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Ligand Specific Efficiency (LSE) Index for PET Tracer Optimization

Yves P. Auberson,*[a] Emmanuelle Briard,[a] David Sykes,[b] John Reilly,[c] and Mark Healy[c]

Ligand efficiency indices are widely used to guide chemical optimization in drug discovery, due to their predictive value in the early steps of optimization. At later stages, however, as more complex properties become critical for success, indices relying on calculated, rather than experimental, parameters become less informative. This problem is particularly acute when developing positron emission tomography (PET) imaging agents, for which nonspecific binding (NSB) to membranes and non-target proteins is a frequent cause of failure. NSB cannot be predicted using in silico parameters. To address this gap, we explored the use of the experimentally determined chromatographic hydrophobicity index on immobilized artificial membranes, CHI(IAM), to guide the optimization of NSB. The ligand specific efficiency (LSE) index was defined as the ratio between affinity (pIC_{50} or pK_d) and the logarithmic value of CHI(IAM). It allows for quantification of binding affinity to the target of interest, relative to NSB. Its use was illustrated by the optimization of PET tracer candidates for the prostacyclin receptor.

Introduction

The use of ligand efficiency (LE) indices in medicinal chemistry has become widespread over the last decade, providing a convenient way to evaluate the quality of a drug candidate at an early stage. These indices provide information about structural efficacy with regard to affinity and lipophilicity and about the enthalpic contribution to binding thermodynamics, in addition to allowing anticipation of some in vivo properties, such as oral bioavailability or expected in vivo efficacy.[1] As such, they have become indispensable tools for medicinal chemists in the early stages of optimization. Their weakness, unfortunately, is that with the exception of affinity and lipophilicity measurements, they rely exclusively on calculated properties, such as the number of non-hydrogen atoms (N_{heavy}), topological polar surface area (TPSA), or molecular weight (M_r). This is an advantage in early stages of optimization when little experimental data is available; however, these computational indices increasingly show limitations when approaching more advanced stages of drug optimization. At this point, candidates differ in a more subtle manner, where additional parameters come to the forefront and more complex properties become critical for success.

Within the field of positron emission tomography (PET) tracer optimization, multi-parameter optimization (MPO) metrics are useful for refining the properties of tracer candidates for imaging in the central nervous system.[2] Based on preferred values for clog*P*, log*D* at pH 7.4, M_r, TPSA, hydrogen donor count, and pK_a, these MPO metrics allow for initial in silico prioritization of tracer candidates. A limitation, however, is that these metrics do not take nonspecific binding (NSB) into account. NSB is the tendency of a molecule to bind indistinctly to cell membranes, and it is a property that is difficult to predict. It cannot be quantified by current in silico methods and remains a challenge in tracer optimization. NSB is clearly one of the main causes of failure for novel PET tracer candidates. This property is related in part to lipophilicity, but this parameter does not tell the full story. Therefore, we pursued the development of an index that would take measured NSB into account to guide our efforts to discover clinically successful PET imaging agents through the whole optimization process.

With this in mind, we decided to explore the use of the experimentally determined chromatographic hydrophobicity index on immobilized artificial membranes, CHI(IAM). Our motivation came from the previous observation that the CHI(IAM) value of PET tracers is statistically correlated to the extent of their NSB.[3] It is a standardized value derived from the high-performance liquid chromatography (HPLC) retention time, which was originally developed to characterize the interactions of drugs with an immobilized artificial membrane.[4] For practical reasons, we decided to use CHI(IAM) measurements in place of the formerly proposed vesicle electrokinetic chromatography (VEKC) assay.[3] CHI(IAM) measurements are easier to set up, can be automated in higher throughput, and lead to less variation between individual laboratories. Results obtained

[a] *Dr. Y. P. Auberson, Dr. E. Briard*
Novartis Institutes for BioMedical Research,
Klybeckstrasse 141, 4057 Basel (Switzerland)
E-mail: yves.auberson@novartis.com

[b] *D. Sykes*
University of Nottingham, Cell Signalling Department, Nottingham,
NG7 2RD (UK)

[c] *Dr. J. Reilly, Dr. M. Healy*
Novartis Institutes for BioMedical Research,
250 Massachusetts Avenue, Cambridge MA, 02139 (USA)

The ORCID identification number(s) for the author(s) of this article can be found under http://dx.doi.org/10.1002/cmdc.201600112.

with CHI(IAM) are not significantly different from those provided by the VEKC method.

In analogy to the LE index,[5] which relates the affinity of a drug to the number of its non-hydrogen atoms, we explored the usefulness of the ligand specific efficiency (LSE) index, which we defined as the ratio between affinity (expressed as the log of its affinity [e.g., pIC_{50} or pK_d] for a specific target), and the logarithmic value of the experimental nonspecific binding measurement, CHI(IAM) [Eq. (1)]:

$$LSE = pK_d/\log(CHI(IAM)) \quad (1)$$

LSE provides a measure of affinity normalized to nonspecific binding. It shows how efficiently the ligand specifically binds the desired target, compared with all other nonspecific binding partners.

An alternative method to determine NSB with a lipid membrane binding assay (LIMBA) has been described recently.[6] Even though only a small number of tracers were tested in both assays, a preliminary comparison of LIMBA and CHI(IAM) indicates that both methods might provide comparable results. As a consequence, it is conceivable that an index based on LIMBA $\log D_{brain}$, as an alternative to CHI(IAM) measurements, might lead to similar conclusions.

Results and Discussion

Setting the threshold

In a first phase, we evaluated a series of sixteen successful clinical PET tracers to get an understanding of what a desirable LSE value would be. In all cases, the LSE value was ≥ 5.0 (Table 1), with the dopamine antagonist fallypride reaching a value of 7.0, due to a very high affinity for the D_2 ($K_d = 0.03$ nM at room temperature) and D_3 ($K_d = 0.3$ nM at room temperature) dopamine receptors,[7] combined with a moderate propensity for NSB (CHI(IAM) = 31.9). Of note, this value is possibly overestimated, as another study measured the affinity of fallypride for the D_2 receptor at 37 °C at $K_d = 2$ nM.[8] Taking this lower affinity value into account, the LSE of fallypride would be 5.8, still within the range of other successful PET imaging agents. Differences in methods used for the determination of affinity made it impossible to exactly compare all tracers in Table 1; however, variations remained within a range that did not affect the general trend. It is interesting to compare fallypride with the opioid antagonist diprenorphine, which also has a high affinity for its target ($K_d = 0.2$ nM)[9] but a less favorable CHI(IAM) value of 46.9. This leads to a difference of one LSE unit and illustrates the importance of NSB on tracer quality. The two tracers with the lowest LSE values (donepezil: 5.1 and nomifensine: 5.0) had moderate CHI(IAM) values of 42 and 40, respectively, but comparatively modest affinities for PET tracers ($IC_{50} = 5.7$ nM for AChE,[10] $IC_{50} = 10$ nM for norepinephrine reuptake,[11] and $K_i = 17$ nM for dopamine reuptake inhibition[12]). PK11195, a radioligand well known for its high NSB, has an LSE value of 5.1. Overall, only two successful PET tracers have LSE values below 5.4, with an average for all tracers in Table 1 of 5.7. We therefore accepted that a minimum objective to achieve in PET tracer optimization was LSE > 5, preferably LSE ≥ 5.4.

Based on data published by Jiang et al.,[3] CHI(IAM) correlates with the NSB ratio according to the formula CHI(IAM) = 59.4 − 24.8 × NSB ratio, with a threshold for successful PET tracers of CHI(IAM) ≤ ~50. The NSB ratio[13] is defined such that a compound with very low NSB in a brain membrane vs. buffer dialysis assay has a value close to one, whereas a compound with high NSB yields a value close to zero. A CHI(IAM) ≤ ~50 corresponds to a NSB ratio ≤ 0.38, beyond which the specific signal appears to decrease beyond usefulness.

At the other end of the range, an NSB ratio of 1 corresponds to a molecule distributing to the membrane compartment but having no NSB. This translates into a theoretical CHI(IAM) value of 34.6. Compounds with CHI(IAM) values significantly below this number are unlikely to be useful as PET tracers, as this implies that they preferentially distribute outside of the membrane compartment. This might happen, for example, with very hydrophilic compounds, and is likely to occur in parallel with low organ penetration. Based on these considerations, the target CHI(IAM) range for successful PET tracers appears to be roughly between 35 and 50. This corresponds to the observed CHI(IAM) values of successful tracers (Table 1), all of which were between 31.9 and 51.9.

Calculating backward from the preferred LSE value of 5.4, it follows that the target affinity should be at least 0.7 nM for a CHI(IAM) value of 50 and 4.6 nM for a CHI(IAM) value of 35. This provides useful guidance in the optimization process, in the form of a convenient, quantitative relationship between affinity and NSB measurements.

A similar analysis to confirm that failed tracers have a lower LSE value could not be performed, as it was impossible to tease out the role of NSB among the multiple parameters that lead to tracer failure. While a certain threshold must be reached in terms of specific binding for success, the inverse argument cannot be made, as unsuccessful tracer candidates do not necessarily fail due to high NSB.

Table 1. Affinity for target (pK_d), experimental CHI(IAM) values, and calculated LSE values of sixteen successful clinical PET tracers.

Compound	pK_d	CHI(IAM)	LSE
ABP688	8.46	39.1	5.3
AC-5216	9.52	38.5	6.0
diprenorphine	9.70	46.9	5.8
donepezil	8.24	42.2	5.1
fallypride	10.52	31.9	7.0
GR103545	9.30	42.2	5.7
MPPF	9.47	37.4	6.0
NMeSpiperone	9.60	51.9	5.6
nomifensine	8.00	40.3	5.0
NPA	9.57	42.9	5.9
PK11195	8.35	42.6	5.1
PPHT	9.19	48.7	5.4
raclopride	8.74	35.4	5.6
rolipram	8.75	28.9	6.0
SCH23390	10.0	47.4	6.0
WAY100635	9.40	38.9	5.9

Target expression can vary greatly (from ~1 to 1000 nM), depending on the target itself, the tissue, and the animal species.[14] LSE does not contain any information with regard to the imaging target, only information regarding the intrinsic properties of PET tracer candidates. The desirable LSE value is therefore independent of target expression (B_{max}). Clearly, a tracer for a target expressed at a lower level will require a higher affinity for the same signal intensity. If all other factors are proportional, the tracer will therefore benefit from a lower NSB. The required affinity for a PET tracer has been discussed elsewhere and as a rule of thumb is given by the formula $K_d < B_{max}/5$.[15] Moreover, the concentration of radiotracer available for binding to the receptor is dependent on its free fraction, which can be measured using, for example, tissue homogenates and LC–MS. It also depends on the ratio between specific and nonspecific binding, a property that can be quantified and optimized using LSE.

Applying LSE to tracer optimization

The human prostacyclin receptor (hIPR) is a member of the G-protein-coupled receptor family. Prostacyclin, the major product of cyclooxygenase in the macrovascular endothelium, elicits potent vasodilation activity and inhibition of platelet aggregation through binding to hIPR. We were interested in evaluating whether we could develop a PET tracer to study the pharmacological properties of hIPR ligands, which are of potential interest for the treatment of pulmonary arterial hypertension (PAH).[16]

Our chemical starting point was Ro 1138452 (**1**, Figure 1),[17] which we selected based on encouraging overall properties, including a high affinity for hIPR (K_i = 0.9 nM, M_r = 309, PSA = 45 Å). In contrast, its CHI(IAM) value of 58 and LSE of 4.9 clearly indicated a high tendency for NSB. This was confirmed in a binding assay using [^{3}H]**1** and membranes of Chinese hamster ovary (CHO) cells expressing hIPR (Figure 1). To better illustrate how a higher LSE value translated into better imaging properties, comparative data is provided for [^{3}H]*N*-methylscopolamine ([^{3}H]NMS). Although not a candidate PET tracer due to limited brain penetration, [^{3}H]NMS has a very low NSB (Figure 1) and is a useful tracer for in vitro autoradiography studies.[18] It has a low CHI(IAM) value of 26.5, which, combined with a high affinity to muscarinic receptors (pK_i = 9.2), led to an LSE value of 6.4. The PET tracer ABP688, which despite good imaging properties displays some NSB,[19] has an intermediate LSE value of 5.3.

Despite suffering from high NSB, the LSE value of **1** was close to the minimum value that would be expected for a successful PET tracer. This raised hope that some improvement in binding specificity would allow the use of a close derivative for imaging purposes. The published structure–activity relationship (SAR) focused on modifications of the substituent on the left aromatic ring, as well as replacement of the CH_2 bridge between the aromatic moieties and limited variations around the imidazoline moiety. This left ample space for further structural modifications to improve the LSE.

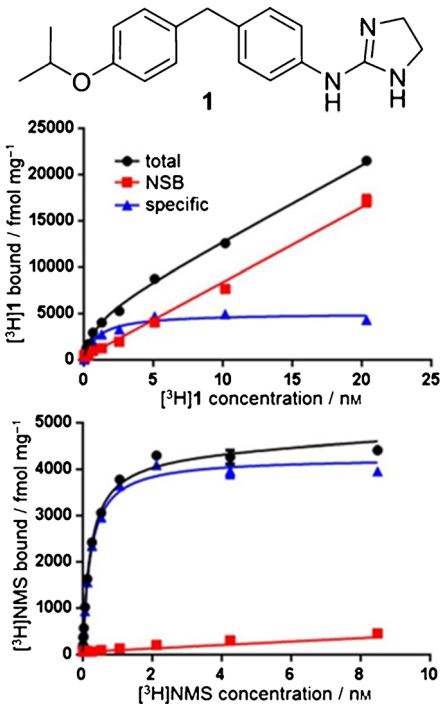
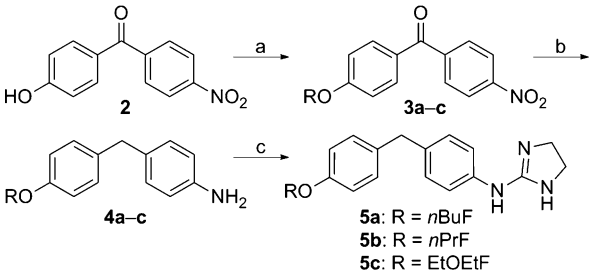


Figure 1. Structure of **1** and saturation binding curves of (upper panel): [^{3}H]**1** binding to hIPR receptors expressed in CHO cell membranes, and for comparison (lower panel): binding of [^{3}H]NMS to CHO-M_3 receptor. Data represent at least three separate experiments performed in triplicate.

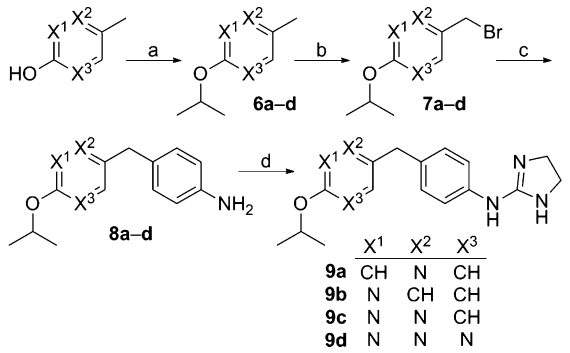
Chemistry

Analogues with fluorinated side chains were obtained in a manner similar to the original synthesis of **1**,[17] starting from *p*-substituted phenol **2** (Scheme 1). Introduction of the desired fluoroalkyl side chain, followed by simultaneous reduction of the ketone and nitro groups, led to amines **4a–c**, which were transformed into the desired products **5a–c** by alkylation with 2-chloro-4,5-dihydro-1*H*-imidazole.

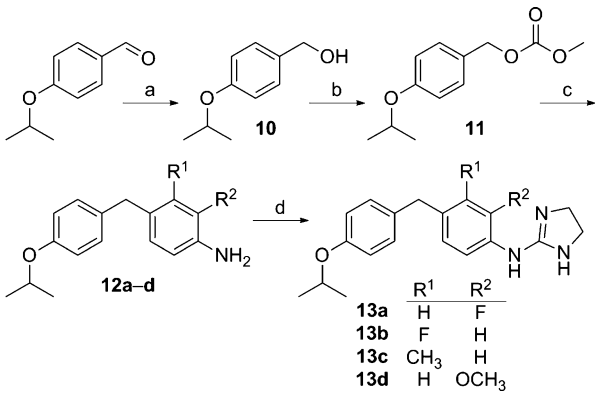


Scheme 1. *Reagents and conditions*: a) RX, K_2CO_3, DMF, 5 h, 70 °C, quant.; b) H_2, 10% Pd/C, EtOH, HCl, 4.5 h, 50 °C, 35–91%; c) 2-chloro-4,5-dihydro-1*H*-imidazole, *i*PrOH, 2 h, 85 °C, 37–90%.

Pyridine, pyridazine, and triazine derivatives (Scheme 2) were obtained from the corresponding methyl heteroaromatic alcohols by alkylation with 2-bromopropane to **6a–d**, followed by bromination of the methyl group using *N*-bromosuccinimide and AIBN. Compounds **7a–d** were then coupled to 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline using a Pd-mediat-



Scheme 2. *Reagents and conditions*: a) For **6a** (21%) and **6c** (35%): 2-bromopropane, K_2CO_3, NaI, DMF, 80 °C, 22 h, 21–35%; for **6b** (41%) and **6d** (47%): 2-bromopropane, Ag_2CO_3, *n*-hexane, 85 °C, 16 h; b) NBS, AIBN, CCl_4, 80 °C, 16 h, 10–90%; c) 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, $[Pd(PPh_3)_4]$, K_3PO_4, EtOH/H_2O (1:1), DME, microwave, 150 °C, 20 min, 49%–quant.; d) 2-chloro-4,5-dihydro-1*H*-imidazole, *i*PrOH, 1 h, 85 °C, 34–55%.

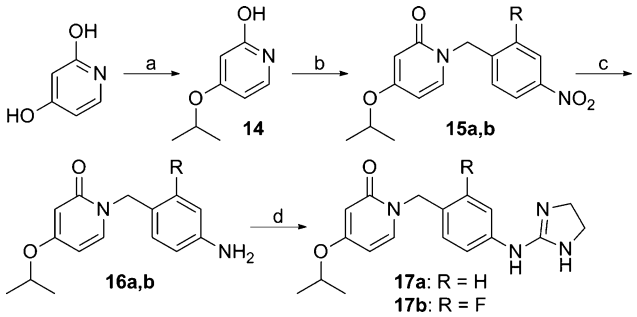


Scheme 3. *Reagents and conditions*: a) $NaBH_4$, THF/H_2O (1:1), 0 °C to RT, 4 h, 92%; b) methyl chloroformate, pyridine, THF, 0 °C to RT, 16 h, 90%; c) allylpalladium(II) chloride dimer, dppe, substituted 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, K_2CO_3, DMF, 65 °C, 15 h, 38–51%; d) 2-chloro-4,5-dihydro-1*H*-imidazole, *i*PrOH, 2 h, 85 °C, 35–86%.

ed cross-coupling reaction, and the dihydroimidazole group was introduced by alkylation, as above, to yield **9a**–**d** in good overall yields.

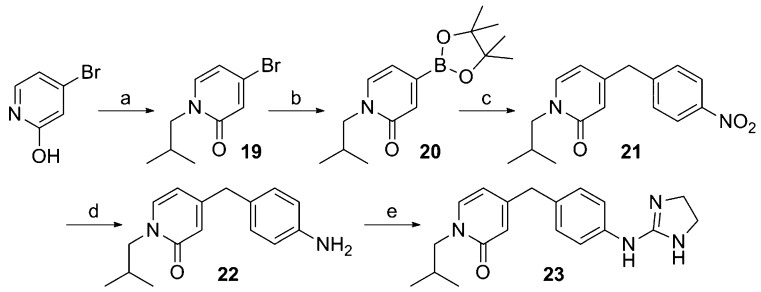
The introduction of substituents on the middle aromatic ring started from 4-isopropoxybenzaldehyde (Scheme 3), which was reduced to alcohol **10** and transformed into carbonate **11** using methyl chloroformate and pyridine as a base. Pd-mediated coupling with an appropriately substituted boronate, followed by the introduction of the dihydroimidazole, led to **13a**–**d**.

Pyridinones **17a**–**b** (Scheme 4) were prepared starting from pyridine-2,4-diol, which was selectively alkylated in position 4 with isopropyl bromide. The ring nitrogen was then alkylated with the corresponding substituted benzyl bromide to yield **15a**–**b**. Reduction of the nitro group and introduction of the dihydroimidazole as above led to **17a**–**b** in high yields.



Scheme 4. *Reagents and conditions*: a) 2-bromopropane, K_2CO_3, DMF, 65 °C, 16 h, 48%; b) K_2CO_3, 1-(bromomethyl)-4-nitrobenzene or 1-(bromomethyl)-2-fluoro-4-nitrobenzene, THF, 16 h, reflux, 75%; c) Pd/C (10%), NEt_3, MeOH, RT, 2 h, 86%; d) 2-chloro-4,5-dihydro-1*H*-imidazole, *i*PrOH, 2 h, 85 °C, 76%.

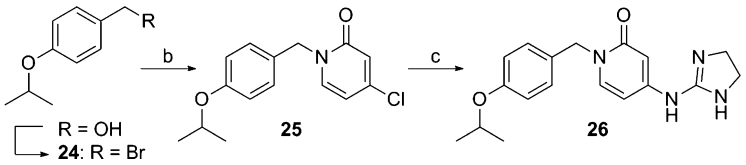
Compound **23** (Scheme 5), with an inverted pyridinone in the left ring, was prepared from 4-bromopyridin-2-ol, first by N-alkylation with 1-bromo-2-methylpropane to yield **19**, then transformation into the corresponding pinacolato boronate and Pd-mediated coupling to 1-(bromomethyl)-4-nitrobenzene to yield **21**. Reduction of the nitro group and introduction of the dihydroimidazole, under the same conditions as previously used, provided **23**.



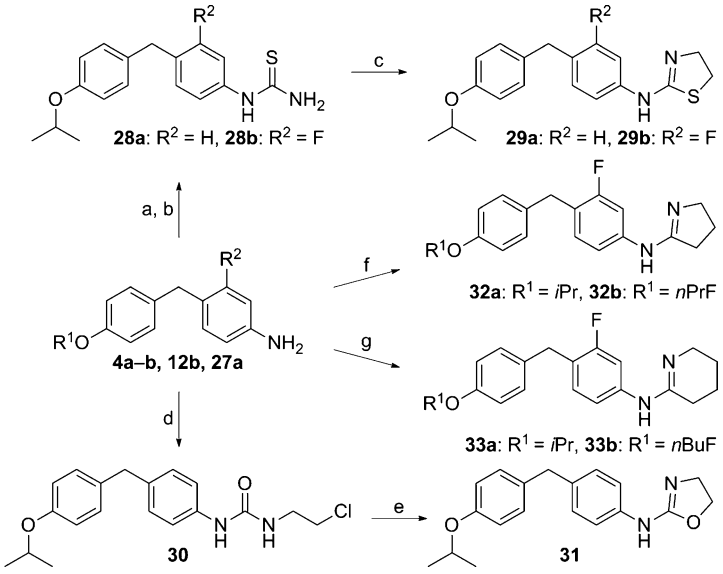
Scheme 5. *Reagents and conditions*: a) 1-bromo-2-methylpropane, K_2CO_3, NaI, DMF, 16 h, 60 °C, 61%; b) bis(pinacolato)diboron, $PdCl_2(dppf)\cdot CH_2Cl_2$, KOAc, dioxane, microwave, 120 °C, 1 h, 95%; c) 1-(bromomethyl)-4-nitrobenzene, $[PdCl_2(dppf)\cdot CH_2Cl_2]$, K_2CO_3, DME, EtOH/H_2O, microwave, 120 °C, 10 min, 34%; d) Pd/C (10%), H_2, NEt_3, EtOH, RT, 2 h, 90%; e) 2-chloro-4,5-dihydro-1*H*-imidazole, *i*PrOH, 40 min, 85 °C, 39%.

Compound **26** (Scheme 6), with a pyridinone in the center of the molecule, was prepared by bromination of (4-isopropoxyphenyl)methanol, followed by alkylation of 4-chloropyridin-2(1*H*)-one to yield **25**. The dihydroimidazolamine group was introduced by Pd-mediated coupling and yielded the desired product in low yield. No attempt was made to further optimize this step.

Finally, replacement of the dihydroimidazolamine group was achieved by the following procedures (Scheme 7). A dihydrothiazolamine was introduced by treating **12b** (R^1 = *i*Pr, R^2 = F) and **27a**[20] (R^1 = *i*Pr, R^2 = H) with benzoyl isothiocyanate, followed by alka-



Scheme 6. *Reagents and conditions*: a) CBr_4, PPh_3, CH_2Cl_2, RT, 1 h, 42%; b) 4-chloropyridin-2(1*H*)-one, K_2CO_3, THF, microwave, 130 °C, 30 min; c) 4,5-dihydro-1*H*-imidazol-2-amine, [BrettPhos-Pd^{II}], BrettPhos, LiHMDS (1 M in THF), THF, microwave, 130 °C, 30 min, 7%.



Scheme 7. *Reagents and conditions*: a) benzoyl isothiocyanate, acetone, RT, 1 h, 77%; b) aq NaOH (5%), 90 °C, 20 min, quant.; c) 2-bromoethylamine, EtOH/H_2O (8:1), reflux, 16 h, 32%; d) 1-chloro-2-isocyanatoethane, CH_2Cl_2, RT, 16 h, quant.; e) SiO_2·KF, CH_3CN, reflux, 4 h, 70%; f) 5-chloro-3,4-dihydro-2*H*-pyrrole, toluene, reflux, 2 h, 55%; g) 6-chloro-2,3,4,5-tetrahydropyridine, toluene, reflux, 2 h, 49–60%.

line hydrolysis and cyclization with 2-bromoethylamine to yield **29a**–**b**. The dihydrooxazolamine derivative **31** was obtained by treating 4-(4-isopropoxybenzyl)aniline[17] with 1-chloro-2-isocyanatoethane, followed by cyclization with KF, in the presence of SiO_2 as a phase-transfer catalyst. The fluorinated anilines **4a** (R^1 = *n*BuF, R^2 = F) and **4b** (R^1 = *n*PrF, R^2 = F) were alkylated with five- and six-membered imidoyl chlorides to yield the dihydroaminopyrrole and tetrahydroaminopyridine derivatives **32a**–**b** and **33a**–**b**.

Optimization and pharmacological evaluation

Before engaging in a broader exploration program, we first ensured that a fluorine atom could be introduced on the oxyalkyl side chain of **1** without deleterious effects. We selected side chains known to be convenient for preparation of precursors and later radiolabeling by classical nucleophilic substitution using $^{18}F^-$ (**5a**–**c**, Table 2). Encouragingly, the first two variations had no effect on LSE, while **5c** appeared less attractive, as the decrease in its CHI(IAM) value did not compensate for its lower affinity for the receptor.

We then tried to introduce polarity in the left aromatic ring, a measure which effectively spread polarity across the molecule and was expected to improve LSE. Compounds **9a**–**b**, the pyridine analogues of **1**, however, showed no significant improvement. Introducing two nitrogen atoms into the ring (**9c**–**d**) led to a marked decrease in CHI(IAM) values but was paralleled by a strong reduction in affinity and did not improve LSE.

Suspecting that the imidazoline might play an important role in driving not only affinity but also NSB, we then tried to influence the latter by modulating its basicity. The pK_b value of **1** in its protonated form was 10.5; introducing a fluorine in the ortho or meta position on the ring adjacent to the imidazoline decreased it slightly, to 9.6 (**13a**) and 9.8 (**13b**), with no effect on LSE. Other substituents (**13c**–**d**) did not have a stronger influence on basicity. While unsuccessful at influencing this property, these derivatives provided interesting SAR information. In particular, the introduction of a fluorine atom in the meta position (**13b**) was well-tolerated and led to a slight increase in affinity for hIPR.

To further explore the potential of increasing LSE by introducing polarity in the left part of the molecule, we prepared pyridinone derivatives **17a** and **23**. Both showed a clear improvement in CHI(IAM) values but lost affinity for hIPR. Compound **17a**, nevertheless, retained an LSE value of 4.9. Encouraged by this result, we tried to regain potency by introducing a fluorine atom on the left ring (**17b**), in analogy to **13b**. To our surprise, the affinity for hIPR actually decreased, while a concomitant increase in NSB led to an LSE value of only 3.5.

A first hint that the imidazoline group could be replaced while retaining some affinity was obtained with **29a**. In contrast, the CHI(IAM) value of dihydrooxazole analogue **31** did not improve enough to compensate for the compound's loss in affinity. Compound **29b**, the fluoro analogue of **29a**, was only slight improved. In contrast, **32a** and **33a** regained potency, and thanks to lower CHI(IAM) values, again reached LSE values equivalent to those of compound **1**.

Despite synthesizing a number of additional derivatives, we struggled to identify compounds with significantly higher LSE

Table 2. Affinity for hIPR (pK_i), experimental CHI(IAM) values, and calculated LSE values of hIPR ligands.

Compound	pK_i	CHI(IAM)	LSE
1	8.68	57.7	4.9
5a	9.09	56.8	5.2
5b	8.75	55.0	5.0
5c	7.56	50.0	4.4
9a	7.33	56.6	4.2
9b	7.91	54.0	4.6
9c	5.10	45.1	3.1
9d	7.87	49.4	4.6
13a	8.03	56.7	4.6
13b	9.28	60.0	5.2
13c	7.27	64.6	4.0
13d	8.33	56.3	4.8
17a	7.06	28.0	4.9
17b	5.92	36.3	3.8
23	5.88	33.7	3.8
26	4.93	31.2	3.3
29a	7.48	47.8	4.5
29b	7.66	48.1	4.6
31	6.78	39.8	4.2
32a	8.44	49.7	5.0
32b	8.46	46.6	5.0
33a	8.06	49.2	4.8
33b	8.51	50.0	5.0

values in this series. We hence decided to prepare fluoroalkyl analogues of the best candidates to assess their potential, including compounds **32b** and **33b**. The affinity and CHI(IAM) values of these compounds did not differ significantly, leading to identical LSE values and showing only marginal improvement over the lead molecule. We therefore concluded that this series did not have enough potential for the development of PET imaging agents. To confirm this result, we nevertheless decided to tritiate **33b** and test it in an autoradiography study looking at lung tissue in the presence and absence of a blocker. We expected to see at least a modest signal if **33b** had properties deserving further evaluation. The images obtained in this experiment (not shown) did not display any significant differences in signal intensity with or without blocking, confirming that **33b** did not have potential for development as a PET imaging agent.

Conclusions

The use of LSE as an index of suitability for PET imaging is based on a series of clinically validated tracers and is an easily applied tool that proved useful in this and other projects. Clearly, its application to a diverse series of PET tracer candidates will be needed to definitively confirm its predictive value. Nevertheless, LSE is based on a rather intuitive concept, in the sense that a good PET tracer candidate should have a balanced mix of affinity and binding specificity. It is a convenient index to evaluate and compare molecules based on measured, rather than in silico, values and is applicable independently of target and chemotype. It can be used to compare diverse starting points for optimization programs, as well as for candidate prioritization and selection.

Experimental Section

Chemistry

General methods: All chemicals, reagents, and solvents were of analytical grade, purchased from commercial sources, and used without purification, unless otherwise specified. ^{1}H NMR spectra were acquired on a Bruker (400 MHz) or Bruker Advance (600 MHz) spectrophotometer. δ values are given in parts per million (ppm) relative to the residual solvent peak. Analytical LCMS/HPLC conditions (% = percent by volume): UPLC-ZQ2000, Acquity HSS-T3 (2.1 × 50 mm, 1.8 μm) column; room temperature; mobile phase: water + 0.5–1.0% HCO_2H (A)/5% acetonitrile + 0.5–1.0% HCO_2H (B); gradient: from 2% to 98% B in 4.3 min + 0.7 min isocratic; flow rate: 1.0 mL min^{-1}.

(4-(4-Fluorobutoxy)phenyl)(4-nitrophenyl)methanone (3a): A suspension of **2**[17] (108 mg, 0.44 mmol), 1-bromo-4-fluorobutane (195 μL, 1.78 mmol), K_2CO_3 (92 mg, 0.67 mmol), and NaI (3.7 mg, 0.02 mmol) in DMF (600 μL) was stirred at 65–70 °C in a sealed glass tube for 5 h. The reaction mixture was diluted in EtOAc and washed with water. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated. The crude product was purified by flash chromatography on silica gel (cyclohexane/EtOAc, 100:0 to 50:50) to give **3a** (144 mg, quant.) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.26–8.22 (m, 2H), 8.27 (d, J = 8.7 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 7.74 (d, J = 8.8 Hz, 2H), 6.91 (d, J = 8.8 Hz, 2H), 4.8 (dt, J = 48 Hz, J = 5.6 Hz, 2H), 4.05 (t, J = 6.0 Hz, 2H), 1.76–1.96 ppm (m, 4H); UPLC–MS: t_R = 1.2 min, m/z: 318.0 [M + H].

4-(4-(4-Fluorobutoxy)benzyl)aniline (4a): Concentrated aq HCl (0.22 mL, 7.40 mmol) was added to a suspension of **3a** (150 mg, 0.47 mmol) and Pd/C (15 mg, 0.014 mmol) in EtOH (2.1 mL). The reaction mixture was stirred under hydrogen (3.5 bar) for 4.5 h at 50 °C, cooled to room temperature, and filtrated through Celite. The Celite cake was washed with EtOH, and the filtrate was concentrated and dissolved in a mixture of EtOAc and saturated aq $NaHCO_3$. The aqueous layer was extracted twice with EtOAc, and the combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated to give **4a** (117 mg, 91%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.00 (d, J = 8.56 Hz, 2H), 6.89 (d, J = 8.19 Hz, 2H), 6.73 (d, J = 8.0 Hz, 2H), 6.55 (d, J = 8.0 Hz, 2H), 4.50 (dt, J = 48 Hz, J = 5.55 Hz, 2H), 3.90 (t, J = 5.75 Hz, 2H), 1.66–1.86 ppm (m, 4H); UPLC–MS: t_R = 1.08 min, m/z: 274.1 [M + H].

***N*-(4-(4-(4-Fluorobutoxy)benzyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (5a)**: A suspension of **4a** (117 mg, 0.41 mmol) and 2-chloro-4,5-dihydro-1*H*-imidazole (109 mg, 0.54 mmol) in isopropanol (1.5 mL) was stirred at 80–85 °C in a sealed glass tube for 2 h. The reaction mixture was cooled to room temperature, diluted in EtOAc, and washed with diluted aq $NaHCO_3$. The aqueous layer was extracted twice with EtOAc. The combined organic layer was washed with brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH:NH_3 [9:1], from 100:0 to 90:10), to give **5a** (126 mg, 90%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.05–6.97 (m, 4H) 6.84 (d, J = 8.31 Hz, 2H) 6.74 (d, J = 7.67 Hz, 2H) 4.45 (dt, J = 48 Hz, J = 5.6 Hz, 2H) 3.91 (t, J = 5.81 Hz, 2H) 3.79 (s, 2H) 3.45 (s, 4H) 2.22–1.90 (br s, 2H) 1.76–1.89 ppm (m, 4H); UPLC–MS: t_R = 0.84 min, m/z: 342.4 [M + H].

(4-(3-Fluoropropoxy)phenyl)(4-nitrophenyl)methanone (3b): Compound **3b** was prepared from **2** (108 mg, 0.44 mmol), according to the procedure described for the synthesis of **3a**. After purification by flash chromatography, **3b** was obtained as a white powder (144 mg, quant.): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.35 (d, J = 8.68 Hz, 2H), 7.89 (d, J = 8.68 Hz, 2H), 7.81 (d, J = 8.80 Hz, 2H), 6.99 (d, J = 8.80 Hz, 2H), 4.55 (dt, J = 44 Hz, 5.6 Hz, 2H), 4.12 (t, J = 6.05 Hz, 2H), 1.86–2.02 ppm (m, 2H); UPLC–MS: t_R = 1.20 min, m/z: 318.0 [M + H].

4-(4-(3-Fluoropropoxy)benzyl)aniline (4b): Compound **4b** was prepared from **3b** (229 mg, 0.38 mmol), according to the procedure described for the synthesis of **4a**, and was obtained as a white powder (81 mg, 83%): UPLC–MS: t_R = 1.01 min, m/z: 260.3 [M + H].

***N*-(4-(4-(3-Fluoropropoxy)benzyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (5b)**: Compound **5b** was prepared from **4b** (81 mg, 0.31 mmol), according to the procedure described for the synthesis of **5a**, and was obtained as a white powder (39 mg, 37%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 (dd, J = 19.75, 8.38 Hz, 4H), 6.90 (d, J = 8.31 Hz, 2H), 6.83 (d, J = 8.56 Hz, 2H), 4.65 (dt, J = 44 Hz, J = 5.81 Hz, 2H), 4.08 (t, J = 6.11 Hz, 2H), 3.86 (s, 2H), 3.51 (s, 4H), 2.09–2.23 ppm (m, 2H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 157.8, 157.1, 147.5, 135.2, 134.0, 129.9 (2C), 129.6 (2C), 122.9, 114.4 (2C), 81.4, 80.3, 63.5, 42.5, 40.5 (2C), 30.5 ppm; UPLC–MS: t_R = 0.73 min, m/z: 328.1 [M + H].

(4-(2-(2-Fluoroethoxy)ethoxy)phenyl)(4-nitrophenyl)methanone (3c): TBAF (3.94 mL, 3.94 mmol) was added to a solution of 2-(2-(4-(4-nitrobenzoyl)phenoxy)ethoxy)ethyl 4-methylbenzenesulfonate (239 mg, 0.49 mmol) in THF (4.10 mL), and the resulting deep purple solution was stirred at 65 °C for 2 h in a sealed glass tube under nitrogen. The solvent was then evaporated. The residue was dissolved in EtOAc, and the organic layer was washed with water. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The crude was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 85:15) to give **3c** (103.8 mg, 63.3%) as a yellow liquid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34 (d, J = 8.80 Hz, 2H), 7.89 (d, J = 8.68 Hz, 2H), 7.81 (d, J = 8.7 Hz, 2H), 7.03 (d, J = 8.7 Hz, 2H), 4.65–4.70 (m, 1H), 4.49–4.62 (m, 1H), 4.22–4.28 (m, 2H), 3.92–3.99 (m, 2H), 3.86–3.91 (m, 1H), 3.76–3.85 ppm (m, 1H); UPLC–MS: t_R = 1.07 min, m/z: 334.0 [M+H].

The precursor was prepared in the following manner:

2-(2-(4-(4-Nitrobenzoyl)phenoxy)ethoxy)ethyl 4-methylbenzenesulfonate: *p*-Tolylsulfonyl chloride (133 mg, 0.69 mmol) followed by triethylamine (230 μL, 1.65 mmol) were added successively at 0–5 °C under nitrogen to a stirred solution of (4-(2-(2-hydroxyethoxy)ethoxy)phenyl)(4-nitrophenyl)methanone (218.6 mg, 0.66 mmol) in CH_2Cl_2 (528 μL). The resulting solution was stirred at room temperature for 18 h, then diluted with CH_2Cl_2. The organic layer was washed with water, brine, dried over anhyd Na_2SO_4, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (cyclohexane/EtOAc 100:0 to 45:55) to give the desired product (239 mg, 72.5%) as transparent liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.35 (d, J = 8.68 Hz, 2H), 7.89 (d, J = 8.68 Hz, 2H), 7.73–7.85 (m, 4H), 7.33 (d, J = 8.31 Hz, 2H), 7.00 (d, J = 8.80 Hz, 2H), 4.19 (dt, J = 12.26, 4.69 Hz, 4H), 3.82–3.89 (m, 2H), 3.75–3.81 (m, 2H), 2.44 ppm (s, 3H); UPLC–MS: t_R = 1.21 min, m/z: 486.0 [M+H].

(4-(2-(2-Hydroxyethoxy)ethoxy)phenyl)(4-nitrophenyl)methanone: A suspension of **2** (150 mg, 0.62 mmol), 2-(2-chloroethoxy)-ethan-1-ol (267 μL, 2.47 mmol), K_2CO_3 (128 mg, 0.92 mmol) and NaI (5.2 mg, 0.035 mmol) in DMF (833 μL) was stirred at 65–70 °C in a sealed glass tube for 16 h. After cooling to room temperature, the reaction mixture was diluted in EtOAc and washed with water. The aqueous layer was extracted twice with EtOAc. The combined organic layers were washed with brine, dried over anhyd Na_2SO_4, filtered and concentrated. The crude was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 20:80) to provide the desired product as a white solid (218.6 mg, 100%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.34 (d, J = 8.80 Hz, 2H), 7.89 (d, J = 8.80 Hz, 2H), 7.82 (d, J = 8.80 Hz, 2H), 7.02 (d, J = 8.80 Hz, 2H), 4.18–4.29 (m, 2H), 3.86–3.97 (m, 2H), 3.76–3.83 (m, 2H), 3.66–3.73 (m, 2H), 1.99–2.05 ppm (m, 1H); UPLC–MS: t_R = 0.90 min, m/z: 332.1 [M+H].

4-(4-(2-(2-Fluoroethoxy)ethoxy)benzyl)aniline (4c): Compound **4c** was prepared from **3c** (83 mg, 0.25 mmol), according to the procedure described for the synthesis of **4a**, and was obtained as a white oil (25.6 mg, 35%): UPLC–MS: t_R = 0.91 min, m/z: 290.1 [M+H].

***N*-(4-(4-(2-(2-Fluoroethoxy)ethoxy)benzyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (5c)**: Compound **5c** was prepared from **4c** (25 mg, 0.0.9 mmol), according to the procedure described for the synthesis of **5a**, and was obtained as a colorless oil (14 mg, 42.7%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09 (dd, J = 17.91, 8.38 Hz, 4H), 6.79–6.95 (m, 4H), 4.62–4.69 (m, 1H), 4.49–4.56 (m, 1H), 4.08–4.17(m, 2H), 3.83–3.92 (m, 5H), 3.76–3.81 (m, 1H), 3.47–3.54 ppm (m, 4H); UPLC–MS: t_R = 0.68 min, m/z: 358.4 [M+H].

5-Isopropoxy-2-methylpyridine (6a): A suspension of 6-methylpyridin-3-ol (1.0 g, 9.16 mmol), 2-bromopropane (2.6 mL, 27.5 mmol), K_2CO_3 (1.9 g, 13.75 mmol), and NaI (0.076 g, 0.50 mmol) in DMF (10.00 mL) was stirred in a sealed glass tube at 80 °C for 22 h under argon. The reaction mixture was diluted with EtOAc, washed with water and brine, dried over a phase separator, and concentrated. The residue was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 0:100) to afford **6a** (288 mg, 20.8%) as a colorless liquid: ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.10 (d, J = 2.93 Hz, 1H), 7.26 (dd, J = 8.50, 3.00 Hz, 1H), 7.15 (d, J = 8.56 Hz, 1H), 4.62 (dt, J = 12.07, 6.01 Hz, 1H), 2.38 (s, 3H), 1.26 ppm (d, J = 5.99 Hz, 6H).

2-(Bromomethyl)-5-isopropoxypyridine (7a): NBS (339 mg, 1.90 mmol) and AIBN (31.3 mg, 0.19 mmol) were added to a solution of **6a** (288 mg, 1.90 mmol) in CCl_4 (6.35 mL) at room temperature under argon. The reaction mixture was stirred at 80 °C for 60 h. The reaction mixture was then cooled to room temperature and extracted with CH_2Cl_2 and water. The organic layer was dried over a phase separator and concentrated. The crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 70:30) to afford **7a** (85 mg, 9.7%) as an orange oil with 50% purity. The product was used as such in the next step.

4-((5-Isopropoxypyridin-2-yl)methyl)aniline (8a): DME (1.7 mL) and EtOH/H_2O (0.4 mL each) were added to a mixture of **7a** (85 mg, 0.18 mmol), 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (50.1 mg, 0.22 mmol), [$Pd(PPh_3)_4$] (21.34 mg, 0.018 mmol), and K_3PO_4 (78 mg, 0.369 mmol). Argon was bubbled through the stirred reaction mixture for 5 min, then the mixture was heated in a microwave at 120 °C for 40 min, cooled to room temperature, concentrated, and purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 50:50) to afford **8a** as a yellow oil (32 mg, 49%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 8.01–8.36 (m, 1H); 7.41–7.76 (m, 3H), 6.98–7.34 (m, 2H), 6.87 (dd, J = 8.19, 2.32 Hz, 1H), 6.11–6.67 (m, 2H), 4.85 (br s, 1H), 4.60 (ddt, J = 8.94, 6.01, 3.16, 3.16 Hz, 1H), 3.79 (d, J = 2.20 Hz, 1H), 1.24 ppm (dd, J = 5.99, 2.69 Hz, 6H); UPLC–MS: t_R = 0.70 min, m/z: 243.4 [M+H].

***N*-(4-((5-Isopropoxypyridin-2-yl)methyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (9a)**: Compound **8a** (32 mg, 0.13 mmol) and 2-chloro-4,5-dihydro-1*H*-imidazole (37.2 mg, 0.17 mmol) in isopropanol (1.8 mL) were stirred at 85 °C in a sealed glass tube for 1 h under argon. The reaction mixture was cooled to room temperature and concentrated, and the residue was extracted with CH_2Cl_2 and 2 M aq NaOH. The organic layer was washed with brine, dried over a phase separator, and concentrated. The crude product was purified on silica gel by flash chromatography ([CH_2Cl_2:MeOH]/10% NH_4OH, 100:0 to 80:20) to afford **9a** as an orange oil (17 mg, 33.5%): ^{1}H NMR (400 MHz, CD_3OD): δ = 8.08 (d, J = 2.81 Hz, 1H), 7.33 (dd, J = 8.68, 2.93 Hz, 1H), 7.13–7.27 (m, 3H), 6.94–7.06 (m, 2H), 4.64 (dt, J = 12.07, 6.01 Hz, 1H), 4.03 (s, 2H), 3.53 (d, J = 2.57 Hz, 4H), 1.34 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 0.61 min, m/z: 311.4 [M+H].

2-Isopropoxy-5-methylpyridine (6b): 2-bromopropane (2.58 mL, 27.5 mmol), Ag_2CO_3 (3.79 g, 13.75 mmol), and NaI (0.069 g, 0.46 mmol) were added to 5-methylpyridin-2-ol (1.00 g, 9.16 mmol) in *n*-hexane (40 mL) at 85 °C under argon. After cooling to room temperature, the reaction mixture was stirred in a sealed glass tube at 85 °C for 16 h. The insoluble residue was filtered through Celite, the Celite was washed with n-hexane, and the filtrate was concentrated and purified on silica gel by flash chromatography

(cyclohexane/EtOAc, 100:0 to 95:5) to provide **6b** as a colorless liquid (630 mg, 41%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.94 (d, J = 2.08 Hz, 1H), 7.49 (dd, J = 8.31, 2.45 Hz, 1H), 6.63 (d, J = 8.44 Hz, 1H), 5.18 (dt, J = 12.35, 6.17 Hz, 1H), 2.19 (s, 3H), 1.26 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 1.03 min, m/z: 152.1 [M+H].

5-(Bromomethyl)-2-isopropoxypyridine (7b): Compound **7b** was prepared from **6b** (464 mg, 3.07 mmol), according to the procedure described for the synthesis of **7a**, and was obtained as a colorless oil (159 mg, 13.5%). The product was not stable and was used as such in the next step.

4-((6-Isopropoxypyridin-3-yl)methyl)aniline (8b): Compound **8b** was prepared from **7b** (159 mg, 0.43 mmol), according to the procedure described for the synthesis of **8a**, and was obtained as a yellow oil (13 mg, 12.6%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.97 (d, J = 2.20 Hz, 1H), 7.43 (dd, J = 8.44, 2.45 Hz, 1H), 6.85 (d, J = 8.19 Hz, 2H), 6.62 (d, J = 8.44 Hz, 1H), 6.47 (d, J = 8.31 Hz, 2H), 5.18 (dt, J = 12.35, 6.17 Hz, 1H), 4.87 (s, 2H), 3.65 (s, 2H), 1.25 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 0.98 min, m/z: 243.1 [M+H].

***N*-(4-((6-Isopropoxypyridin-3-yl)methyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (9b)**: Compound **9b** was prepared from **8b** (108 mg, 0.44 mmol), according to the procedure described for the synthesis of **9a**, and was obtained as a white oil (14 mg, 54.6%): ^{1}H NMR (400 MHz, CD$_3$OD): δ = 7.97 (d, J = 2.20 Hz, 1H), 7.50 (dd, J = 8.56, 2.45 Hz, 1H), 7.17 (d, J = 8.19 Hz, 2H), 7.01 (d, J = 8.44 Hz, 2H), 6.66 (d, J = 8.56 Hz, 1H), 5.16 (dt, J = 12.35, 6.17 Hz, 1H), 3.87 (s, 2H), 3.55 (s, 4H), 1.32 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 0.75 min, m/z: 311.4 [M+H].

3-Isopropoxy-6-methylpyridazine (6c): Compound **6c** was prepared from 6-methylpyridazin-3-ol (1 g, 9.08 mmol), according to the procedure described for the synthesis of **6a**, and was obtained as an orange oil (489 mg, 35.4%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.29 (d, J = 9.41 Hz, 1H), 6.83 (d, J = 9.54 Hz, 1H), 5.11 (quin, J = 6.66 Hz, 1H), 2.28 (s, 3H), 1.25 ppm (d, J = 6.72 Hz, 6H); UPLC–MS: t_R = 0.68 min, m/z: 153.0 [M+H].

3-(Bromomethyl)-6-isopropoxypyridazine (7c): Compound **7c** was prepared from **6c** (489 mg, 3.21 mmol), according to the procedure described for the synthesis of **7a**, and was obtained as an orange oil (446 mg, 60%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.53 (d, J = 9.66 Hz, 1H), 6.96 (d, J = 9.54 Hz, 1H), 5.12 (quin, J = 6.63 Hz, 1H), 4.59 (s, 2H), 1.27 ppm (d, J = 6.60 Hz, 6H); UPLC–MS: t_R = 0.83 min, m/z: 232.9 [M+H].

4-((6-Isopropoxypyridazin-3-yl)methyl)aniline (8c): Compound **8c** was prepared from **7c** (45 mg, 0.19 mmol), according to the procedure described for the synthesis of **8a**, and was obtained as a yellow oil (34 mg, 56.7%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.60–7.67 (m, 2H), 7.52–7.58 (m, 1H), 7.19 (d, J = 9.41 Hz, 1H), 6.89 (d, J = 8.31 Hz, 2H), 6.80 (d, J = 9.54 Hz, 1H), 6.50 (d, J = 8.31 Hz, 2H), 5.12 (quin, J = 6.63 Hz, 1H), 3.71 (s, 2H), 1.16–1.35 ppm (m, 6H); UPLC–MS: t_R = 0.72 min, m/z: 243.9 [M+H].

***N*-(4-((6-Isopropoxypyridazin-3-yl)methyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (9c)**: Compound **9c** was prepared from **8c** (34 mg, 0.11 mmol), according to the procedure described for the synthesis of **9a**, and was obtained as a white powder (18 mg, 51.8%): ^{1}H NMR (400 MHz, CD$_3$OD): δ = 7.17 (d, J = 9.41 Hz, 1H), 7.06 (d, J = 8.31 Hz, 2H), 6.88 (d, J = 8.31 Hz, 2H), 6.74 (d, J = 9.54 Hz, 1H), 5.14 (quin, J = 6.63 Hz, 1H), 3.81 (s, 2H), 3.39 (s, 4H), 1.28 ppm (d, J = 6.60 Hz, 6H); ^{13}C NMR (151 MHz, CD$_3$OD): δ = 161.5, 161.1, 150.1, 147.8, 134.3, 133.0, 130.7 (2C), 130.1, 124.6 (2C), 50.8, 44.0 (2C), 41.3, 21.3 ppm (2C); UPLC–MS: t_R = 0.56 min, m/z: 311.8 [M+H].

2-Isopropoxy-5-methylpyrazine (6d): Compound **6d** was prepared from 5-methylpyrazin-2-ol (45 mg, 0.19 mmol), according to the procedure described for the synthesis of **6b**, and was obtained as an orange liquid (395 mg, 46.9%). It was used in the next step without further purification.

2-(Bromomethyl)-5-isopropoxypyrazine (7d): Compound **7d** was prepared from **6d** (489 mg, 3.21 mmol), according to the procedure described for the synthesis of **7a**, and was obtained as an orange oil (338 mg, 68%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.34 (d, J = 0.98 Hz, 1H), 8.23 (d, J = 1.10 Hz, 1H), 5.23 (dt, J = 12.35, 6.17 Hz, 1H), 4.73 (s, 2H), 1.25–1.36 ppm (m, 6H); UPLC–MS: t_R = 1.04 min, m/z: 232.9 [M+H].

4-((5-Isopropoxypyrazin-2-yl)methyl)aniline (8d): Compound **8d** was prepared from **7d** (85 mg, 0.34 mmol), according to the procedure described for the synthesis of **8a**, and was obtained as an orange oil (81 mg, 72%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 8.09–8.14 (m, 1H), 8.04 (s, 1H), 6.89 (d, J = 8.31 Hz, 2H), 6.47 (d, J = 8.31 Hz, 2H), 5.07–5.25 (m, 1H), 3.82 (s, 2H), 1.29 ppm (d, J = 6.24 Hz, 6H); UPLC–MS: t_R = 0.89 min, m/z: 244.1 [M+H].

***N*-(4-((5-Isopropoxypyrazin-2-yl)methyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (9d)**: Compound **9d** was prepared from **8d** (81 mg, 0.25 mmol), according to the procedure described for the synthesis of **9a**, and was obtained as a yellow oil (40 mg, 50.4%): ^{1}H NMR (400 MHz, CD$_3$OD): δ = 8.03 (d, J = 9.90 Hz, 2H), 7.19 (d, J = 8.31 Hz, 2H), 6.93–7.06 (m, 2H), 5.26 (dt, J = 12.35, 6.17 Hz, 1H), 4.03 (s, 2H), 3.52 (s, 4H), 1.35 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 0.71 min, m/z: 312.4 [M+H].

(4-Isopropoxyphenyl)methanol (10): A suspension of 4-isopropoxybenzaldehyde (1.0 g, 6.09 mmol) in THF (8 mL) and water (8 mL) was cooled to 0 °C, and $NaBH_4$ (0.69 g, 18.27 mmol) was added portion-wise. The reaction mixture was stirred for 4 h, allowing the reaction to warm to room temperature. The solvent was removed under reduced pressure, water (20 mL) was added, and the mixture was extracted with EtOAc. The combined organic layers were washed with water and brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The crude product was purified on silica gel by flash chromatography (hexanes/EtOAc, 100:0 to 80:20) to give **10** as a colorless oil (950 mg, 92%): UPLC–MS: t_R = 0.71 min, m/z: 149.0 [M+H−H$_2$O].

4-Isopropoxybenzyl methyl carbonate (11): Methyl chloroformate (0.920 mL, 11.91 mmol) was added slowly to a solution of **10** (900 mg, 5.41 mmol) in THF (30 mL) and pyridine (1.14 mL, 14.08 mmol) at 0 °C. The resulting white suspension was stirred overnight at room temperature. A solution of aq HCl (6 M) was added until the mixture reached pH 1, and the mixture was then extracted with methyl-*tert*-butyl ether. The combined organic layers were washed with brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The crude product was purified on silica gel by flash chromatography (hexanes/EtOAc, 100:0 to 90:10) to give **11** as a colorless oil (1.1 g, 90%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.18–7.25 (m, 2H), 6.77–6.82 (m, 2H), 5.02 (s, 2H), 4.48 (dt, J = 12.10, 6.05 Hz, 1H), 3.71 (s, 3H), 1.26 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 1.08 min.

2-Fluoro-4-(4-isopropoxybenzyl)aniline (12a): A solution of **11** (200 mg, 0.89 mmol) and 3-fluoro-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline (233 mg, 0.981 mmol) in DMF (2 mL) was degassed using an argon stream for 10 min. Potassium carbonate (370 mg, 2.68 mmol), allylpalladium chloride dimer (48.9 mg, 0.13 mmol), and 1,5-bis(diphenylphosphino)pentane (118 mg, 0.27 mmol) were added to the solution at room temperature

under argon, then the suspension was stirred at 65 °C for 16 h. After cooling to room temperature, EtOAc (20 mL) and water (20 mL) were added, and the biphasic suspension was filtered over Celite. The filter cake was washed with EtOAc and water. The organic layer was washed with brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The crude product was purified on silica gel by flash chromatography (hexanes/EtOAc, 100:0 to 90:10) to provide **12a** as a yellow oil (130 mg, 51.7%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.08 (d, J = 8.56 Hz, 2H), 6.69–6.87 (m, 5H), 4.52 (quin, J = 6.05 Hz, 1H), 3.82 (s, 2H), 1.34 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 1.19 min, m/z: 260.1 [M+H].

***N*-(2-Fluoro-4-(4-isopropoxybenzyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (13a)**: A suspension of **12a** (100 mg, 0.39 mmol) and 2-chloro-4,5-dihydro-1*H*-imidazole (69.3 mg, 0.66 mmol) in 2-propanol (4 mL) was stirred at reflux for 3 h under argon. The reaction mixture was concentrated, and the residue was extracted with methyl-*tert*-butyl ether and aq NaOH (2 M), the organic layer was washed with brine, dried over anhyd Na_2SO_4, filtered, and concentrated. The crude product was purified on silica gel (CH_2Cl_2/MeOH/NH_4OH, 99:1:1 to 80:20:1) to provide **13a** as a white powder (45 mg, 35%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.10 (d, J = 8.56 Hz, 2H), 6.96 (t, J = 8.25 Hz, 1H), 6.80–6.89 (m, 4H), 4.53 (dt, J = 12.13, 6.10 Hz, 2H), 3.85 (s, 2H), 3.55 (s, 4H), 1.53–1.77 (br s, 2H), 1.35 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 0.83 min, m/z: 328.4 [M+H].

3-Fluoro-4-(4-isopropoxybenzyl)aniline (12b): Compound **12b** was prepared from **11** (200 mg, 0.89 mmol), according to the procedure described for the synthesis of **12a**. After purification by flash chromatography, **12b** was obtained as a yellow oil (130 mg, 51.7%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.08 (m, J = 8.44 Hz, 2H), 6.89 (t, J = 8.19 Hz, 1H), 6.77–6.82 (m, J = 8.44 Hz, 2H), 6.38 (d, J = 9.66 Hz, 2H), 4.49 (dt, J = 12.10, 6.05 Hz, 1H), 3.81 (s, 2H), 3.65 (br s, 2H), 1.31 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 1.17 min, m/z: 260.1 [M+H].

***N*-(3-Fluoro-4-(4-isopropoxybenzyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (13b)**: Compound **13b** was prepared from **12b** (120 mg, 0.46 mmol), according to the procedure described for the synthesis of **13a**. After purification by flash chromatography, **13b** was obtained as a yellow oil (105 mg, 68.8%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.09–7.14 (m, 2H), 6.99 (t, J = 8.13 Hz, 1H), 6.78–6.83 (m, J = 8.44 Hz, 2H), 6.64–6.70 (m, 2H), 4.46–4.55 (m, 2H), 3.86 (s, 2H), 3.52 (s, 4H), 1.64 (br s, 2H), 1.32 (d, J = 6.11 Hz, 6H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 161.3 (d, J = 238 Hz, 1C), 157.9, 156.2, 150.1, 132.4, 131.1, 129.7 (2C), 121.6, 118.7, 115.8 (2C), 109.8, 69.9, 42.4, 33.6 (2C), 22.2 ppm (2C); UPLC–MS: t_R = 0.84 min, m/z: 328.4 [M+H].

4-(4-Isopropoxybenzyl)-3-(trifluoromethyl)aniline (12c): Compound **12c** was prepared from **11** (100 mg, 0.45 mmol), according to the procedure described for the synthesis of **12a**. After purification by flash chromatography, **12c** was obtained as a yellow oil (65 mg, 43.8%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 6.92–7.02 (m, 3H), 6.88 (d, J = 2.32 Hz, 1H), 6.81 (d, J = 8.56 Hz, 2H), 6.73 (dd, J = 8.31, 2.08 Hz, 1H), 5.40 (s, 2H), 4.53 (dt, J = 12.07, 6.01 Hz, 1H), 3.85 (s, 2H), 1.24 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 1.30 min, m/z: 310.0 [M+H].

***N*-(4-(4-Isopropoxybenzyl)-3-(trifluoromethyl)phenyl)-4,5-dihydro-1*H*-imidazol-2-amine (13c)**: Compound **13c** was prepared from **12c** (65 mg, 0.19 mmol), according to the procedure described for the synthesis of **13a**, and was obtained as a yellow oil (65 mg, 86%): ^{1}H NMR (400 MHz, CD_3OD): δ = 7.30 (s, 1H), 7.14 (d, J = 0.98 Hz, 2H), 7.05 (d, J = 8.56 Hz, 2H), 6.83 (d, J = 8.68 Hz, 2H), 4.56 (dt, J = 12.07, 6.01 Hz, 1H), 4.03 (s, 2H), 3.52 (s, 4H), 1.30 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 1.01 min, m/z: 378.5 [M+H].

4-(4-Isopropoxybenzyl)-2-methoxyaniline (12d): Compound **12d** was prepared from **11** (100 mg, 0.45 mmol) and 2-methoxy-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline,[21] according to the procedure described for the synthesis of **12a**, and was obtained as a yellow oil (56 mg, 38%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.08 (d, J = 8.56 Hz, 2H), 6.80 (d, J = 8.56 Hz, 2H), 6.66 (d, J = 1.10 Hz, 1H), 6.40–6.58 (m, 2H), 4.51–4.57 (m, 1H), 3.72 (s, 3H), 3.70 (s, 2H), 1.23 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 1.17 min, m/z: 272.4 [M+H].

***N*-(4-(4-Isopropoxybenzyl)-2-methoxyphenyl)-4,5-dihydro-1*H*-imidazol-2-amine (13d)**: Compound **13d** was prepared from **12d** (55 mg, 0.17 mmol), according to the procedure described for the synthesis of **13a**, and obtained as a yellow oil (23 mg, 40.4%): ^{1}H NMR (400 MHz, CD_3OD): δ = 7.11 (d, J = 8.68 Hz, 2H), 7.01 (d, J = 7.95 Hz, 1H), 6.78–6.85 (m, 3H), 6.74 (dd, J = 7.95, 1.59 Hz, 1H), 4.55 (dt, J = 12.10, 6.05 Hz, 1H), 3.86 (s, 2H), 3.78 (s, 3H), 3.50 (s, 4H), 1.30 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 0.90 min, m/z: 340.4 [M+H].

4-Isopropoxypyridin-2-ol (14): 2-Bromopropane (0.84 mL, 9.0 mmol) and K_2CO_3 (1.49 g, 10.8 mmol) were added to a solution of pyridine-2,4-diol (1.0 g, 9.0 mmol) in anhydrous DMF (10 mL) at room temperature under argon. The reaction mixture was stirred overnight at 65 °C. The off-white suspension was cooled to room temperature, water was added, and the product extracted with CH_2Cl_2/2-propanol (70:30). The organic layer was dried over a phase separator and concentrated. The crude product was purified on silica gel by flash chromatography (hexanes/EtOAc, 50:50 to 0:100 then CH_2Cl_2/MeOH 100:0 to 80:20) to provide **14** as a white powder (668 mg, 48%), in addition to 2-isopropoxypyridin-4-ol (175 mg, 13%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 11.02 (br s, 1H), 7.20 (d, J = 7.34 Hz, 1H), 5.79 (dd, J = 7.34, 2.32 Hz, 1H), 5.65 (d, J = 2.32 Hz, 1H), 4.58 (dt, J = 12.01, 6.04 Hz, 1H), 1.24 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 0.57 min, m/z: 154.0 [M+H].

4-Isopropoxy-1-(4-nitrobenzyl)pyridin-2(1*H*)-one (15a): K_2CO_3 (361 mg, 2.61 mmol) and 1-(bromomethyl)-4-nitrobenzene (367 mg, 1.697 mmol) were slowly added to a solution of **14** (200 mg, 1.306 mmol) in THF (8 mL). The reaction mixture was stirred overnight at reflux. The mixture was cooled to room temperature, water was added, and the product was extracted with methyl-*tert*-butyl ether. The organic layer was dried over a phase separator and concentrated to provide **15a** as a white powder (285 mg, 75%): ^{1}H NMR (400 MHz, CDCl3): δ = 8.19 (m, J = 8.56 Hz, 2H), 7.43 (d, J = 8.56 Hz, 2H), 7.13 (d, J = 7.21 Hz, 1H), 5.89–5.94 (m, 2H), 5.16 (s, 2H), 4.52 (dt, J = 12.17, 6.02 Hz, 1H), 1.35 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 0.93 min, m/z: 289.3 [M+H].

1-(4-Aminobenzyl)-4-isopropoxypyridin-2(1*H*)-one (16a): A solution of **15a** (71 mg, 0.25 mmol), triethylamine (0.34 mL, 2.46 mmol) and Pd/C (14.7 mg, 0.014 mmol) in MeOH (5.0 mL) was stirred at room temperature under hydrogen (0.1 bar) for 2 h. The reaction mixture was filtered over Celite, and the Celite pad was washed with EtOAc. The filtrate was concentrated, and the residue was dissolved with EtOAc and washed with water, dried over a phase separator, and concentrated. The crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 0:100) to afford **16a** as a colorless oil (55 mg, 86%): ^{1}H NMR (400 MHz, [D$_6$]DMSO): δ = 7.54 (d, J = 7.58 Hz, 1H), 6.99 (d, J = 8.31 Hz, 2H), 6.49 (d, J = 8.31 Hz, 2H), 5.86 (dd, J = 7.58, 2.57 Hz, 1H), 5.76 (d, J = 2.57 Hz, 1H), 5.05 (s, 2H), 4.80 (s, 2H), 4.58 (dt, J = 11.98, 5.99 Hz,

1H), 1.24 ppm (d, $J=5.99$ Hz, 6H); UPLC–MS: $t_R=0.73$ min, *m/z*: 259.1 [$M+H$].

1-(4-((4,5-Dihydro-1*H*-imidazol-2-yl)amino)benzyl)-4-isopropoxypyridin-2(1*H*)-one (17a): A mixture of **16a** (55 mg, 0.21 mmol) and 2-chloro-4,5-dihydro-1*H*-imidazole (59.9 mg, 0.28 mmol) in isopropanol (2.8 mL) was stirred at 85 °C in a sealed glass tube for 1 h under an argon atmosphere. The reaction mixture was cooled to room temperature, concentrated, and the residue extracted with CH_2Cl_2/aq NaOH (2 M), the organic layer was washed with brine, dried over a phase separator, and concentrated. The crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH/14.7 M NH_4OH, 100:0:0 to 90:10:1) to afford **17a** as a white foam (53 mg, 76%): ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.55$ (d, $J=7.58$ Hz, 1H), 7.24 (d, $J=8.31$ Hz, 2H), 7.00 (d, $J=8.31$ Hz, 2H), 6.06 (dd, $J=7.58$, 2.69 Hz, 1H), 5.93 (d, $J=2.69$ Hz, 1H), 5.06 (s, 2H), 4.53–4.71 (m, 1H), 3.51 (s, 4H), 1.34 ppm (d, $J=5.99$ Hz, 6H); ^{1}C NMR (400 MHz, CD_3OD): $\delta=168.8$, 166.4, 161.2, 149.0, 139.9, 132.0, 130.0 (2C), 124.6 (2C), 103.6, 99.0, 72.1, 52.3, 43.9 (2C), 21.9 ppm (2C); UPLC–MS: $t_R=0.62$ min, *m/z*: 327.2 [$M+H$].

1-(2-Fluoro-4-nitrobenzyl)-4-isopropoxypyridin-2(1*H*)-one (15b): Compound **15b** was prepared from **14** (50 mg, 0.33 mmol) and 1-(bromomethyl)-2-fluoro-4-nitrobenzene,[22] according to the procedure described for the synthesis of **15a** (86 mg, 85%): ^{1}H NMR (400 MHz, CDCl3): $\delta=8.00$ (d, $J=8.75$ Hz, 1H), 7.94 (d, $J=9.69$ Hz, 1H), 7.59 (t, $J=7.89$ Hz, 1H), 7.19–7.25 (m, 1H), 5.86–5.93 (m, 2H), 5.15 (s, 2H), 4.50 (dt, $J=12.17$, 6.02 Hz, 1H), 1.34 ppm (d, $J=5.99$ Hz, 6H); UPLC–MS: $t_R=0.95$ min, *m/z*: 307.4 [$M+H$].

1-(4-Amino-2-fluorobenzyl)-4-isopropoxypyridin-2(1*H*)-one (16b): A saturated solution of $Cu(OAc)_2$ (47,4 mg, 0.26 mmol) in MeOH (2.5 mL) was added to a solution of **15b** (80 mg, 0.26 mmol) in MeOH (7 mL). The reaction mixture was cooled to 0 °C and $NaBH_4$ (148 mg, 3.92 mmol) was added in four portions. The reaction mixture was stirred at 0 °C for 15 min and filtered over Celite, then the filtrate was concentrated. The residue was dissolved in EtOAc, washed with water, dried over a phase separator, and concentrated. The crude product was purified on silica gel by flash chromatography (hexanes/EtOAc, 50:50 to 0:100) to provide **16b** as colorless oil (34 mg, 46.6%): ^{1}H NMR (400 MHz, CDCl3): $\delta=7.17$–7.26 (m, 2H), 6.34–6.43 (m, 2H), 5.79–5.88 (m, 2H), 4.98 (s, 2H), 4.48 (dt, $J=12.13$, 6.10 Hz, 1H), 3.79 (br s, 2H), 1.32 ppm (d, $J=5.99$ Hz, 6H); UPLC–MS: $t_R=0.82$ min, *m/z*: 277.1 [$M+H$].

1-(4-((4,5-Dihydro-1*H*-imidazol-2-yl)amino)-2-fluorobenzyl)-4-isopropoxypyridin-2(1*H*)-one (17b): Compound **17b** was prepared from **16b** (30 mg, 0.11 mmol), according to the procedure described for the synthesis of **17a** (29 mg, 77%): ^{1}H NMR (400 MHz, CDCl3): $\delta=7.12$–7.26 (m, 2H), 6.59–6.68 (m, 2H), 5.74–5.81 (m, 2H), 4.95 (s, 2H), 4.36–4.45 (m, 1H), 3.46 (s, 4H), 1.25 ppm (d, $J=6.11$ Hz, 6H); UPLC–MS: $t_R=0.59$ min, *m/z*: 345.4 [$M+H$].

4-Bromo-1-isobutylpyridin-2(1*H*)-one (19): A suspension of 4-bromopyridin-2(1*H*)-one (0.5 g, 2.87 mmol), 1-bromo-2-methylpropane (0.37 mL, 3.45 mmol), K_2CO_3 (0.67 g, 4.89 mmol), and NaI (0.02 g, 0.14 mmol) in DMF (5.01 mL) was stirred in a sealed glass tube at 60 °C for 16 h under argon. The reaction mixture was diluted with EtOAc at room temperature. The organic layer was washed with water and brine, dried over a phase separator, and concentrated. The crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 0:100) to afford the major product **19** as a colorless oil (407 mg, 61%), and 4-bromo-2-isobutoxypyridine (120 mg, 18%): ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta=7.64$ (d, $J=7.21$ Hz, 1H), 6.71 (d, $J=2.20$ Hz, 1H), 6.45 (dd, $J=7.21$, 2.20 Hz, 1H), 3.69 (d, $J=7.46$ Hz, 2H), 2.03 (dt, $J=13.60$, 6.59 Hz, 1H), 0.84 ppm (d, $J=6.72$ Hz, 6H); UPLC–MS: $t_R=0.87$ min, *m/z*: 232.0 [$M+2$].

1-Isobutyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)pyridin-2(1*H*)-one (20): A mixture of **19** (224 mg, 0.97 mmol), bis(pinacolato)diboron (49 mg, 1.95 mmol), and KOAc (287 mg, 2.92 mmol) in 1,4-dioxane (5.0 mL) was degassed with argon for 5 min. $PdCl_2$(dppf)/CH_2Cl_2 adduct (79 mg, 0.09 mmol) was added, and the vial was capped. The reaction mixture was heated in a microwave at 120 °C for 1 h. The reaction mixture was filtered over Celite and concentrated. The crude product was purified by flash chromatography (cyclohexane/EtOAc, 100:0 to 0:100) to give **20** as brown oil (320 mg, 95%): ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta=7.58$ (d, $J=6.72$ Hz, 1H), 6.61 (s, 1H), 6.26 (d, $J=6.72$ Hz, 1H), 3.69 (d, $J=7.34$ Hz, 2H), 1.94–2.13 (m, 1H), 1.25–1.30 (m, 12H), 0.84 ppm (d, $J=6.72$ Hz, 6H); UPLC–MS: $t_R=0.54$ min.

1-Isobutyl-4-(4-nitrobenzyl)pyridin-2(1*H*)-one (21): $PdCl_2$(dppf)/CH_2Cl_2 adduct (56.7 mg, 0.069 mmol) was added to a solution of **20** (289 mg, 0.833 mmol), 1-(bromomethyl)-4-nitrobenzene (150 mg, 0.694 mmol), and K_2CO_3 (221 mg, 2.083 mmol) in dimethoxyethane (2.3 mL), and EtOH/water (0.46 mL, 1:1) was added at room temperature under argon. Argon was bubbled through the stirred reaction mixture for 5 min before microwaving at 120 °C while stirring for 10 min. The reaction mixture was filtered over Celite, the filtrate was concentrated, and the crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 30:70) to afford **21** as an orange oil (67 mg, 33.7%): ^{1}H NMR (400 MHz, [D_6]DMSO): $\delta=8.19$ (d, $J=8.44$ Hz, 2H), 7.55 (dd, $J=10.70$, 7.76 Hz, 3H), 6.25 (s, 1H), 6.08 (d, $J=6.97$ Hz, 1H), 3.92 (s, 2H), 3.60–3.68 (m, 2H), 2.01 (dt, $J=13.79$, 6.86 Hz, 1H), 0.82 ppm (d, $J=6.72$ Hz, 6H); UPLC–MS: $t_R=0.96$ min, *m/z*: 287.1 [$M+H$].

4-(4-Aminobenzyl)-1-isobutylpyridin-2(1*H*)-one (22): A solution of **21** (67 mg, 0.23 mmol), triethylamine (0.33 mL, 2.34 mmol), and Pd/C (12.45 mg, 0.012 mmol) in EtOH (5.00 mL) was stirred at room temperature under H_2 atmosphere (0.1 bar) for 2 h. The reaction mixture was filtered over Celite, and the Celite pad was washed with EtOH. The filtrate was concentrated, and the crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH/14.7 M NH_4OH, 99:1:0.1 to 90:10:1) to afford **22** as a yellow oil (54 mg, 90%): ^{1}H NMR (600 MHz, [D_6]DMSO): $\delta=7.47$ (d, $J=6.97$ Hz, 1H), 6.88 (d, $J=8.25$ Hz, 2H), 6.50 (d, $J=8.44$ Hz, 2H), 6.12 (s, 1H), 6.01 (dd, $J=6.97$, 1.83 Hz, 1H), 4.94 (s, 2H), 3.62 (d, $J=7.34$ Hz, 2H), 3.54 (s, 2H), 1.94–2.05 (m, 1H), 0.82 ppm (d, $J=6.79$ Hz, 6H); UPLC–MS: $t_R=0.73$ min, *m/z*: 257.1 [$M+H$].

4-(4-((4,5-Dihydro-1*H*-imidazol-2-yl)amino)benzyl)-1-isobutylpyridin-2(1*H*)-one (23): A mixture of **22** (54 mg, 0.21 mmol) and 2-chloro-4,5-dihydro-1*H*-imidazole (59.3 mg, 0.28 mmol) in isopropanol (2.8 mL) was stirred at 85 °C in a sealed glass tube for 40 min under argon. After cooling to room temperature, the reaction mixture was evaporated, and the residue was extracted with CH_2Cl_2 and aq NaOH (2 M). The combined organic layers were washed with brine, dried over a phase separator, and concentrated. The crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH/14.7 M NH_4OH, 100:0:0 to 90:10:1) to afford **23** as a white foam (27 mg, 38.7%): ^{1}H NMR (400 MHz, CD_3OD): $\delta=7.48$ (d, $J=6.97$ Hz, 1H), 7.17 (d, $J=8.07$ Hz, 2H), 7.01 (d, $J=8.19$ Hz, 2H), 6.36 (s, 1H), 6.27 (d, $J=6.85$ Hz, 1H), 3.70–3.86 (m, 4H), 3.48–3.57 (m, 4H), 2.13 (dt, $J=13.63$, 6.88 Hz, 1H), 0.93 ppm (d, $J=6.72$ Hz, 6H); UPLC–MS:]0.62 min, *m/z*: 325.1 [$M+H$].

1-(Bromomethyl)-4-isopropoxybenzene (24): Polymer-bound triphenylphosphine, (2.4 g, 7.67 mmol) and CBr_4 (2.54 g, 7.67 mmol)

were added to a solution of **10** (1.0 g, 5.11 mmol) in CH_2Cl_2 (17 mL) at 0 °C under argon. The reaction mixture was allowed to warm to room temperature and was stirred for 1 h, then filtered over Celite, and the Celite pad was washed with CH_2Cl_2. The filtrate was concentrated, and the crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 95:5) to afford **24** as an orange oil (842 mg, purity 60%), which was used in the next step without further purification.

4-Chloro-1-(4-isopropoxybenzyl)pyridin-2(1*H*)-one (25): A mixture of 4-chloropyridin-2(1*H*)-one (50 mg, 0.39 mmol), **24** (115 mg, 0.50 mmol), and K_2CO_3 (107 mg, 0.77 mmol) in THF (2.5 mL) was heated in a microwave at 130 °C for 30 min. After cooling to room temperature, water was added to the reaction mixture, and the product was extracted with CH_2Cl_2. The organic layer was dried over a phase separator and concentrated, and the crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 70:30) to afford **25** as a colorless oil (60 mg, 56%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.13 (dd, J = 12.90, 7.89 Hz, 3H), 6.78 (d, J = 8.56 Hz, 2H), 6.53–6.60 (m, 1H), 6.08 (dd, J = 7.27, 2.26 Hz, 1H), 4.95 (s, 2H), 4.46 (dt, J = 12.07, 6.01 Hz, 1H), 1.25 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 1.05 min, m/z: 278.1 [M+H].

4-((4,5-Dihydro-1*H*-imidazol-2-yl)amino)-1-(4-isopropoxybenzyl)-pyridin-2(1*H*)-one (26): 4,5-Dihydro-1*H*-imidazol-2-amine (34.6 mg, 0.234 mmol) and LHMDS (1 M in THF) (0.42 mL, 0.42 mmol) were added to a solution of [BrettPhos-Pd^{II}] precatalyst (8.48 mg, 10.62 μmol), BrettPhos (5.70 mg, 10.62 μmol), and **25** (59 mg, 0.212 mmol) in anhydrous THF (2.1 mL) at room temperature under argon. The reaction mixture was heated in a microwave at 130 °C for 30 min, then cooled to 0 °C, quenched with saturated aq NH_4Cl, diluted with water, and extracted with EtOAc. The organic layer was dried over a phase separator and concentrated, and the crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH/14.7 M NH_4OH, 100:0:0 to 50:50:5). The fractions were concentrated, and the residue was treated with 5% MeOH in CH_2Cl_2, filtered, and concentrated to give **26** as a colorless oil (5 mg, 7%): ^{1}H NMR (400 MHz, CD_3OD): δ = 7.63 (d, J = 7.70 Hz, 1H), 7.27 (d, J = 8.56 Hz, 2H), 6.83–6.97 (m, 3H), 6.25 (d, J = 2.57 Hz, 1H), 5.08 (s, 2H), 4.59 (quin, J = 5.99 Hz, 1H), 3.85–3.99 (m, 2H), 3.58 (t, J = 8.01 Hz, 2H), 1.30 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 0.65 min, m/z: 327.2 [M+H].

1-(4-(4-Isopropoxybenzyl)phenyl)thiourea (28a): A solution of benzoyl isothiocyanate (0.11 mL, 0.83 mmol) in anhydrous acetone (1.6 mL) was added dropwise to a stirred solution of **27a** (200 mg, 0.83 mmol) in anhydrous acetone (2.5 mL) at room temperature under argon. The reaction mixture was stirred at room temperature for 1 h and concentrated. The residue was filtered and washed with a small amount of cold anhydrous acetone, and the residual solvent was evaporated. The crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 90:10) to afford N-((4-(4-isopropoxybenzyl)phenyl)carbamothioyl)benzamide (258 mg, 77%) as a white solid: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 12.45 (br s, 1H), 8.97 (br s, 1H), 7.82 (d, J = 7.58 Hz, 2H), 7.53–7.65 (m, 3H), 7.44–7.52 (m, 2H), 7.16 (d, J = 8.31 Hz, 2H), 7.02 (d, J = 8.44 Hz, 2H), 6.75 (d, J = 8.56 Hz, 2H), 4.44 (dt, J = 12.10, 6.05 Hz, 1H), 3.86 (s, 2H), 1.25 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 1.45 min, m/z: 405.1 [M+H]. This intermediate (250 mg, 0.62 mmol) was then added in one portion to a 5% aq NaOH (1 mL) solution while stirring at 90 °C. The reaction mixture was stirred at 90 °C for 20 min, cooled to room temperature, and acidified with 15% aq HCl. The pH was adjusted to 8.0 using aq ammonia, and the crude product was filtered. The solid was washed with water and dried to give 135 mg of **28a** as a yellow solid, which was used in the next step without further purification.

***N*-(4-(4-Isopropoxybenzyl)phenyl)-4,5-dihydrothiazol-2-amine (29a)**: Compound **28a** (135 mg, 0.449 mmol) in EtOH (1.8 mL) was added to a solution of 2-bromoethanamine hydrobromide (92 mg, 0.45 mmol) in water (0.22 mL) at room temperature under argon. The reaction mixture was stirred at reflux for 16 h. After cooling to room temperature, the EtOH was evaporated, and the residue was basified with diluted aq ammonia and extracted with CH_2Cl_2. The combined organic layers were dried over a phase separator and concentrated. The crude product was purified on silica gel by flash chromatography (cyclohexane/EtOAc, 100:0 to 80:20) to afford **29a** (34 mg, 22.9%) as a white solid: ^{1}H NMR (400 MHz, CD_3OD): δ = 7.01–7.18 (m, 6H), 6.77–6.86 (m, 2H), 4.55 (dt, J = 12.10, 6.05 Hz, 1H), 3.82–3.91 (m, 4H), 3.28–3.31 (m, 2H), 1.30 ppm (d, J = 6.11 Hz, 6H); ^{13}C NMR (151 MHz, CD_3OD): δ = 157.6, 147.4, 138.0, 135.1, 130.8 (2C), 130.2 (2C), 122.9 (2C), 117.1 (2C), 121.9, 71.0, 41.4, 33.0, 30.8, 22.4 ppm (2C); UPLC–MS: t_R = 0.94 min, m/z: 327.4 [M+H].

1-(3-Fluoro-4-(4-isopropoxybenzyl)phenyl)thiourea (28b): Compound **28b** was prepared from **12b** (200 mg, 0.77 mmol), according to the procedure described for the synthesis of **28a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.07 (br s, 1H), 7.20 (t, J = 8.09 Hz, 1H), 7.08–7.13 (m, 2H), 6.90–7.00 (m, 2H), 6.80–6.85 (m, 2H), 6.14 (br s, 2H), 4.46–4.56 (m, 1H), 3.92 (s, 2H), 1.32 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 1.06 min, m/z: 319.1 [M+H].

***N*-(3-Fluoro-4-(4-isopropoxybenzyl)phenyl)-4,5-dihydrothiazol-2-amine (29b)**: Compound **29b** was prepared from **28b** (100 mg, 0.31 mmol), according to the procedure described for the synthesis of **29a**: ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.03 (d, J = 8.31 Hz, 2H), 6.82–6.97 (m, 2H), 6.67–6.76 (m, 3H), 4.42 (dt, J = 12.07, 6.01 Hz, 1H), 3.80 (s, 2H), 3.71–3.77 (m, 2H), 3.25 (t, J = 7.03 Hz, 2H), 1.22 ppm (d, J = 6 Hz, 6H); UPLC–MS: t_R = 0.96 min, m/z: 345.4 [M+H].

1-(2-Chloroethyl)-3-(4-(4-isopropoxybenzyl)phenyl)urea (30): 1-Chloro-2-isocyanatoethane (0.13 mL, 1.49 mmol) was added dropwise to a solution of **27a** (300 mg, 1.24 mmol) in CH_2Cl_2 (5 mL) at room temperature under argon. The reaction mixture was stirred overnight at room temperature, and the precipitate was collected by filtration. The filter cake was washed with cold Et_2O and dried. The product was obtained as a white powder (305 mg, 70%) and used in the next step without further purification: UPLC–MS: t_R = 1.16 min, m/z: 347.1 [M+H].

***N*-(4-(4-Isopropoxybenzyl)phenyl)-4,5-dihydrooxazol-2-amine (31)**: A mixture of SiO_2·KF (60:40) (511 mg, 4.32 mmol) was added to a solution of **30** (150 mg, 0.43 mmol) in CH_3CN (6 mL) at room temperature under argon. The reaction mixture was stirred at reflux for 4 h, cooled to room temperature and filtered, and the solvent was evaporated. The crude product was purified by preparative HPLC (column: Waters Sunfire C_{18}, 5 μm, 30×100 mm, solvent A: H_2O + 0.1%TFA, solvent B: CH_3CN + 0.1%TFA, flow: 50 mL min^{-1}, gradient: 20 to 40% B over 16 min) to afford **31** as a white powder (130 mg, 69.4%): ^{1}H NMR (400 MHz, [D_6]DMSO): δ = 7.21–7.34 (m, 4H), 7.13 (d, J = 8.31 Hz, 2H), 6.83 (d, J = 8.44 Hz, 2H), 4.85 (t, J = 8.56 Hz, 2H), 3.88 (s, 2H), 3.81–3.86 (m, 2H), 1.24 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 0.88 min, m/z: 311.4 [M+H].

***N*-(3-Fluoro-4-(4-isopropoxybenzyl)phenyl)-3,4-dihydro-2*H*-pyrrol-5-amine (32a)**: A solution of phosphorus oxychloride (0.3 mL, 3.20 mmol) in toluene (2 mL) was added dropwise to a so-

lution of pyrrolidin-2-one (0.31 mL, 4.0 mmol) in toluene (2 mL) at 0 °C under argon, and the reaction mixture was stirred overnight at room temperature. A solution of **12b** (50 mg, 0.400 mmol) in toluene (2 mL) was added, and the reaction mixture was stirred at reflux for 2 h. MeOH (0.5 mL) was added at room temperature. The product was extracted with 10% aq NaOH and EtOAc, and the combined organic layers were dried over a phase separator and concentrated. The crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH/14.7 M NH_4OH, 99:1:0.1 to 90:10:1) to give **32a** as colorless oil (35 mg, 26.6%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.04 (d, J = 8.31 Hz, 2H), 6.94 (t, J = 8.38 Hz, 1H), 6.58–6.76 (m, 4H), 4.42 (dt, J = 12.10, 6.05 Hz, 1H), 3.79 (s, 2H), 3.32–3.46 (m, 2H), 2.48 (t, J = 7.89 Hz, 2H), 1.94–2.05 (m, 2H), 1.24 ppm (d, J = 5.99 Hz, 6H); UPLC–MS: t_R = 0.88 min, m/z: 327.2 [M+H].

***N*-(3-Fluoro-4-(4-(3-fluoropropoxy)benzyl)phenyl)-3,4-dihydro-2*H*-pyrrol-5-amine (32b)**: Compound **32b** was prepared from **4b** (70 mg, 0.25 mmol) according to the procedure described for the synthesis of **32a** (50 mg, 55.8%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.11–7.16 (m, 2H), 7.01 (br t, J = 8.19 Hz, 1H), 6.84 (br d, J = 7.46 Hz, 2H), 6.75 (br d, J = 9.41 Hz, 1H), 6.67 (br d, J = 8.07 Hz, 1H), 4.64 (dt, J = 48 Hz, 5.32 Hz, 2H), 4.08 (br t, J = 5.69 Hz, 2H), 3.88 (s, 2H), 3.41–3.50 (m, 2H), 2.42–2.59 (m, 2H), 2.02–2.22 ppm (m, 3H, 1.25 (br t, J = 6.79 Hz, 1H); UPLC–MS: t_R = 0.83 min, m/z: 345.2 [M+H].

***N*-(3-Fluoro-4-(4-isopropoxybenzyl)phenyl)-3,4,5,6-tetrahydropyridin-2-amine (33a)**: A solution of phosphorus oxychloride (0.14 mL, 1.54 mmol) in toluene (2 mL) was added dropwise to a solution of piperidin-2-one (191 mg, 1.93 mmol) in toluene (2 mL) at 0 °C under argon. The reaction mixture was stirred at room temperature overnight. A solution of **12b** (50 mg, 0.193 mmol) in toluene (2 mL) was added, and the reaction mixture was stirred at reflux for 2 h. After adding MeOH (0.2 mL) at room temperature, the product was extracted with 10% aq NaOH and methyl-*tert*-butyl ether. The combined organic layers were dried over a phase separator and concentrated. The crude product was purified on silica gel by flash chromatography (CH_2Cl_2/MeOH/14.7 M NH_4OH, 99:1:0.1 to 90:10:1) to give **33a** as a white powder (34 mg, 49.2%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.15 (t, J = 8.4 Hz, 1H), 7.08 (d, J = 8.5 Hz, 2H), 6.91 (d, J = 8.56 Hz, 2H), 6.82 (d, J = 8.44 Hz, 2H), 4.51 (dt, J = 12.07, 6.01 Hz, 1H), 3.91 (s, 2H), 3.51–3.56 (m, 2H), 2.47 (br t, J = 6.17 Hz, 2H), 1.76–1.92 (m, 4H), 1.32 ppm (d, J = 6.11 Hz, 6H); UPLC–MS: t_R = 0.88 min, m/z: 341.0 [M+H].

***N*-(3-Fluoro-4-(4-(4-fluorobutoxy)benzyl)phenyl)-3,4,5,6-tetrahydropyridin-2-amine (33b)**: Compound **33b** was prepared from **4a** (200 mg, 0.51 mmol), according to the procedure described for the synthesis of **33a** (183 mg, 96%): ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.14 (d, J = 8.44 Hz, 2H), 7.03 (t, J = 8.25 Hz, 1H), 6.83 (d, J = 8.68 Hz, 2H), 6.56–6.65 (m, 2H), 4.52 (dt, J = 48 Hz, 5.56 Hz, 2H), 3.99 (t, J = 5.75 Hz, 2H), 3.88 (s, 2H), 3.23 (br s, 2H), 2.45 (br s, 2H), 1.69–1.94 ppm (m, 8H); ^{13}C NMR (151 MHz, $CDCl_3$): δ = 165.9, 160.7, 156.6, 133.7, 131.8, 130.6, 129.8 (2C), 129.3, 122.0, 116.0 (2C), 113.6, 83.9 (d, J = 165 Hz, 1C), 67.3, 42.1, 33.7, 27.3, 26.3, 25.2, 20.7, 18.1 ppm; UPLC–MS: t_R = 0.85 min, m/z: 373.7 [M+H].

Pharmacology

Saturation binding assays: Binding was performed with a range of concentrations of [^{3}H]**1** to construct saturation binding curves, as described previously.[23] Experiments were conducted at room temperature in 96-well deep-well plates in assay binding buffer (HBSS containing 5 mM HEPES, 0.02% pluronic acid, and 2% DMSO, pH 7.4). Membranes of Chinese hamster ovary (CHO) cells expressing hIPR (2 μg per well) were incubated in 96-well deep-well plates at 37 °C in assay binding buffer (final volume 0.5 mL) with gentle agitation for 2 h to ensure equilibrium was reached. [^{3}H]NMS binding to CHO-M_3 membranes (10 μg per well) was similarly carried out in HBSS buffer at 37 °C (final volume 1.5 mL) with gentle agitation for 2 h. Following this incubation period, bound and free radioligand were separated by rapid vacuum filtration using a FilterMate Cell Harvester (PerkinElmer, Beaconsfield, UK) onto 96-well GF/B filter plates pre-treated with assay buffer and rapidly washed three times with ice-cold 20 mM HEPES containing 1 mM $MgCl_2$, pH 7.4. After drying (>4 h), 40 μL of Microscint 20 (PerkinElmer) was added to each well, and radioactivity was quantified using single photon counting on a TopCount microplate scintillation counter (PerkinElmer). Aliquots of [^{3}H]**1** and [^{3}H]NMS were also quantified accurately to determine how much radioactivity was added to each well using liquid scintillation spectrometry on a Hidex 300SL scintillation counter (LabLogic, Sheffield, UK). In all experiments, total binding never exceeded more than 10% of ligand added, therefore limiting significant depletion of the free radioligand concentration.[24]

Data analysis: [^{3}H]**1** saturation binding assays were analyzed by nonlinear regression using Prism 6.0 (GraphPad Software, San Diego, USA). Specific binding, obtained by subtracting NSB from total binding, was fitted to a one-site binding model which describes a rectangular hyperbola or binding isotherm, leading to individual estimates for total receptor number and radioligand equilibrium dissociation constant.

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Keywords: imaging agents · inhibitor design · ligand efficiency · medicinal chemistry · prostacyclin receptor

[1] M. M. Hann, G. M. Keserü, *Nat. Rev. Drug Discovery* **2012**, *11*, 355–365, and references therein.

[2] a) T. T. Wager, R. Y. Chandrasekaran, X. Hou, M. D. Troutman, P. R. Verhoest, A. Villabolos, Y. Will, *ACS Chem. Neurosci.* **2010**, *1*, 420–434; b) T. T. Wager, X. Hou, P. R. Verhoest, A. Villabolos, *ACS Chem. Neurosci.* **2010**, *1*, 435–449; c) L. Zhang, A. Villalobos, E. M. Beck, T. Bocan, T. A. Chappie, L. Chen, S. Grimwood, S. D. Heck, C. J. Helal, X. Hou, J. M. Humphrey, J. Lu, M. B. Skaddan, T. J. McCarthy, P. R. Verhoest, T. T. Wager, K. Zasadny, *J. Med. Chem.* **2013**, *56*, 4568–4579; d) Q. Guo, M. Brady, R. N. Gunn, *J. Nucl. Med.* **2009**, *50*, 1715–1723.

[3] Z. Jiang, J. Reilly, B. Everatt, E. Briard, *J. Pharm. Biomed. Anal.* **2011**, *54*, 722–729.

[4] K. Valko, C. M. Du, C. D. Bevan, D. P. Reynolds, M. H. Abraham, *J. Pharm. Sci.* **2000**, *89*, 1085–1096.

[5] M. D. Shultz, *Bioorg. Med. Chem. Lett.* **2013**, *23*, 5980–5991.

[6] a) F. Assmus, A. Seelig, L. Gobbi, E. Borroni, P. Glaentzlin, H. Fischer, *Eur. J. Pharm. Sci.* **2015**, *79*, 27–35; b) S. Belli, F. Assmus, B. Wagner, M. Honer, H. Fischer, F. Schiuler, R. Alvarez-Sanchez, *Mol. Pharm.* **2015**, *12*, 4529–4541.

[7] J. Mukherjee, Z. Y. Yang, M. K. Das, T. Brown, *Nucl. Med. Biol.* **1995**, *22*, 283–296; J. Mukherjee, Z. Y. Yang, T. Brown, R. Lew, M. Wernick, X. Ouyang, N. Yasillo, C. T. Chen, R. Mintzer, M. Cooper, *Nucl. Med. Biol.* **1999**, *26*, 519–527.

[8] M. Slifstein, D. R. Hwang, Y. Huang, N. Guo, Y. Sudo, R. Narendran, P. Talbot, M. Laruelle, *Psychopharmacology* **2004**, *175*, 274 – 286.
[9] J. R. Bunzow, G. Zhang, C. Bouvier, C. Saez, O. K. Ronnekleiv, M. J. Kelly, D. K. Grandy, *J. Neurochem.* **1995**, *64*, 14 – 24.
[10] H. Sugimoto, Y. Iimura, Y. Yamanishi, K. Yamatsu, *J. Med. Chem.* **1995**, *38*, 4821 – 4829.
[11] H. Sugimoto, Y. Yamanishi, Y. Iimura, Y. Kawakami, *Current Med. Chem.* **2000**, *7*, 303 – 339.
[12] B. Giros, S. el Mestikawy, N. Godinot, K. Zheng, H. Han, T. Yang-Feng, M. G. Caron, *Mol. Pharmacol.* **1992**, *42*, 383 – 390.
[13] C. V. Cella, S. M. Woodhouse, R. A. Lewis, A. E. Fray, *Abstracts: 4th Forum of European Neuroscience,* Lisbon (Portugal), 2004, *2*, A059.4.
[14] M. Schou, V. W. Pike, C. Halldin, *Current Top. Med. Chem.* **2007**, *7*, 1806 – 1816.
[15] P. Kannan, V. W. Pike, C. Halldin, O. Langer, M. M. Gottesman, R. B. Innis, M. D. Hall, *Mol. Pharm.* **2013**, *10*, 2222 – 2229.
[16] M. Healy, *Abstracts: 250th National Meeting of the American Chemical Society,* Boston, MA (USA), August 16 – 20, 2015, ACS, Washington DC, MEDI 18.
[17] R. D. Clark, A. Jahangir, D. Severance, R. Salazar, T. Chang, D. Chang, M. F. Jett, S. Smith, K. Bley, *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1053 – 1056.
[18] G. P. Donahich, A. E. Johnson, B. Nock, B. S. McEwen, H. H. Feder, *Eur. J. Pharmacol.* **1985**, *119*, 9 – 16.
[19] S. Hintermann, I. Vranesic, H. Allgeier, A. Brülisauer, D. Hoyer, M. Lemaire, T. Moenius, S. Urwyler, S. Whitebread, F. Gasparini, Y. P. Auberson, *Bioorg. Med. Chem.* **2007**, *15*, 903 – 914.
[20] K. R. Bley, R. D. Clark, A. Jahangir, B. A. Kowalczyk, F. J. Lopez-Tapala, A. V. Muehldorf, C. O'Yang, T. W. Sun (F. Hoffmann – La Roche AG), Eur. Pat. No. EP0902018A3, **1999**.
[21] M. Nonnenmacher, T. Busch (Euticals S.P.A.), Int. PCT Pub. No. WO 2014180735 A1, **2014**.
[22] R. Frank, T. Christoph, K. Schiene, V. J. De, N. Damann, B. Lesch, G. Bahrenberg, D. J. Saunders, H. Stockhausen, Y.-S. Kim, M.-S. Kim, J. Lee (Grünenthal GmbH), Int. PCT Pub. No. WO2013068461, **2013**.
[23] D. A. Sykes, M. R. Dowling, S. J. Charlton, *Curr. Protoc. Pharmacol.* **2010**, *50*, 9.14.1 – 9.14.30.
[24] C. M. Carter, J. R. Leighton-Davies, S. J. Charlton, *J. Biomol. Screening* **2007**, *12*, 255 – 266.

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Y. P. Auberson, E. Briard, D. Sykes, J. Reilly, M. Healy*

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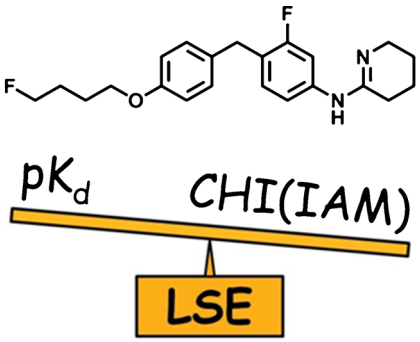
Ligand Specific Efficiency (LSE) Index for PET Tracer Optimization



Striking a balance: Nonspecific binding cannot be predicted using in silico parameters and remains a major cause of failure for candidate PET imaging agents. We defined the ligand specific efficiency (LSE) index as a measure of affinity, normalized to nonspecific binding, and determined the minimal value for a successful PET tracer. The use of LSE to guide chemical optimization is illustrated with tracer candidates for prostacyclin receptors.