obtained in yields from 72-92%.

Having the desired amidines in hands, we were able to execute the Groebke-Blackburn-Bienaymé reaction. Based on the docking studies, we selected phenyl-containing isocyanides for the possibility of generating π-stacking with Tyr-123 in the pocket of the PD-L1 dimer. As aldehyde component we chose polar substrates to increase polarity, solubility, and the possibility of potential hydrogen bonding. Therefore, we used tert-butyl (2-oxoethyl)carbamate and both enantiomers of the Garner aldehyde (S)-(−)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde and (R)-(−)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde.

![Scheme 3. GBB-reaction. Reagents and conditions: a) Sc(OTf)₃ 5mol%, DCM:MeOH (2:1, 0.3M), 1h, 120 °C, microwave assisted heating; B: 7N HCl in 2-propanol, RT, 20h.](image)

We found the optimal reaction conditions for the used substrates to be Scandium triflate (10 mol%) as catalyst, DCM/MeOH (2:1) as the solvent system, a concentration of 0.3 M in regard to the amidine, and 1.7 equivalents of isocyanide and aldehyde components. Microwave assisted heating for 1h generated the corresponding GBB product in good to excellent yields (48-86%). Subsequently, a chromatographic purification on silica was performed. This purification step yielded generally in still slightly impure compounds. Therefore, we continued with the deprotection of the tert-butyl carbamate group and, regarding compounds 9d & 9e, of the additional aminal protecting group. Performing the deprotection with 7N HCl in 2-propanol at room temperature for 20h exhibited full conversion. Following an additional chromatographic purification over silica, the final compounds 9a-k (Chart 1) were obtained and were analyzed via HRMS and NMR (¹H and ¹³C).
For compound 9f, an adjusted synthesis route was chosen where instead of (3-bromo-2-methylphenyl)methanol we used 1-(benzyloxy)-3-bromo-2-methylbenzene (A) for the Suzuki reaction yielding 3-(benzyloxy)-2-methyl-1,1'-biphenyl (B). Additionally, a hydrogenation was performed with Pd/C and H₂ (1 bar) in methanol for 2h at 40 °C reaching full conversion to 2-methyl-[1,1'-biphenyl]-3-ol (C). The remainder of the synthesis route was performed analogous to the other compounds leading to compound 9f.

Moreover, we also explored a post-modification of compound 9h. Hereby, we formed a tetrazole from the cyanide function on the isocyanide substrate resulting in compound 9i. Therefore, compound 9h was reacted with sodium azide and zinc chloride in n-propanol at 95 °C for 20h. After chromatographic purification and subsequent deprotection we achieved a yield of 39% of compound 9i. Conceptually, we expected that this modification will increase the affinity via hydrogen bonding between the tetrazole, being a carboxylic acid isostere, with Arg125.

To support our binding hypothesis from protein-ligand docking we performed binding studies, which included NMR studies of ligand-PD-L1 binding and Homogeneous Time Resolved Fluorescence (HTRF) assay with PD-1 and PD-L1 proteins. Their binding to PD-L1 was verified using NMR titration and HTRF assay (SI). Determination of binding of compounds 9 to PD-L1 was carried out with the ¹H NMR. The line width broadening in the proton NMR of PD-L1 suggests that the compounds induce protein oligomerization. In all the cases, the well resolved
narrow resonance peaks in the aliphatic region of $^1$H NMR spectrum of apo-PD-L1 exhibited significant broadening upon addition of each compound 9 indicating significant increase in the molecular weight of the complex (Figure 2A). The molecular weight of each complex estimated from relaxation time analysis, which can only be explained by the compound induced PD-L1 dimerization. No significant changes were observed upon addition of the PD-1 with the tested compounds. The results of the NMR titration experiment demonstrated that our synthesized compounds generally disrupt the PD-1/PD-L1 complex and bind to PD-L1, as well as induce dimerization of PD-L1.

Figure 2. Binding studies. A) The aliphatic part of $^1$H NMR spectrum of PD-L1 (blue) and PD-L1 with compounds 9a (red), 9b (green), 9c (purple), 9f (orange) in the molar 1:1. B) HTRF scouting based on 50 µM concentration. C) HTRF inhibition assay and calculated IC$_{50}$ values for the best compounds from HTRF scouting.
HTRF experiments were conducted to further prove the ability of our compounds to disrupt the PD-1/PD-L1 complex and for IC\textsubscript{50} value determination. The results of the HTRF experiment proved that all the tested compounds were capable of disrupting PD-1/PD-L1 complex showing the potential of this scaffold. HTRF scouting revealed that parts of GBB-3CR scaffold selected as binding parts in docking and co-crystallization studies (represented here as compounds 3a, 6, B) have some disruption potential, however full structure is needed for effective PD-L1 blocking, which we report in compounds 9a-9e and 9j-9k. Phenylether (9f) and benzylether (9a) analogues show different affinity to the target protein - presence of linking methyl group increase the binding potential. Fluorine substitution in biphenyl core is not tolerated (9g-9i), whereas dioxane addition is accepted (9j-9k). Additionally, methyl group present in imidazopyridine ring (9j) improves inhibitory activity of GBB-3CR scaffold. IC\textsubscript{50} values were determined for selected compounds. Exhibiting IC\textsubscript{50} values in the range of 1.8 – 22.9 µM, the compounds with the lowest IC\textsubscript{50} were 9b (9.3 µM) and 9j (1.8 µM).

Next, the molecular basis of the interaction between 9c and the PD-L1 dimer was elucidated by X-ray structure analysis (Figure 2). The resolution is 2.45 Å and the electron density of 9c shows well, with the exception of the phenyl moiety of the phenylethyl fragment. 9c is mostly buried in the deep and elongated receptor site which is composed of two PD-L1 monomers. In the absence of 9c the protein dimer complex has C\textsubscript{2} symmetry. The binding of the small molecule however breaks the symmetry. The biphenyl methylene moiety is embedded by two and three antiparallel \(\beta\)-sheets of monomer A and B, respectively. Monomer A (in green) contributes with the 11 amino acids Lys124, Tyr56, Ala121, Ser93, Ser117, Ile54, Met115, Tyr123, Arg125, Lys124, and Asp 122 to the binding pocket. Monomer B (in red) contributes with the 12 amino acids Tyr56, Met115, Tyr123, Ala121, Ser117, Gln66, Ile54, Asp122, Ala121, Ser117, Ile54, and Gln66 to the binding pocket. The imidazopyridine moiety is embedded by Tyr56 and Gln66 of monomer B and Tyr123, Asp122, and Ala121 of monomer A. The phenylethylamine substituent is surrounded by Lys124, Arg125, and Tyr123 of monomer A and the hydroxy group of Tyr56 of monomer B. Finally, the methanamine is largely water-exposed.
Figure 3. 9c binding to PD-L1 dimer. A) 2D structure of 9c. B) Extracted 3D structure of 9c. C) Overall view of 9c at the interface of two PD-L1 monomers (A and B, shown in green and red colors). D-F) Scorpion software ligand-receptor interaction analysis. D) vdW interactions between 9c and the PD-L1 dimer. E) π-Stacking interactions. F) Charge-charge interactions between Asp122 and the solvent exposed methylene amine substituent.

9c undergoes a diversity of hydrophobic, π-stacking, and charge-charge interactions (Figure 3D). Ring A of the biphenyl moiety is the deepest buried part of 9c. The ring makes a short T-shaped π-stacking contact of 3.8 Å with the amino acid Tyr56 of monomer A (Figure 3E). Plenty of hydrophobic contacts between ring A and Tyr123(B), Ile54(A), Ala121(B), Ser117(A), and Met115(A) can be observed. The twisted C-C bond connecting rings A and B is approximately at the position of the C2 axis of the imaginary PD-L1 dimer complex (A-B). The dihedral angle defined by the two planes of the biphenyl is 124° (Figure 3B). This is comparable to other dihedral angles of similar molecules, e.g. BMS1166. The ortho-methyl group helps to keep the biphenyl moiety in a twisted shape close to the receptor conformation. In other compound designs the ortho-methyl group was replaced by a bromide, chloride, or nitrile in ortho position. The oxymethylene linker between the biphenyl and
imidazopyridine fragments is rather flexible and in other designs can be an alkene, however missing structural data.  

Why is the imidazopyridine compound 9c showing a rather low PD-L1 affinity? Detailed analysis of the cocrystal structure reveals that the π-stacking interaction of pyridine and the amino acid Tyr56(A) is suboptimal due to the low overlap of the two aromatic rings. Also, the electrostatic of this interaction is poor as the directions of the two dipole moments are parallel-aligned (Figure 3E). The distance between Asp122(B) and the solvent exposed methylene amine substituent is rather long with 4.2 Å and 4.8Å, resulting in a poor charge-charge interaction (Figure 3F). Moreover, the largely hydrophobic pocket formed by Ile54(A), Val68(A), and Gin66(A) is not filled.

To understand the molecular basis accounting for the differences in affinity between 9c and 9j, we docked compound 9j to the dimer of PD-L1 (6R3K). The predicted binding mode of 9j matches perfectly the overall orientation of 9c and reveals the origins of greater potency of 9j (Figure 4). First, the presence of extended biphenyl is predicted to induce structural rearrangement of Tyr56 from Monomer B. Thus, 9c occupies larger surface of PD-L1 dimer and forms an open channel between monomers as it has been previously shown for compounds bearing ‘extended’ biphenyl ring system with the 2,3-dihydrobenzo[ ]1,4-dioxine moiety. Second, the application of shorter linker between imidazopyridine core and ring C locates the latter one closer to the surface of Monomer B and allows for additional π-stacking with Tyr123, which are not observed in case of 9c (Figure 4).

![Figure 4. Comparison of binding modes of 9c and 9j. Superimposition of 9c from co-crystal structure with anticipated orientation of 9j obtained from molecular docking; additional π-π stacking interactions highlighted as red dashes, transparent amino acids denote residues anticipated to change their conformation upon binding of 9j to PD-L1 dimer.](image-url)
CONCLUSIONS

Inspired by our previously published cocrystal structures of PD-L1, we designed a novel scaffold, imidazopyridine, as a PD-L1 dimer. Exploiting the principal of MCR, imidazopyridines can be conveniently synthesized by the GBB-3CR with high variations of aldehydes, aminopyridines, and isocyanides. A small library consisting of 11 compounds was synthesized and tested on their efficacy through various biological assays, exhibiting IC50 values between 16.8–1.8 µM. We were further able to co-crystallize compound 9c with PD-L1, providing an explanation for the rather low affinities of the compound series. Overall, 9c can be regarded as a model compound which indicates the need of further improvements to achieve practical affinities.
REFERENCES


EXPERIMENTAL SECTION

GENERAL EXPERIMENTAL PROCEDURES

Procedure A: General procedure for the Suzuki-reaction

A mixture of (3-bromo-2-methylphenyl)methanol (1 eq.), boronic acid (1.5 eq.) in toluene:ethanol:sat. aq. sodium bicarbonate solution (5:1:5, 0.3 M) was placed under nitrogen and degassed for 10 minutes. [1,1’-Bis(diphenylphosphino)ferrocene]palladium(II) chloride (0.5 mol%) was added and the reaction mixture was heated at 85 °C for 12 h. Ethyl acetate and water were added to the reaction mixture. The organic phase was washed with 1M sodium hydroxide solution and brine. The organic extract was dried over magnesium sulfate and concentrated by rotatory evaporation. The residue was purified by silica gel flash chromatography using EA/PE as eluent.

Procedure B: General procedure for the nucleophilic aromatic substitution & reduction

A solution of the hydroxide component (1 eq.) and para-fluoro-nitropyridine component (1 eq.) in DMSO (1 M) was stirred at 0 °C. Potassium hydroxide (2 eq.) was finely ground and added to the reaction mixture. The reaction mixture was stirred at RT until complete conversion (TLC monitoring, 10-60 min.). Ethyl acetate was added to the reaction mixture followed by cold water. The water phase was extracted thrice with ethyl acetate. The combined organic phases were washed with brine and dried over magnesium sulfate. Concentration of the organic phases resulted in orange oil. The crude product was used without further purification for the next reaction.

A suspension of nitropyridine component (1 eq.), ethanol:water (5:1, 0.1 M), iron (10 eq.) and hydrochloric acid (37%, 3 eq.) was stirred at reflux for 2 h (TLC monitoring). After completion, the reaction mixture was concentrated by rotatory evaporation, diluted with sat. bicarbonate solution and ethyl acetate where added. The water phase has been extracted thrice with ethyl acetate. The combined organic phase was filtrated over magnesium sulfate and silica, concentration resulted in an orange solid. The residue was purified by silica gel flash chromatography using MeOH/DCM as eluent.

Procedure C: General procedure for Groebke–Blackburn–Bienaymé reaction & deprotection reaction

A mixture of aminopyridine component (1 eq.), aldehyde (1.7 eq.), scandium triflate (0.1 eq.) and isocyanide (1.7 eq.) in DCM/MeOH (2:1, 0.3 M) was placed in a glass microwave vial and sealed. The reaction vial was heated in a microwave for 1 hour at 120 °C. Evaporation of the solvents was followed by purification by silica gel flash chromatography using EA/PE as eluent. The still slightly impure product was used directly in the further step. Afterwards the crude product was treated with 50 eq. 7N HCl in 2-Propanol for 20h at rt. Concentration of
the reaction mixture under vacuum resulted in an orange oil. Chromatographic purification on silica gel (DCM/MeOH) resulted in the corresponding products.

1-(benzyloxy)-3-bromo-2-methylbenzene (A)

Benzyl alcohol (20 mmol) was added to a suspension of NaH (20 mmol) in N-methylpyrrolidone. The freshly prepared solution was added to 1-bromo-3-fluoro-2-methylbenzene (5.41 mmol) in N-methylpyrrolidone. The reaction was heated at 100 °C until complete conversion of the starting material (TLC monitoring). Ethyl acetate was added to the reaction mixture followed by water. The water phase was extracted thrice with ethyl acetate. The combined organic phases were washed with brine and dried over magnesium sulfate. Concentration and chromatographic purification over silica using EA/PE as eluent afforded A (1.38g, 4.98 mmol, 92%) as a colorless oil, $^1$H NMR (500 MHz, chloroform-d) $\delta = 7.39 – 7.32$ (m, 4H), $7.30 – 7.26$ (m, 1H), $7.13$ (dd, $J = 8.1, 1.1$ Hz, 1H), $6.93$ (t, $J = 8.1$ Hz, 1H), $6.76$ (dd, $J = 8.1, 1.1$ Hz, 1H), $4.98$ (s, 2H), $2.36$ (s, 3H), $^{13}$C NMR (126 MHz, chloroform-d) $\delta = 157.5$, $137.0$, $128.6$, $128.0$, $127.4$, $127.3$, $127.2$, $126.0$, $124.9$, $110.6$, $70.4$, $16.0$, HRMS (APCI) m/z calculated for C$_{14}$H$_{14}$OBr [M+H]+: 277.0223, found [M+H]+: 277.0221.

3-(benzyloxy)-2-methyl-1,1′-biphenyl (B)

Synthesis according to procedure A using compound A (1.00 g, 3.6 mmol) and phenylboronic acid (658 mg, 5.41 mmol) afforded B (770 mg, 2.81 mmol, 78%) as an colorless amorphous solid, $^1$H NMR (500 MHz, chloroform-d) $\delta = 7.47$ (dd, $J = 8.3, 1.2$ Hz, 2H), $7.43 – 7.37$ (m, 4H), $7.36 – 7.30$ (m, 4H), $7.19$ (t, $J = 7.8$ Hz, 1H), $6.92$ (dd, $J = 8.3, 1.2$ Hz, 1H), $6.89$ (dd, $J = 7.8, 1.2$ Hz, 1H), $5.13$ (s, 2H), $2.20$ (s, 3H), $^{13}$C NMR (126 MHz, chloroform-d) $\delta = 157.2$, $143.6$, $142.0$, $137.6$, $129.5$, $128.7$, $128.1$, $127.9$, $127.3$, $126.9$, $126.1$, $124.9$, $122.6$, $110.4$, $70.3$, $13.7$, HRMS (APCI) m/z calculated for C$_{20}$H$_{19}$O [M+H]+: 275.1430, found [M+H]+: 275.1430.

2-methyl-[1,1′-biphenyl]-3-ol (C)

3- (benzyloxy) -2-methyl-1,1′-biphenyl (500 mg, 1.82 mmol) was hydrogenated under atmospheric hydrogen with 10% Pd/C on carbon (containing 50% water, 50 mg) as catalyst in MeOH (5 ml) and THF (2 ml) at room temperature for 72 hours. Filtration of precipitated, wash with ethyl acetate, then concentration gave the afforded C (319 mg, 1.73 mmol, 95%) as a colorless oil, $^1$H NMR (500 MHz, chloroform-d) $\delta = 7.40 – 7.33$ (m, 2H), $7.34 – 7.23$ (m, 3H), $7.07$ (t, $J = 7.9$ Hz, 1H), $6.83$ (dd, $J = 7.5, 1.3$ Hz, 1H), $6.74$ (dd, $J = 7.9, 1.3$ Hz, 1H), $5.11$ (s, 1H), $2.13$ (s, 3H), $^{13}$C NMR (126 MHz, chloroform-d) $\delta = 157.2$, $143.6$, $142.0$, $137.6$, $129.5$, $128.7$, $128.1$, $127.9$, $127.3$, $126.9$, $126.1$, $124.9$, $122.6$, $110.4$, $70.3$, $13.7$, HRMS (APCI) m/z calculated for C$_{20}$H$_{18}$O [M+H]+: 275.1430, found [M+H]+: 275.1430.
126.1, 124.9, 122.6, 110.4, 70.3, 13.7., HRMS (APCI) m/z calculated for C_{13}H_{13}O [M+H]^+: 185.0961, found [M+H]^+: 185.0960.

(2-methyl-[1,1'-biphenyl]-3-yl)methanol (3a)

Synthesis according to procedure A using (3-bromo-2-methylphenyl)methanol (2.7 g, 13.4 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride ( 51 mg, 70 µmol), and phenylboronic acid (2.46 g, 20.2 mmol) afforded 3a (2.52 g, 12.7 mmol, 95%) as a colorless solid, \(^1\)H NMR (500 MHz, chloroform-d) \(\delta = 7.37 (q, J = 8.6, 7.7 \text{ Hz}, 2H), 7.33 - 7.29 (m, 1H), 7.27 - 7.23 (m, 2H), 7.23 - 7.20 (m, 1H), 7.16 (dd, J = 7.7, 1.6 Hz, 1H), 4.74 (d, J = 5.6 Hz, 2H), 2.20 (s, 3H), 1.62 (t, J = 5.6 Hz, 1H), \(^{13}\)C NMR (126 MHz, chloroform-d) \(\delta = 142.8, 142.1, 139.3, 133.6, 129.5, 129.5, 128.1, 126.9, 126.7, 125.6, 63.9, 15.9\).

HRMS (APCI) m/z calculated for C_{14}H_{13}[M+H-H_2O]^+: 181.1012, found [M+H-H_2O]^+: 181.1009.

(3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylphenyl)methanol (3b)

Synthesis according to procedure A using (3-bromo-2-methylphenyl)methanol (5.2 g, 26 mmol), [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride ( 95 mg, 130 µmol), and (2,3-dihydrobenzo[b][1,4]dioxin-6-yl)boronic acid (6.5 g, 39 mmol) afforded 3b (6.00 g, 23.4 mmol, 90%) as a colorless solid, \(^1\)H NMR (500 MHz, chloroform-d) \(\delta = 7.35 (dd, J = 7.6, 1.6 \text{ Hz}, 1H), 7.21 (t, J = 7.6 \text{ Hz}, 1H), 7.16 (dd, J = 7.6, 1.6 \text{ Hz}, 1H), 6.89 (d, J = 8.2 \text{ Hz}, 1H), 6.80 (d, J = 2.1 \text{ Hz}, 1H), 6.75 (dd, J = 8.2, 2.1 \text{ Hz}, 1H), 4.73 (s, 2H), 4.28 (s, 3H), 2.24 (s, 3H).) \(^{13}\)C NMR (126 MHz, chloroform-d) \(\delta = 143.1, 142.6, 142.3, 139.3, 135.5, 133.7, 129.6, 126.6, 125.6, 122.6, 118.3, 116.9, 77.4, 77.2, 76.9, 64.5, 64.6, 64.0, 16.0, HRMS (APCI) m/z calculated for C_{16}H_{15}O_2 [M+H-H_2O]^+: 239.1067, found [M+H-H_2O]^+: 239.1066.

(4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methanol (3c)

Synthesis according to procedure A using (3-bromo-2-methylphenyl)methanol (2.0 g, 10 mmol) [1,1'-Bis(diphenylphosphino)ferrocene]palladium(II) chloride (37 mg, 50 µmol), and (4-fluorophenyl)boronic acid (2.1 mg, 15 mmol) afforded 3c (2.2 g, 9.7 mmol, 97%) as a colorless solid, \(^1\)H NMR (500 MHz, chloroform-d) \(\delta = 7.38 (dd, J = 7.5, 1.5 \text{ Hz}, 1H), 7.26 - 7.21 (m, 3H), 7.16 (dd, J = 7.5, 1.5 Hz, 1H), 7.12 - 7.05 (m, 2H), 4.74 (s, 2H), 2.20 (s, 3H), 1.84 (s, 1H), \(^{13}\)C NMR (126 MHz, chloroform-d) \(\delta = 162.04 (d, J_{CF} = 245.6 \text{ Hz}) 141.91, 139.42, 138.04 (d, J_{CF} = 3.5 \text{ Hz}), 133.78, 131.00 (d, J_{CF} = 7.9 \text{ Hz}), 129.70, 127.01, 125.77, 115.08 (d, J_{CF} = 21.3 Hz), 64.10, 15.96, HRMS (APCI) m/z calculated for C_{13}H_{12}F [M+H-H_2O]^+: 199.0918, found [M+H-H_2O]^+: 199.0915.
5-fluoro-4-methyl-2-nitropyridine (4b)

Sulfuric acid (15 ml, 97%) was mixed with 30% hydrogen peroxide solution (13.2 ml) at 0°C. After addition of 5-fluoro-4-methylpyridin-2-amine (2.70 g, 21.4 mmol) the reaction mixture was let warm up to RT and stirred for 18 h. Neutralization with Sodium bicarbonate at 0°C was followed by extraction of the reaction mixture thrice with ethyl acetate. The combined organic phase was filtrated over magnesium sulfate, concentration resulted in a yellow solid. The residue was purified by silica gel flash chromatography using PE–EA as eluent afforded 4b (3.1 g, 19.8 mmol, 93%) as a yellow solid, $^1$H NMR (500 MHz, DMSO-$d_6$) $\delta$ = 8.59 (s, 1H), 8.43 (d, $J = 5.3$ Hz, 1H), 2.43 (d, $J = 1.8$ Hz, 3H), $^{13}$C NMR (126 MHz, chloroform-$d$) $\delta$ = 161.33 (d, $J_{CF} = 263$ Hz), 152.23, 138.34 (d, $J_{CF} = 17.0$ Hz), 136.19 (d, $J_{CF} = 28.6$ Hz), 121.38 (d, $J_{CF} = 4.60$ Hz), 14.61 (d, $J_{CF} = 2.70$ Hz), HRMS (APCI) m/z calculated for C$_6$H$_6$FN$_2$O$_2$ [M+H]$^+$: 157.0408, found [M+H]$^+$: 157.0408.

5-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)pyridin-2-amine (6a)

Synthesis according to procedure B using 3a (800 g, 4.04 mmol) and 5-Fluoro-2-nitropyridine (574 mg, 4.04 mmol) afforded 6a (1.02 g, 3.51 mmol, 87%) as an orange solid, $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ = 7.89 (dd, $J = 3.0$, 0.7 Hz, 1H), 7.45 – 7.37 (m, 3H), 7.36 – 7.32 (m, 1H), 7.31 – 7.28 (m, 2H), 7.27 – 7.21 (m, 2H), 7.17 (dd, $J = 8.8$, 3.0 Hz, 1H), 6.47 (dd, $J = 8.8$, 0.7 Hz, 1H), 5.03 (s, 2H), 4.25 (s, 2H), 2.24 (s, 3H), $^{13}$C NMR (126 MHz, chloroform-$d$) $\delta$ = 153.4, 148.9, 143.0, 142.0, 135.3, 134.8, 134.3, 130.3, 129.5, 128.2, 128.3, 126.9, 126.8, 125.7, 109.4, 70.7, 16.2, HRMS (ESI) m/z calculated for C$_{19}$H$_{19}$ON$_2$ [M+H]$^+$: 291.1492, found [M+H]$^+$: 291.1490.

5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methyl(benzyl)oxy)pyridin-2-amine (6b)

Synthesis according to procedure B using 3b (3.56 g, 13.9 mmol) and 5-Fluoro-2-nitropyridine (1.98 g, 13.9 mmol) afforded 6b (4.31 g, 12.4 mmol, 89%) as an orange solid, $^1$H NMR (500 MHz, chloroform-$d$) $\delta$ = 7.89 (dd, $J = 3.0$, 0.7 Hz, 1H), 7.37 (dd, $J = 6.3$, 2.8 Hz, 1H), 7.24 – 7.19 (m, 2H), 7.18 (dd, $J = 8.8$, 3.0 Hz, 1H), 6.90 (d, $J = 8.2$ Hz, 1H), 6.83 (d, $J = 2.1$ Hz, 1H), 6.77 (dd, $J = 8.2$, 2.1 Hz, 1H), 6.48 (dd, $J = 8.8$, 0.7 Hz, 1H), 5.02 (s, 2H), 4.30 (s, 4H), 4.22 (s, 2H), 2.26 (s, 3H), $^{13}$C NMR (126 MHz, chloroform-$d$) $\delta$ = 153.3, 148.9, 143.1, 142.7, 142.5, 135.4, 135.2, 134.5, 134.4, 130.3, 127.9, 127.0, 125.6, 122.7, 118.3, 117.0, 109.6, 70.7, 64.5, 64.5, 16.3, HRMS (ESI) m/z calculated for C$_{21}$H$_{21}$O$_2$N$_2$ [M+H]$^+$: 349.1547, found [M+H]$^+$: 349.1545.
5-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl)methoxy)pyridin-2-amine (6c)

Synthesis according to procedure B using 3c (1.08 g, 5.00 mmol) and 5-fluoro-2-nitropyridine (711 mg, 5.00 mmol) afforded 6c (1.42 g, 4.6 mmol, 92%) as an orange solid. 

$^1$H NMR (500 MHz, chloroform-d) $\delta = 7.89 (d, J = 2.9 Hz, 1H), 7.40 (dd, J = 7.5, 1.6 Hz, 1H), 7.28 – 7.22 (m, 3H), 7.21 – 7.16 (m, 2H), 7.13 – 7.07 (m, 2H), 6.49 (d, J = 8.8 Hz, 1H), 5.03 (s, 2H), 4.24 (bs, 2H), 2.23 (s, 3H),

$^{13}$C NMR (126 MHz, chloroform-d) $\delta = 161.98 (d, J_{CF} = 245.7 Hz), 153.31, 148.76, 141.96, 137.82 (d, J_{CF} = 3.3 Hz), 135.33, 134.41, 134.36, 130.94 (d, J_{CF} = 7.9 Hz), 130.24, 128.20, 126.90, 125.69, 115.02 (d, J_{CF} = 21.2 Hz), 109.48, 70.57, 16.15

HRMS (ESI) m/z calculated for C$_{19}$H$_{18}$FN$_{2}$O $[M+H]^{+}$: 309.1398, found [M+H]$^{+}$: 309.1397.

5-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-4-methylpyridin-2-amine (6d)

Synthesis according to procedure B using 3a (1.15 g, 4.50 mmol) and 4b (703 mg, 4.50 mmol) afforded 6d (1.17 g, 3.24 mmol, 72%) as a pale white solid.

$^1$H NMR (500 MHz, chloroform-d) $\delta = 7.73 (s, 1H), 7.40 (dd, J = 7.0, 2.1 Hz, 1H), 7.26 – 7.18 (m, 2H), 6.91 (d, J = 8.2 Hz, 1H), 6.83 (dd, J = 2.1 Hz, 1H), 6.78 (dd, J = 8.2, 2.1 Hz, 1H), 6.38 (s, 1H), 5.03 (s, 2H), 4.30 (s, 4H), 4.16 (bs, 2H), 2.26 (s, 3H), 2.19 (s, 3H),

$^{13}$C NMR (126 MHz, chloroform-d) $\delta = 153.2, 148.0, 143.1, 142.7, 142.4, 140.0, 135.7, 135.4, 134.2, 131.4, 130.1, 127.5, 125.6, 122.7, 118.4, 117.0, 111.1, 70.6, 64.6, 64.5, 16.3, 16.2, HRMS (ESI) m/z calculated for C$_{22}$H$_{23}$O$_3$N$_2$ $[M+H]^{+}$: 363.1701, found [M+H]$^{+}$: 363.1703.

(3-benzylamino)-6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9a)

Synthesis according to procedure C using 6a (145 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded 9a (175 mg, 0.36 mmol, 72%) as a colorless solid.

$^1$H NMR (500 MHz, Methanol-d$_4$) $\delta = 7.82 (d, J = 2.3 Hz, 1H), 7.51 (d, J = 9.7 Hz, 1H), 7.46 – 7.38 (m, 3H), 7.37 – 7.33 (m, 1H), 7.33 – 7.22 (m, 9H), 7.20 (dd, J = 7.7, 1.5 Hz, 1H), 5.06 (s, 2H), 4.20 (s, 2H), 3.97 (s, 2H), 2.24 (s, 3H),

$^{13}$C NMR (126 MHz, Methanol-d$_4$) $\delta = 150.38, 144.48, 143.22, 141.00, 138.62, 135.78, 135.65, 131.36, 130.36, 129.81, 129.77, 129.28, 128.77, 128.05, 127.30, 126.67, 124.66, 114.82, 116.68, 107.97, 107.75, 71.44, 53.33, 53.21, 53.08, 35.57, 16.52, 16.48, HRMS (ESI) m/z calculated for C$_{29}$H$_{28}$ON$_4$ $[M+H]^{+}$: 449.2336, found [M+H]$^{+}$: 449.2335.
(6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-((1-phenylethyl)amino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9b)

Synthesis according to procedure C using 6a (145 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and (1-isocyanatoethyl)benzene (112 mg, 0.85 mmol) afforded 9b (120 mg, 0.24 mmol, 48%) as a pale white solid, ¹H NMR (500 MHz, Methanol-d₄) δ = 7.90 (d, J = 2.3 Hz, 1H), 7.66 (d, J = 9.7 Hz, 1H), 7.55 (dd, J = 9.7, 2.3 Hz, 1H), 7.43 (tt, J = 8.2, 1.6 Hz, 3H), 7.39 – 7.34 (m, 1H), 7.33 – 7.21 (m, 9H), 5.20 (d, J = 11.6 Hz, 1H), 5.13 (d, J = 11.6 Hz, 1H), 4.27 (q, J = 6.8 Hz, 1H), 4.01 (d, J = 14.6 Hz, 1H), 3.93 (d, J = 14.6 Hz, 1H), 2.27 (s, 3H), 1.67 (d, J = 6.8 Hz, 3H), ¹³C NMR (126 MHz, Methanol-d₄) δ = 151.20, 145.46, 144.58, 143.14, 136.80, 135.71, 135.48, 131.64, 131.49, 130.36, 129.92, 129.39, 129.25, 128.98, 128.09, 127.90, 126.75, 124.42, 115.26, 108.83, 108.70, 71.69, 59.54, 59.01, 58.49, 34.43, 16.61, 16.49, HRMS (ESI) m/z calculated for C₃₀H₃₁O₂N₄ [M+H]⁺: 463.2492, found [M+H]⁺: 463.2495.

(6-((2-methyl-[1,1'-biphenyl]-3-yl)methoxy)-3-(phenethylamino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9c)

Synthesis according to procedure C using 6a (145 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and (2-isocyanatoethyl)benzene (112 mg, 0.850 mmol) afforded 9c (172 mg, 0.255 mmol, 51%) as a pale white solid, ¹H NMR (500 MHz, Methanol-d₄) δ = 7.77 (d, J = 2.3 Hz, 1H), 7.67 (d, J = 9.7 Hz, 1H), 7.56 (dd, J = 9.7, 2.3 Hz, 1H), 7.47 – 7.41 (m, 3H), 7.38 – 7.32 (m, 1H), 7.31 – 7.20 (m, 8H), 7.17 – 7.10 (m, 1H), 5.06 (s, 2H), 4.31 (s, 2H), 3.41 (t, J = 6.8 Hz, 2H), 2.95 (t, J = 6.8 Hz, 2H), 2.25 (s, 3H), ¹³C NMR (126 MHz, Methanol-d₄) δ = 151.44, 144.59, 143.16, 140.92, 136.40, 135.94, 135.34, 132.86, 131.62, 130.34, 130.15, 129.77, 129.51, 129.30, 128.09, 127.47, 126.75, 122.18, 115.22, 114.99, 108.49, 108.25, 71.97, 50.03, 37.72, 34.52, 16.57, 16.51, HRMS (ESI) m/z calculated for C₂₀H₂₁ON₄ [M+H]⁺: 463.2492, found [M+H]⁺: 463.2490.
(S)-1-(3-(benzylamino)-6-((2-methyl-[1,1’-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)-2-hydroxyethan-1-aminium chloride (9d)

Synthesis according to procedure C using 6a (145 mg, 0.5 mmol), (R)-(−)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde (195 mg, 0.85 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded 9d (160 mg, 0.31 mmol, 62%) as a pale white solid, $^1$H NMR (500 MHz, Methanol-d$_4$) $\delta$ = 7.95 (s, 1H), 7.71 (d, $J$ = 9.7 Hz, 1H), 7.56 (d, $J$ = 9.7 Hz, 1H), 7.48 - 7.39 (m, 3H), 7.38 - 7.19 (m, 10H), 5.12 (s, 2H), 4.50 (dd, $J$ = 5.6, 4.2 Hz, 1H), 4.30 (d, $J$ = 13.9 Hz, 1H), 4.21 (d, $J$ = 13.9 Hz, 1H), 3.79 (dd, $J$ = 11.5, 5.6 Hz, 1H), 3.74 (dd, $J$ = 11.5, 4.2 Hz, 1H), 4.30, 4.21 (d, $J$ = 13.9 Hz, 1H), 2.25 (s, 3H), $^{13}$C NMR (126 MHz, Methanol-d$_4$) $\delta$ = 151.1, 144.5, 143.2, 140.8, 137.4, 135.7, 135.5, 131.7, 131.4, 130.3, 129.9, 129.8, 129.5, 129.2, 128.9, 128.1, 126.7, 115.8, 115.6, 108.5, 71.6, 62.2, 53.1, 49.1, 16.6. HRMS (ESI) m/z calculated for C$_{30}$H$_{31}$O$_2$N$_4$ [M+H]$^+$: 479.2442, found [M+H]$^+$: 479.2441.

(R)-1-(3-(benzylamino)-6-((2-methyl-[1,1’-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)-2-hydroxyethan-1-aminium chloride (9e)

Synthesis according to procedure C using 6a (145 mg, 0.5 mmol), (S)-(−)-3-Boc-2,2-dimethyloxazolidine-4-carboxaldehyde (195 mg, 0.85 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded 9e (149 mg, 0.29 mmol, 58%) as a pale white solid, $^1$H NMR (500 MHz, Methanol-d$_4$) $\delta$ = 7.68 (d, $J$ = 2.3 Hz, 1H), 7.45 - 7.39 (m, 4H), 7.37 - 7.33 (m, 1H), 7.32 - 7.23 (m, 8H), 7.20 (dd, $J$ = 7.7, 1.6 Hz, 1H), 7.15 (dd, $J$ = 9.7, 2.3 Hz, 1H), 5.02 (s, 2H), 4.40 (dd, $J$ = 8.6, 4.5 Hz, 1H), 4.24 - 4.10 (m, 2H), 3.84 (dd, $J$ = 11.5, 8.6 Hz, 1H), 3.61 (dd, $J$ = 11.5, 4.5 Hz, 1H), 2.24 (s, 3H), $^{13}$C NMR (126 MHz, Methanol-d$_4$) $\delta$ = 149.6, 144.5, 143.3, 141.2, 140.1, 136.0, 135.6, 131.3, 131.1, 130.5, 130.4, 129.8, 129.2, 128.7, 128.0, 126.6, 118.0, 107.5, 107.3, 71.3, 63.2, 53.5, 50.9, 16.5, HRMS (ESI) m/z calculated for C$_{30}$H$_{31}$O$_2$N$_4$ [M+H]$^+$: 479.2442, found [M+H]$^+$: 479.2444.
(6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl) methoxy)-3-(phenethylamino)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9g)

Synthesis according to procedure C using 6c (161 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and (2-Isocyanoethyl)benzene (112 mg, 0.850 mmol) afforded 9g (199 mg, 0.385 mmol, 77%) as a colorless solid. 1H NMR (500 MHz, Methanol-d4) δ = 8.53 (s, 3H), 7.73 (d, J = 2.4 Hz, 1H), 7.48 (dd, J = 7.7, 1.5 Hz, 1H), 7.44 (d, J = 9.7 Hz, 1H), 7.37 – 7.31 (m, 2H), 7.31 – 7.22 (m, 7H), 7.21 (dd, J = 7.7, 1.5 Hz, 1H), 7.08 (dd, J = 9.7, 2.4 Hz, 1H), 5.89 (t, J = 6.3 Hz, 1H), 5.11 (s, 2H), 4.21 (d, J = 6.3 Hz, 2H), 3.91 (s, 2H), 2.21 (s, 3H), 13C NMR (126 MHz, Methanol-d4) δ = 163.4 (d, JCF = 244.4 Hz), 149.8, 143.4, 141.2, 139.8, 139.3 (d, JCF = 3.3 Hz), 136.0, 135.9, 132.2, 131.5, 131.2, 130.1, 129.8, 129.5, 128.9, 127.4, 126.7, 122.7, 117.9, 116.0, 115.8, 107.2, 71.5, 50.6, 37.9, 36.6, 16.6, HRMS (ESI) m/z calculated for C30H29FN4F [M+H]+: 481.2398, found [M+H]+: 481.2398.

(3-((3-cyanobenzyl)amino)-6-((4'-fluoro-2-methyl-[1,1'-biphenyl]-3-yl) methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9h)

Synthesis according to procedure C using 6c (161 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and 3-(isocyanomethyl)benzonitrile (121 mg, 0.85 mmol) afforded 9h (227 mg, 0.43 mmol, 86%) as a colorless solid. 1H NMR (500 MHz, DMSO-d6) δ = 8.49 (s, 3H), 7.92 – 7.86 (m, 2H), 7.73 (ddt, J = 8.3, 6.8, 1.5 Hz, 2H), 7.50 (q, J = 7.7 Hz, 2H), 7.42 – 7.33 (m, 3H), 7.29 – 7.22 (m, 3H), 7.21 (dd, J = 7.7, 1.5 Hz, 1H), 7.08 (dd, J = 9.7, 2.4 Hz, 1H), 5.89 (t, J = 6.3 Hz, 1H), 5.11 (s, 2H), 4.21 (d, J = 6.3 Hz, 2H), 3.91 (s, 2H), 2.21 (s, 3H), 13C NMR (126 MHz, DMSO-d6) δ = 161.4 (d, JCF = 243.7 Hz), 147.3, 141.8, 141.2, 137.6 (d, JCF = 3.2 Hz), 135.0, 134.3, 133.6, 133.5, 132.2, 132.0, 131.2 (d, JCF = 8.0 Hz), 130.9, 130.0, 129.5, 129.5, 128.4, 125.7, 119.5, 118.9, 117.1, 115.1 (d, JCF = 21.2 Hz), 111.1, 69.5, 50.8, 35.1, 16.0, 15.9, HRMS (ESI) m/z calculated for C30H27ON5F [M+H]+: 492.2194, found [M+H]+: 492.2195.
(3-((3-(2H-tetrazol-5-yl)benzyl)amino)-6-((4’-fluoro-2-methyl-[1,1’-biphenyl]-3-yl)methoxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9i)

A mixture of 6c (308 mg, 1 mmol, 1 eq.), N-Boc-2-aminoacetaldehyde (271 mg, 1.7 mmol, 1.7 eq.), scandium triflate (49mg, 0.1 mmol, 0.1 eq.) and 3-(isocyanomethyl)benzonitrile (242mg, 1.7 mmol, 1.7 eq.) in DCM/MeOH (2:1, 3ml, 0.3 M) was placed in a glass microwave vial and sealed. The reaction vial was heated in a microwave for 1 hour at 120°C. Evaporation of the solvents was followed by purification by silica gel flash chromatography using EA/PE as eluent. The still slightly impure product was used directly in the further step. Sodium Azide (mg, mmol, 1,2 eq.), Zink chloride (mg, mmol, 1 eq.), and 5 ml of n-Propanol where added to the crude product. The reaction mixture was heated to 95°C for 20h. After removal of the organic solvents the reaction mixture purified via a reverse-phase purification with H2O/MeOH/NH3(0,1%) as eluent. Afterwards the purified product was directly treated with 2.8 ml 7N HCl in 2-Propanol (3.9 mmol, 50 eq.) for 20h at rt. Concentration of the reaction mixture under vacuum afforded 9i (223 mg, 0.39 mmol 39%) as a yellow solid, 1H NMR (500 MHz, MeOD/CDCl3 (10%)) δ = 8.08 (s, 1H), 7.95 (d, J = 7.7 Hz, 1H), 7.73 (d, J = 2.4 Hz, 1H), 7.35 (d, J = 9.6 Hz, 1H), 7.32 (dt, J = 7.7, 4.0 Hz, 2H), 7.28 – 7.24 (m, 2H), 7.23 – 7.17 (m, 2H), 7.16 – 7.15 (m, 1H), 7.13 (s, 1H), 7.11 (d, J = 7.7 Hz, 1H), 7.08 (dd, J = 9.6, 2.4 Hz, 2H), 4.96 (s, 3H), 4.22 (s, 2H), 3.94 (s, 2H), 2.14 (s, 2H), 13C NMR (126 MHz, MeOD/CDCl3 (10%)) 163.3 (d, JCF = 244.4 Hz), 149.6, 143.2, 141.9, 140.2 139.3 (d, JCF = 3.5 Hz), 135.96, 135.73, 132.13 (d, JCF = 7.8 Hz), 131.62, 131.25, 130.42, 130.33, 130.04, 129.92, 129.54, 127.74, 127.72, 126.94, 126.81, 126.62, 122.29, 118.03, 115.85 (d, JCF = 21.4 Hz), 107.10, 106.88, 71.18, 53.37, 36.52, 16.33, HRMS (ESI) m/z calculated for C30H28N8F [M+H]+: 535.2365, found [M+H]+: 535.2365.

(3-(benzylamino)-6-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)imidazo[1,2-a]pyridin-2-yl)methanaminium chloride (9j)

Synthesis according to procedure C using 6b (174 g, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded 9j (174 mg, 0.32 mmol 64%) as a pale white solid, m.p., 1H NMR (500 MHz, DMSO-d6) δ = 8.91 (s, 3H), 8.23 (d, J = 2.3 Hz, 1H), 7.90 (d, J = 9.7 Hz, 1H), 7.73 (dd, J = 9.7, 2.3 Hz, 1H), 7.45 (dd, J = 7.6, 1.5 Hz, 1H), 7.42 – 7.36 (m, 2H), 7.36 – 7.22 (m, 4H), 7.20 (dd, J = 7.6, 1.5 Hz, 1H), 6.92 (d, J = 8.2 Hz, 1H), 6.79 (d, J = 2.1 Hz, 1H), 6.75 (dd, J = 8.2, 2.1 Hz, 1H), 5.20 (s, 2H), 4.28 (s, 6H), 3.99 (s, 2H), 2.23 (s, 3H), 13C NMR (126 MHz, MeOD) δ = 159.3, 152.5, 151.2, 151.3, 148.7, 143.9, 143.8, 143.7, 142.1, 140.8, 139.7, 138.0, 138.0, 137.7, 137.0, 136.7, 135.1, 131.7, 131.6, 128.5, 127.4, 127.1, 126.4, 126.2,
122.5, 117.4, 79.6, 73.6, 60.1, 41.2, 25.6, 25.5, HRMS (ESI) m/z calculated for C_{31}H_{31}O_{3}N_{4} [M+H]^+: 507.2391, found [M+H]^+: 507.2390.

(3-(benzylamino)-6-((3-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-methylbenzyl)oxy)-7-methylimidazo[1,2-a]pyridin-2-yl) methanaminium chloride (9k)

Synthesis according to procedure C using 6d (181 mg, 0.5 mmol), paraformaldehyde (25.5 mg, 0.850 mmol), Scandium triflate (25 mg, 50 µmol), and benzyl isocyanide (99.6 mg, 0.850 mmol) afforded 9k (175 mg, 0.315 mmol 63%) as an orange solid. "H NMR (500 MHz, Methanol-d_4) δ = 7.60 (s, 1H), 7.40 (dd, J = 7.5, 1.6 Hz, 1H), 7.34 – 7.15 (m, 8H), 6.87 (d, J = 8.2 Hz, 1H), 6.75 (d, J = 2.1 Hz, 1H), 6.72 (dd, J = 8.2, 2.1 Hz, 1H), 4.99 (s, 2H), 4.27 (s, 4H), 4.12 (s, 2H), 3.85 (s, 2H), 2.30 (s, 3H), 2.26 (s, 3H). "C NMR (126 MHz, Methanol-d_4) δ = 149.0, 144.7, 144.2, 144.0, 140.3, 136.4, 136.1, 135.5, 133.1, 131.3, 129.9, 129.8, 129.8, 128.8, 128.7, 126.6, 123.3, 119.2, 119.1, 118.0, 116.7, 116.7, 106.0, 105.8, 71.0, 65.7, 53.7, 36.6, 16.9, 16.9, 16.5, 16.5, HRMS (ESI) m/z calculated for C_{32}H_{33}O_{3}N_{4} [M+H]^+: 521.2547, found [M+H]^+: 521.2546.
NMR BINDING ASSAY

For NMR measurements, the buffer was exchanged by gel filtration to PBS pH 7.4. 10% (v/v) of D₂O was added to the samples to provide the lock signal. All spectra were recorded at 300 K using a Bruker Avance III 600 MHz spectrometer equipped with the nitrogen cryo-probe head.

PD-L1 COCRYSTALLIZATION

PD-L1 expression and purification

The IgV domains of human PD-L1 protein (hPD-L1 residues: 18-134, C-terminal His-tag) was expressed and purified as described previously (Zak et al., 2016). Briefly, protein was expressed in E. coli BL21 (DE3) strain as inclusion bodies which were collected by centrifugation, washed, and dissolved. Protein was refolded by drop-wise dilution into refolding buffer: 0.1 M Tris pH 8.0, 1 M L-Arg hydrochloride, 2 mM EDTA, 0.25 mM oxidized glutathione and 0.25 mM reduced glutathione. Refolded protein was dialyzed 3 times over 48-72 h against buffer containing 10 mM Tris pH 8.0 and 20 mM NaCl. On the final step, PD-L1 was concentrated and loaded to a size exclusion chromatography column HiLoad 26/600 Superdex 75 (GE Healthcare) pre-equilibrated with buffer containing 10 mM Tris pH 8.0 and 20 mM NaCl for crystallization or PBS pH 7.4 for NMR experiments.

PD-L1 cocrystalization

Purified PD-L1 was concentrated to 5 mg/ml, mixed with the inhibitor in 1:3 molar ratio (protein:compound) and clarified by centrifugation at 15 000 × g for 10 min. Supernatant was used for screening using a sitting-drop vapor diffusion method and commercially available buffer sets. Diffraction-quality crystals were obtained at room temperature from the condition containing: 1.2 M sodium citrate tribasic dihydrate 0.01 M sodium borate, pH 8.5. The crystal was flash-cooled in liquid nitrogen.

Crystal structure determination and refinement

The X-ray diffraction data were collected at the BL14.1 beamline operated by the Helmholtz-Zentrum Berlin (HZB) at the BESSY II (Berlin Adlershof, Germany). The data were indexed, integrated, and scaled using XDS, XSCALE, and Aimless. Initial phases were obtained by molecular replacement calculated in Phaser. The model building was performed in Coot and refinement was performed using Phenix or PDB-REDO server. Water molecules were added automatically and inspected manually. Coordinates and structure factors were deposited in the Protein Data Bank under accession code PDB: 7BEA.

Table S1 Data collection and refinement statistics (molecular replacement)

<table>
<thead>
<tr>
<th>Data collection</th>
<th></th>
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<tbody>
<tr>
<td>Wavelength (Å)</td>
<td>0.9184</td>
</tr>
<tr>
<td>Space group</td>
<td>P 2 2 1 21</td>
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<tr>
<td>Cell dimensions</td>
<td></td>
</tr>
<tr>
<td><strong>a, b, c (Å)</strong></td>
<td>32.62 54.62 140.96</td>
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<tr>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td><strong>α, β, γ (°)</strong></td>
<td>90.00 90.00 90.00</td>
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<tr>
<td><strong>Resolution range (Å)</strong></td>
<td>46.99 - 2.45 (2.55 - 2.45)</td>
</tr>
<tr>
<td><strong>Rmerge</strong></td>
<td>0.138 (1.915)</td>
</tr>
<tr>
<td>**</td>
<td>10.9 (0.9)</td>
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<tr>
<td><strong>Completeness (%)</strong></td>
<td>99.8 (98.9)</td>
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<tr>
<td><strong>Redundancy</strong></td>
<td>7.1 (7.4)</td>
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<tr>
<td><strong>Total reflections</strong></td>
<td>69799 (7901)</td>
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<tr>
<td><strong>CC1/2</strong></td>
<td>0.997 (0.474)</td>
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</table>

**Refinement statistics**

<table>
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<tr>
<th><strong>No. reflections</strong></th>
<th>9843 (956)</th>
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<tr>
<td><strong>Rwork/Rfree</strong></td>
<td>0.2741/0.2868 (0.3569)/(0.3895)</td>
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<tr>
<td><strong>Wilson B-factor</strong></td>
<td>49.3</td>
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<tr>
<td><strong>No. atoms</strong></td>
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</tr>
<tr>
<td><strong>Protein</strong></td>
<td>1906</td>
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<tr>
<td><strong>Water</strong></td>
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<tr>
<td><strong>Ramachandran favoured (%)</strong></td>
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<tr>
<td><strong>Ramachandran allowed (%)</strong></td>
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<tr>
<td><strong>Ramachandran outliers (%)</strong></td>
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<tr>
<td><strong>B-factors</strong></td>
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<tr>
<td><strong>Protein</strong></td>
<td>35.91</td>
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<tr>
<td><strong>Water</strong></td>
<td>44.20</td>
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**R.m.s deviations**

<table>
<thead>
<tr>
<th><strong>Bond lengths (Å)</strong></th>
<th>0.011</th>
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</thead>
<tbody>
<tr>
<td><strong>Bond angles (°)</strong></td>
<td>1.555</td>
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</table>
HOMOGENOUS TIME-RESOLVED FLUORESCENCE (HTRF) ASSAY

The HTRF assay was performed using the certified Cis-Bio assay kit (Cis-Bio, Codolet, France) at 20 µL final volume using their standard protocol as described by Musielak et al. Measurements were performed on individual dilution series to determine the half maximal inhibitory concentration (IC$_{50}$) of tested compounds. After mixing all components according to the Cis-Bio protocol, the plate was incubated for 2 h at RT. TR-FRET measurement was performed on the Tecan Spark 20M. Collected data was background subtracted on the negative control, normalized on the positive control, averaged and fitted with normalized Hill’s equation to determine the IC$_{50}$ value using Mathematica 12. For the compounds with IC$_{50}$ values were too high due to e.g., solubility issues a “dissociation value” at the concentration of 50 µM is presented for the sake of comparability to other inhibitors. The dissociation value represents the percentage of the PD-1/PD-L1 complex that is undissolved.

MOLECULAR DOCKING

Structure of 9j compound was prepared and minimized in VEGA ZZ software. Subsequently, the conformational search using Boltzman jump and 5000 steps was performed. The conformation of 9j with lowest energy was selected and used in molecular docking with AutoDock Vina. The structure of compound and PD-L1 dimer (PDB: 6R3K) were prepared in AutoDock Tools. All water molecules and original ligand (BMS-1166) were removed and polar hydrogens atoms were added to the receptor. A grid box of dimensions 20x20x20 Å and following coordinates x: -8.746, y = 18.049 and z = -21.934 was placed at the interface of PD-L1 homodimer. Docking was carried out with exhaustiveness = 16. The obtained binding poses were carefully visually inspected in PyMol.


