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The Discovery of Clinical Candidate (1*R*,4*r*)-4-((*R*)-2-((*S*)-6-Fluoro-5H-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (Navoximod), a Potent and Selective Inhibitor of Indoleamine 2,3-dioxygenase 1

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The Discovery of Clinical Candidate (1*R*,4*r*)-4-((*R*)-2-((*S*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (Navoximod), a Potent and Selective Inhibitor of Indoleamine 2,3-dioxygenase 1.

Sanjeev Kumar[†¤], *Jesse P. Waldo*[‡¤], *Firoz A. Jaipuri*[§¤], *Agnieszka Marcinowicz*[†], *Clarissa Van Allen*[†], *James Adams*[†], *Tanay Kesharwani*[‖], *Xiaoxia Zhang*, *Richard Metz*[†], *Angela J. Oh*[ǂ], *Seth F. Harris*[ǂ], *Mario R. Mautino*[†]*

† NewLink Genetics, Ames, IA 50010, United States

‡ Discovery Chemistry, Janssen Research and Development, LLC, Welsh and McKean Roads, Spring House, PA 19477, United States

§ Medicinal Chemistry, Biocon Bristol-Myers Squibb R&D Center, Biocon Park, Bommasandra Industrial Area, Bengaluru, KA 560099, India

‖ Department of Chemistry, University of West Florida, Pensacola, FL 32514

ǂ Structural Biology, Genentech, Inc. 1 DNA Way, South San Francisco, CA 94080

KEYWORDS. Indoleamine 2,3-dioxygenase 1 inhibitors, Navoximod, Imidazoisoindoles, Phenylimidazoles, Structure-based drug discovery

ABSTRACT. A novel class of 5-substituted 5*H*-imidazo[5,1-*a*]isoindoles are described as potent inhibitors of indoleamine 2,3-dioxygenase 1 (IDO1). A structure-based drug design approach was used to elaborate the 5*H*-imidazo[5,1-*a*]isoindole core and to improve potency and pharmacological properties. Suitably placed hydrophobic and polar functional groups in the lead molecule allowed improvement of IDO1 inhibitory activity while minimizing off-target liabilities. The structure-activity relationship (SAR) studies focused on optimizing IDO1 inhibition potency and a pharmacokinetic profile amenable to oral dosing while controlling CYP450 and hERG inhibitory properties.

Introduction

Indoleamine 2,3-dioxygenase 1 (IDO1) is a 45 kDa cytosolic monomeric heme-dioxygenase that catalyzes the initial and rate limiting oxidative cleavage of the 2,3-indole position of *L*-tryptophan (*L*-Trp) and incorporates both atoms of molecular oxygen (O_2), resulting in the production of *N*-formyl kynurenine. The main function of IDO1 is the regulation of acquired local and peripheral immune tolerance in normal and pathological scenarios. IDO1-expressing cells are found constitutively in many normal tissues where IDO1 regulates local inflammation and moderates responses against pathogens as well as to foreign or uncommon non-pathological antigens. Constitutive IDO1 activity is also found at the maternal-fetal interface, where IDO1 plays a critical role in the induction of maternal immune tolerance to the father-derived allogeneic antigens expressed by the fetus.[1] IDO1 expression can also be induced in antigen-presenting cells (APCs) of the immune system in response to inflammation signals and is subject to complex regulation by

an array of immunological signals. In response to inflammatory signals, IDO1 exerts its immunosuppressive effects by both local depletion of tryptophan (Trp) and by the generation of kynurenine (Kyn). Degradation of tryptophan deprives cells of an essential nutrient, triggering changes in metabolism and protein synthesis that can result in changes of cellular function. Cells have sensing mechanisms to detect deficiency in any amino acid, thus allowing them to transiently stop non-critical protein synthesis and coordinate a survival response. One such amino-acid deficiency sensing mechanism is the stress kinase GCN2, which contains a regulatory domain that binds uncharged amino acid free-tRNA. When GCN2 binds uncharged tRNA this activates its kinase domain, which phosphorylates the ribosomal initiation factor eIF2α.[2] In its phosphorylated configuration, p-eIF2α prevents the translation of most mRNA species, except those involved in compensation responses to the starvation stress.[2-3] In addition to depleting tryptophan, IDO1 generates a series of bioactive downstream metabolites along the kynurenine pathway. Kynurenine and other Trp metabolites are immunologically-active ligands for the aryl hydrocarbon receptor (AhR).[4] The AhR is a ligand-activated transcription factor, which regulates transcription of genes that include IDO1[5] and FoxP3, a key transcription factor that controls differentiation of $Foxp3^+$ regulatory T cells (Tregs)[4], which are a population of cells that play a key role in the regulation of immune responses.[6] The activation of both the GCN2 and AhR pathways leads to differentiation and activation of Tregs, the induction of anergy of effector T cells and modulation of function and phenotype in antigen presenting cells. Together, these IDO1-driven changes mediate local and systemic immunosuppressive effects that block immune response.

Given the important role of IDO1 in control of acquired immune tolerance, it is not surprising that the IDO1 pathway is induced in tumor cells and in host immune cells and contributes to acquired immunologic tolerance towards those tumors.[7] IDO1 expression and activity is observed in many

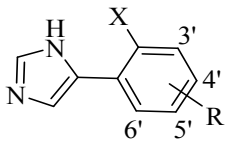
types of tumors including melanoma, leukemia, pancreatic, ovarian, colorectal, endometrial and prostate cancers, and its expression is associated with significantly worse clinical outcomes.[8] IDO1 can also be expressed by cells of the host immune system that are associated with tumors, where it contributes to acquired immunologic tolerance of tumors.

Consequently, inhibition of IDO1 activity to restore immune response against tumors is an area of active interest, triggering the development of numerous classes of IDO1 inhibitory compounds in the last decade.[9] Here, we describe our structure-activity relationship (SAR) strategy and efforts that resulted in the selection of the clinical development candidate navoximod (**NLG-919**), starting from the available crystal structure of IDO1 complexed with the inhibitory compound 4-phenyl imidazole (**1**).[10]

Results and Discussions

Substitution of the phenyl ring of 1. Our present work in this area stems from the published X-ray crystal data of IDO1 complexed with **1**[10] and the preliminary studies by Kumar *et al.* on these structures.[11] In 5-(2-hydroxyphenyl)imidazole (**3**), the increase of 10-fold in potency with respect to **1** (hIDO1 IC_{50} = 28 μM) was attributed to the hydrogen bonding interactions of hydroxyl with Ser167 in the IDO1 active site. Docking studies with **3** showed that small substituents might be accommodated at 3', 5' and 6'-positions of the phenyl ring. By diligently selecting a combination of small hydrophilic and hydrophobic moieties, we were able to increase the potency about 5-fold over **3** to generate **11** (Table 1). **4**-**6**, **8** and **11** showed submicromolar activity, indicating that the IDO1 active site has a small pocket that could accommodate a hydrophobic group *para* to the 2'-OH. Switching the Cl and F positions in **11** resulted in substantial loss of activity (**14**).

Table 1. Effects of Substitution on the Phenyl Ring of **1**.



compound	X	3'	5'	6'	IC_{50} (μM)	EC_{50} (μM)	LE[a]
1	H	-	-	-	28	-	0.58
3	OH	-	-	-	1.7 (4.8)[b]	7.2	0.67
4	OH	-	-Cl	-	0.49	0.94	0.68
5	OH	-	-Me	-	0.64	2.4	0.67
6	OH	-	-Br	-	0.76	1.7	0.66
7	OH	-	-F	-	1.7	-	0.62
8	OH	-F	-F	-	0.96	-	0.60
9	OH	-	-	-OH	4.7[b]	60	0.57
11	OH	-F	-Cl	-	0.3	1.9	0.65
13	OH	-Cl	-	-	32	-	0.48
14	OH	-Cl	-F	-	47	-	0.43

[a]Biochemical LE; [b]Value determined previously[11]

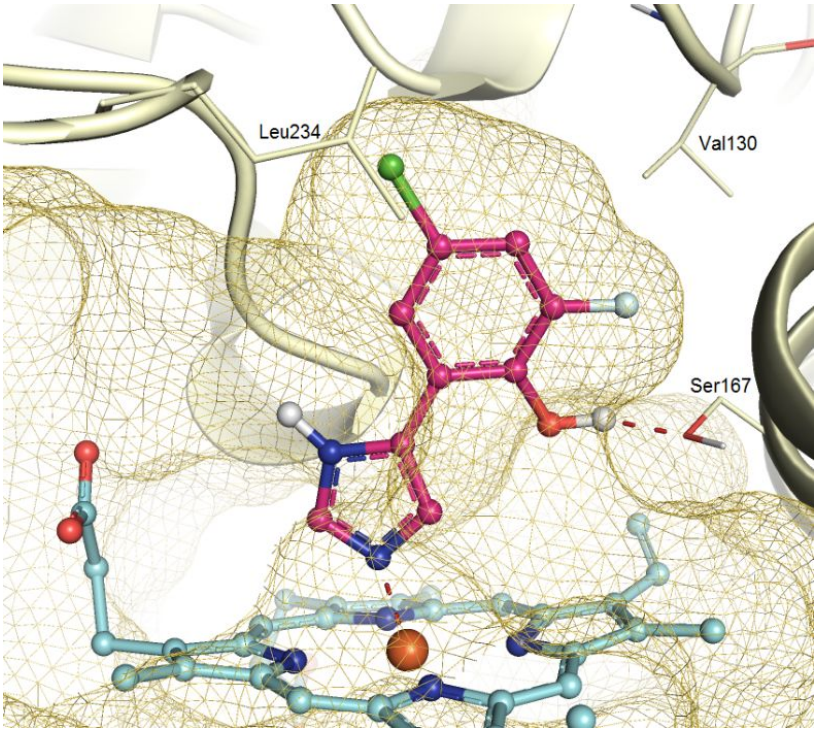
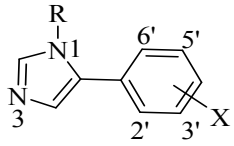


Figure 1. Predicted binding mode for **11** (magenta) minimized in the IDO1 pocket using the crystal IDO1 structure 2D0T (Hydrogen bonding and heme interaction with **11** shown with red dotted line)

11 was the most potent compound of this series and showed very good biochemical ligand efficiency; however, it showed a significant 6-fold shift in cellular potency and had a very short half-life in vivo ($t_{1/2}$ < 1h). The short half-life was attributed to high clearance due to the phase 2 metabolization of phenol (data not reported). Several prodrugs of phenol were synthesized to overcome the metabolic liability, but in vivo PK could not be improved (data not reported). We decided to remove the phenolic hydroxyl group and attempted substitutions on the *N*-1 nitrogen to achieve favorable interactions within the active site. In an attempt to gain hydrogen bond interactions between hydrogen bond donors and the heme propionate, compounds with different linker lengths such as a primary alcohol **15**, amides **18** and **23**-**24** were prepared. However, these attempts led to compounds with only marginal inhibitory activity (**23**) or inactive compounds (**15**, **18** and **24**). Hydrophobic side chains such as 2,2-dimethylbutyl **21** and cyclohexyl **22** were explored in an attempt to gain favorable Van der Waals interactions with Phe226. However, these approaches did not afford any improvement in the activity compared with parent compounds **3** and **4** (Table 2). Nonetheless, they indicated that substitutions on the *N*-1 imidazole could be tolerated, confirming that *N*-1 of imidazole ring points towards the open region of the active site.[11]

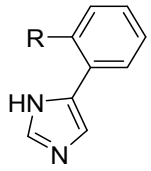
Table 2. Effects of Substitution on the *N*-1 of **1**.

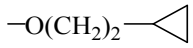
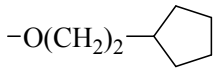
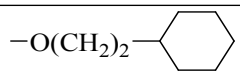
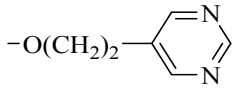
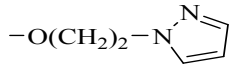


compound	R	2'	5'	IC_{50} (μM)	EC_{50} (μM)
15	$-CH_2CH_2OH$	-	-	>1000	-
18	$-CH_2CH_2NHCOCH_3$	-	-	>1000	-
21	$-CH_2CH_2t$-Bu	OH		23	-
22	Cyclohexyl	OH	Cl	1.9	6.1
23	$-CH_2CONHMe$	-	-	187	-
24	$-CH_2CH_2CONHMe$	-	-	>1000	-

Next, we turned our attention to the hydrophobic pocket formed by Phe163 and Phe226, independently of the interactions between 2-OH and Ser167. In order to access the hydrophobic pocket, ether analogues of **3** were synthesized (Table 3). The active site seems to be able to accommodate a wide range of hydrophobic groups and linker lengths while showing improvement in enzymatic inhibition with respect to the parent compound **1**. A comparison between benzyl, phenethyl and phenpropyl ethers showed that the ethylene linker was more favorable than methylene and propylene linkers (**26**-**28**). *t*-Butyl **29** and *i*-propyl **30** were well tolerated with acceptable biochemical ligand efficiencies. Cycloalkyl compounds with 3-, 5- and 6-membered rings (**31**-**33**) joined to an *O*-ethylene linker were nearly equipotent in biochemical assays and also had good biochemical ligand efficiencies. Notably, an *O*-ethylene linker terminating with a cyclohexyl group (**33**) was nearly equipotent with phenol (**3**) in biochemical assay. Side chains terminating with polar heterocycles such as pyrimidine **34** or pyrazole **35** resulted in the loss of biochemical IDO1 inhibition and decreased ligand efficiencies.

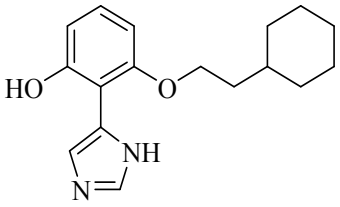
Table 3. Effect of Linker Length and Distal Hydrophobic Group on 2'-Phenylethers.



compound	R	IC_{50} (μM)	EC_{50} (μM)	LE[a]
1	H	27		0.58
26	-OCH_2Ph	18	-	0.35
27	-$O(CH_2)_2Ph$	9.1	65	0.35
28	-$O(CH_2)_3Ph$	16	110	0.32
29	-$O(CH_2)_2$*t*-Bu	7.8	-	0.39
30	-$O(CH_2)_2$*i*-Pr	6.6	-	0.43
31		5.1	-	0.44
32		7.2	-	0.38
33		3.8	26	0.38
34		154	-	0.26
35		44	-	0.32

[a]Biochemical LE. -: Not determined

With these data in hand, we investigated whether a hybrid molecule of **3** and **33** would provide a synergistic effect on improving potency (Figure 2). A combination of 2'-OH and 6'-ethylcyclohexyl ether side chain resulted in slight improvement in biochemical activity (**36**) as compared to the parent **33**, but was equipotent with **3**. This data suggests that only one component, 2'-hydroxyl or the 6'-ethylcyclohexyl ether, is contributing to the binding energy of **36**, and that the optimal conformation adopted within the active site for each group is not compatible with the preferred binding mode provided by the combined substituents. The molecular conformation of **36** shows that due to increased torsional strain between the phenyl and imidazole rings, the phenyl and imidazole rings are at a dihedral angle of 45 degrees.[12] Compound **36** was not considered for further optimization as it exhibited a large shift in cellular activity.



36

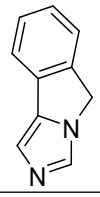
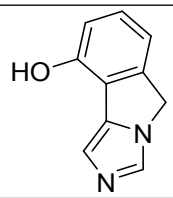
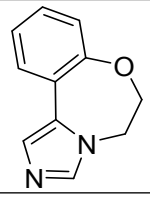
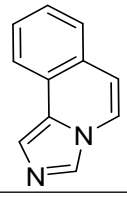
IC_{50} = 1.7 μM

EC_{50} = 80 μM

Figure 2. Effect of Combination on IDO1 Inhibition.

Effect of molecular rigidification on IDO1 activity. One established strategy to increase membrane permeability and cellular potency of small molecule inhibitors is to reduce the number of rotatable bonds.[13] In order to reduce the number of rotatable bonds, we envisioned restricting the rotation of the carbon-carbon single bond of phenyl imidazole by synthesizing fused rigid derivatives (Table 4). A methylene linker fusing the *N*-1 and 2'-position of the phenyl ring (**37**) improved biochemical IDO1 inhibition 4-fold over **1** with improved ligand efficiency. Two carbon linker containing compounds oxazepine derivative **44** and imidazo[5,1-*a*]isoquinoline **45** resulted in the loss of IDO1 inhibition. In order to exploit interactions of the imidazoisoindole ring with Ser167, we explored whether the addition of a 9-OH group would substantially improve binding affinity (**41**). However, **41** was found to be biochemically equipotent with **37**, suggesting that the phenolic OH group on **41** does not interact with Ser167 in a similar orientation as the 2'-OH group of phenylimidazole series. Energy minimization of **37** in the IDO1 active site revealed that the methylene carbon vector towards the opening of the pocket could potentially accommodate various substituents (Figure 3). This presented an excellent opportunity for further modifications to explore 5*H*-substituted imidazoisoindole analogues. Given the comparable biochemical potency of the imidazoisoindoles **37** and **41**, and the potential metabolic sensitivity of phenolic compounds to glucuronidation,[14] we chose to explore substituents on **37** instead of **41**.

Table 4. Effect of Rigidification on Phenylimidazole Derivatives.

compound	Structure	IC_{50} (μM)	EC_{50} (μM)	LE[a]
37		5.7	>25	0.61
41		3.1	7.9	0.59
44		312	-	0.35
45		22	-	0.50

[a]Biochemical LE. -: Not determined

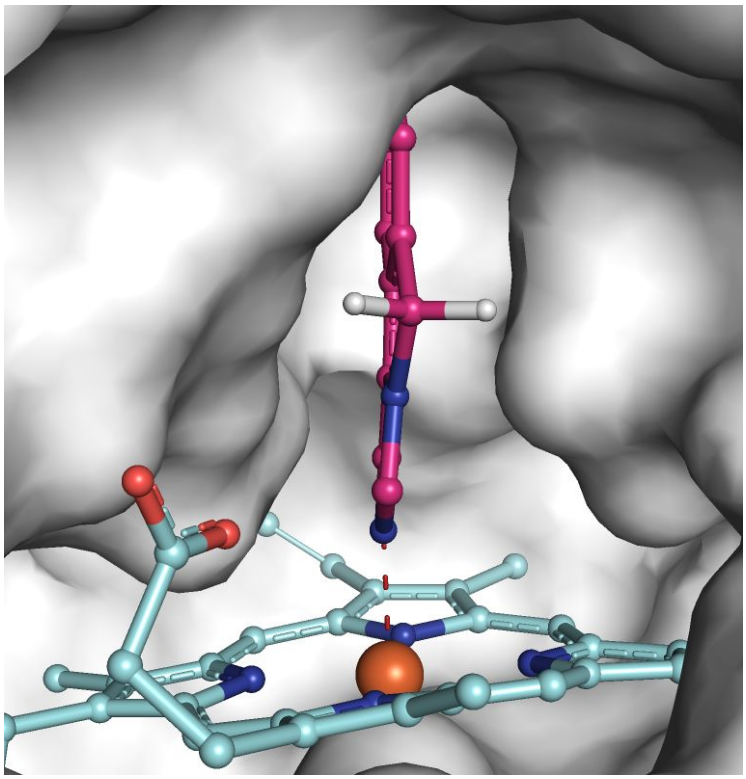


Figure 3. **37** (magenta) minimized in the IDO1 (2D0T) pocket (receptor surface shown in grey, heme and ligand interaction shown by red dashes).

Substitution at the C5-position of the imidazo[5,1-*a*]isoindole core and lead identification. Aiming to engage favorable interactions within the IDO1 active site, our focus turned towards synthesizing imidazo[5,1-*a*]isoindole analogues containing functional groups that might form favorable interactions with the heme propionate side chain or residues Phe226, Ser263, Ser235 or Arg231. We extended **37** by substituting the 5-methylene carbon of 5*H*-imidazo[5,1-*a*]isoindole. Because phenylimidazole **33** containing an ethylcyclohexyl ether side chain had good biochemical IDO1 inhibition as compared to **1**, we synthesized fused analogue **46** by incorporating an ethylcyclohexyl side chain onto the 5-position of the imidazoisoindole core. Racemic imidazoisoindole **46** exhibited superior biochemical (IC_{50} = 0.135 μM) and cellular inhibition of IDO1 (EC_{50} = 1.1 μM) as compared to **33** and to **37** representing a >20-fold improvement in

cellular potency, suggesting that molecular rigidification improved the biochemical and cellular potency. Docking studies suggested that the *S*-enantiomer of **46** would be best accommodated, establishing hydrophobic interactions with Phe226, while the *R*-enantiomer of **46** would likely make an unfavorable clash with Ser263 and Ala264 residues. (Figure 4).

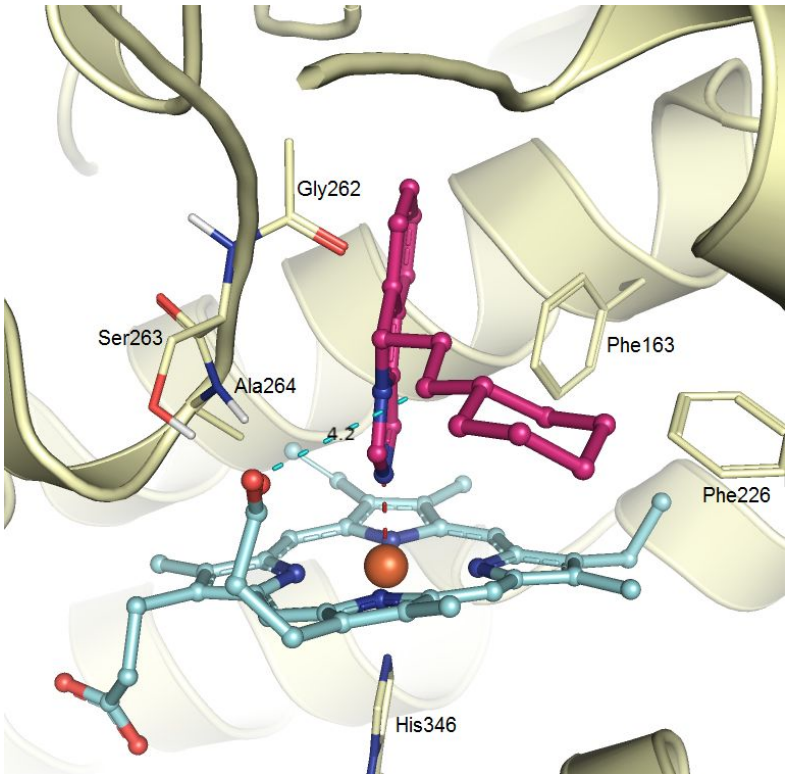


Figure 4. *S*-Isomer of racemate **46** (magenta) minimized in IDO1 (2D0T), cyan dashes shows distance (4.2Å) between heme propionate side chain and methylene carbon linker.

Targeting interactions with heme propionate. In the minimized structure of *S*-**46**, the linker methylene adjacent to the cyclohexyl ring is within close proximity (4.2 Å) of the heme propionate side chain (Figure 4). We envisioned that a suitably placed hydrogen bond donor or basic amine could form a hydrogen bond or ionic interaction respectively with the heme propionate side chain, while at the same time reduce its cLogP to improve solubility and cellular potency. Hydroxyl and amino groups were introduced into **46** giving rise to **52** and **54**. Amine analogue **54** was not

tolerated in the side chain. Interestingly, diastereomeric mixture **52** bearing a hydroxyl group on the side chain was 13-fold more potent (EC_{50} = 83 nM) in cellular IDO1 inhibition assay as compared to parent imidazo[5,1-*a*]isoindole **46**. An analogous non-cyclized derivative **48** was found to be >100-fold less active than **52**, highlighting the importance of restricting conformational freedom and orienting the hydroxyl group vector toward the heme propionate to form a hydrogen bond (Figure 5).

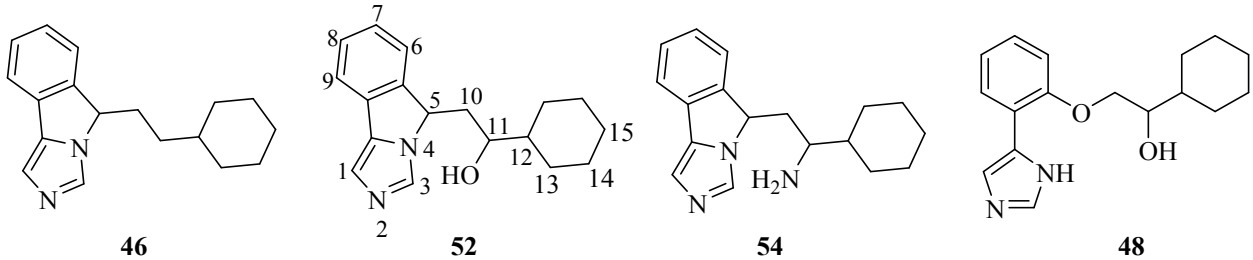
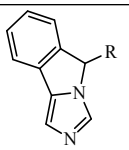
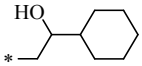
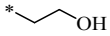
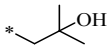
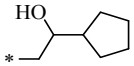
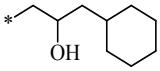
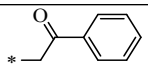
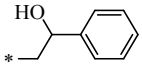
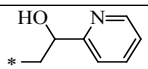
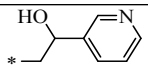
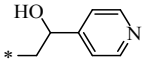
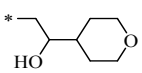
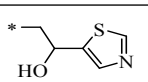
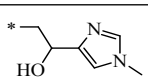
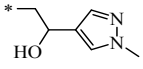
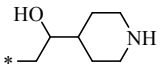
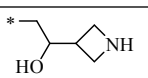
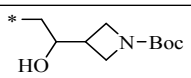
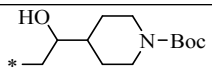


Figure 5. Effect of C11 substitution to target heme propionate interactions

Effect of various substituents at C5 of imidazo[5,1-*a*]isoindole core on IDO1 inhibition. We evaluated different substituents at the C5 position that could afford favorable interactions with the IDO1 enzyme (Table 5). Owing to the difficulty in separating and isolating individual diastereomers for each compound, the initial efforts were focused in the evaluation of IDO1 inhibition for the mixture of diastereomers of each compound. Compounds lacking a cycloalkyl group such as **57** and **58** were 100-fold less potent than **52**, suggesting that a combination of Van der Waals interactions arising from the cyclohexyl moiety and hydrogen bonding by the hydroxyl group is required for potent IDO1 inhibition. Cyclopentyl derivative **61** resulted in a 2.5 fold decrease in biochemical IDO1 inhibition compared to that of **52**. Increasing the linker length by one carbon between the hydroxyl and cyclohexyl of **52** resulted in significant loss in potency (**63**). A ketone functional group (**64**) was not tolerated in the side chain. Presumably, electron pair

repulsion between the carbonyl oxygen's lone pair and the heme propionate side chain decreases the binding efficiency of **64**, resulting in a loss of potency. In case of pyridine analogues **67**, **69** and **71**, the 4-pyridyl derivative **71** was equipotent with the corresponding phenyl analogue **65**, indicating that binding to IDO1 was highly dependent upon the position of heteroatom in the aromatic ring side chain, presumably the heteroatom in the 4-pyridyl analogue occupies the solvent-exposed region. Polar functional groups were not tolerated near hydrophobic residue Phe226 within the IDO1 pocket. Pyran analogue **73** resulted in a 10-fold decrease in IDO1 potency. A similar trend was observed with 5-membered azoles such as thiazole **75**, *N*-methyl-imidazole **77** and *N*-methyl-pyrazole **79** or with basic secondary amines such as piperidine **84** and azetidine **85**. However, the carbamate derivatives of azetidine or piperidine (**81** and **83**) were found to be >25-fold more active as compared to the corresponding free amines. Six-membered ring carbamate **83** was preferred over the corresponding azetidine **81**.

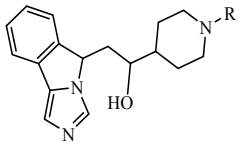
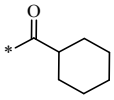
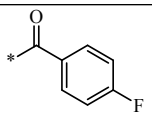
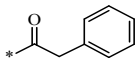
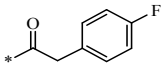
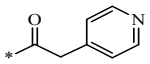
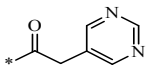
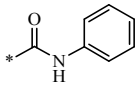
Table 5. Cyclic Side Chain Modification of 5*H*-Imidazo[5,1-*a*]isoindoles

compound		IC_{50} (μM)	EC_{50} (μM)
52		0.060	0.083
57		6.7	5.9
58		7.7	6.7
61		0.16	0.78
63		0.906	0.23
64		5.2	15
65		0.33	0.82
67		8.8	-
69		2.9	-
71		0.37	0.39
73		0.79	0.51
75		1.8	0.76
77		127	-
79		1.2	4.2
84		120	-
85		24	-
81		0.81	0.49
83		0.14	0.24

Optimization of piperidine analogues of 5*H*-imidazo[5,1-*a*]isoindoles. The nitrogen atom in piperidine analogue **84** offered a convenient handle for derivatization to possibly improving

potency and drug-like properties.[15] A library of piperidine amide and urea analogues were prepared and representative examples are shown in Table 6. Acyclic and cyclic aliphatic or aromatic R-groups **87**-**89** resulted in a decrease in potency compared to **83**. A methylene linker between the phenyl ring and amide bond was required to maintain potent IDO1 inhibition as evidenced by a 60-fold improvement in IDO1 inhibition from **89** to **90** and **91**. The urea derivate **94** also exhibited a similar profile. Unfortunately, these compounds showed potent CYP3A4 inhibition. Lowering the cLogP by introducing heteroatoms in the phenyl ring (**92**-**93**) improved the CYP3A4 profile at the expense of a decrease in IDO1 potency. In general, structural modifications that were incorporated to mitigate CYP liabilities resulted in loss of IDO1 potency. Due to their propensity to be potent inhibitors of multiple CYP isoforms, piperidine derivatives were no longer considered for further development as potent IDO1 inhibitors.

Table 6. Analogues of *N*-Piperidine Amides of 5*H*-Imidazo[5,1-*a*]isoindoles

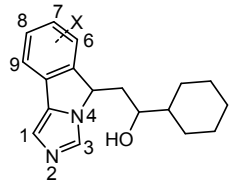
compound		IC_{50} (μM)	EC_{50} (μM)	CYP 450 IC_{50} (μM)				cLogP
				3A4	2D6	1A2	2B6	
87		2.5	1.1	5	208	>100	>100	0.11
88		0.67	0.33	0.70	9.5	>100	61	2.15
89		2.4	-	-	-	-	-	2.15
90		0.031	0.078	0.13	19.5	>100	39	2.38
91		0.040	0.13	0.06	4.8	>100	50	2.53
92		0.23	1.3	3.8	40	28	56	0.88
93		0.7	3.8	8.60	152	>100	>100	-0.07
94		0.045	0.059	0.51	10	93	75	1.98

-: Not determined

Lead optimization of 52. Since optimization of the piperidine series based on **84** did not yield good lead candidates, we focused the lead optimization efforts on cyclohexyl derivative **52**. Due to difficulty in developing methods to synthesize and separate pure stereoisomers for each compound, the initial lead optimization efforts were carried out on mixtures of diastereomers. Compound **52** exhibited high in vitro clearance (HLM Clint = 18.03 mL/min/Kg) and potent CYP3A4 inhibition (0.17 μM). Therefore, we focused our efforts on mitigating CYP3A4 inhibition and improving the metabolic stability of **52** while maintaining potency. First, the effect of small halogen substitutions on the phenyl ring of the imidazo[5,1-*a*]isoindole core was investigated (Table 7). Substitutions with fluoro or chloro at the 7 or 8-position resulted in a loss of potency. Specifically, substitution at the 8-position by fluoro or chloro (**100** and **106**) resulted

in a >60-fold decrease in biochemical inhibition, suggesting tight fitting of the imidazo[5,1-*a*]isoindole core in the IDO1 pocket ceiling and in a different position compared to what was inferred for the mode of binding of phenylimidazole **11**. However, a fluoro group placed at the 6-position in **96** maintained potent IDO1 inhibition and improved the CYP3A4 profile about 10-fold.

Table 7. Effect of Substituents on the Phenyl Ring of the 5*H*-Imidazo[5,1-*a*]isoindole Pharmacophore

compound		IC_{50} (μM)	EC_{50} (μM)	CYP 450 IC_{50} (μM)			
	X			3A4	2D6	1A2	2B6
52	-H	0.06	0.083	0.17	2.2	>100	5.9
96	6-F	0.03	0.2	2.0	2.0	>100	10
98	7-F	0.42	0.34	-	-	-	-
100	8-F	4	2.7	-	-	-	-
102	6-Cl	0.61	1.9	1.70	88	-	4.8
104	7-Cl	0.21	2.2	0.01	4.7	6.1	4.5
106	8-Cl	6.9	5.4	-	-	-	-

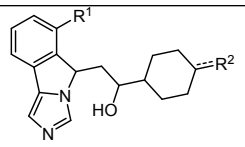
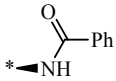
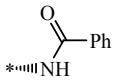
-: Not determined

Mitigating CYP450 and Human Liver Microsomal Clearance. We then focused our efforts on improving the hepatic clearance of lead molecule **96** (18.91 mL/min/Kg). We hypothesized that the high clearance of **96** could be due to poor metabolic stability of the cyclohexyl group. First pass metabolism of cycloalkyl groups is a well-documented pathway.[16] We presumed the C15 of the cyclohexyl side chain to be the hot spot for metabolization, and several strategies were explored to circumvent high clearance in human liver microsomes (Table 8). Blocking the C15-positon by a geminal-difluoro group offered a modest improvement in clearance as observed in compounds

108 and **110**. Other C15-cyclohexyl modifications such as methylcarboxylate **112** and ketone **114** resulted in decreased IDO1 inhibition. Benzamides **116**, **118** derived from *4-trans* and *4-cis* cyclohexyl amines respectively exhibited promising activity against IDO1, but these compounds exhibited poor aqueous solubility near neutral pH and suffered from moderate CYP3A4 and CYP2D6 inhibition.

Modeling of the (C5 *S*, C11 *R*) stereoisomer of **52** within the IDO1 active site revealed that the C15-position of the cyclohexyl ring is within close proximity to Ser235 and Arg231 (Figure 7). We hypothesized that a polar group at the C15-position could improve IDO1 activity by interacting with Ser235 or Arg231. Furthermore, introduction of a polar group would lower cLogP, which could improve CYP3A4 inhibition profile. Several case studies have established that compounds with lower cLogP tend to decrease the risk of potent CYP3A4 inhibition and drug-drug interactions,[17] while also leading to improvement in metabolic stability.[18] Substitution at the C15-position by a hydroxyl group resulted in significant improvement in human microsomal clearance (4.06 mL/min/Kg) and improved CYP profile (**120**) while maintaining IDO1 potency. By decreasing the cLogP of lead candidate **96** (cLogP = 3.64), CYP inhibition and high clearance were greatly improved. Overall, **120** (cLogP = 1.55) had the best balance of potency, metabolic stability, off-target liabilities, and physiochemical properties (Table 8).

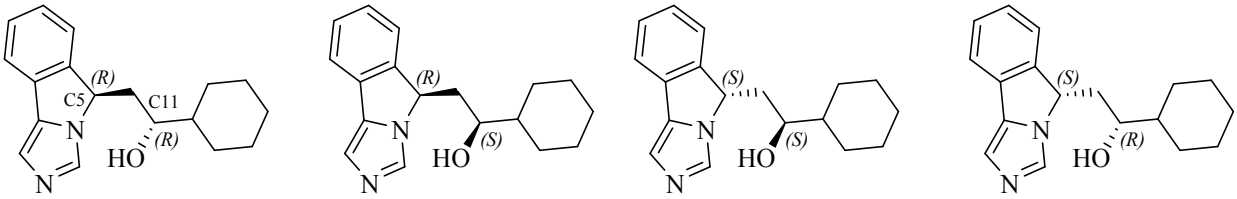
Table 8. Effect of Substituents on the Distal Cyclohexyl Ring

cmpd			IC_{50} (μM)	EC_{50} (μM)	CYP 450 (IC_{50} (μM))				hERG EC_{50} (μM)	HLM	f_u (%)	cLogP
					3A4	2D6	1A2	2B6		Ph1		
	R^1	R^2								mL/min/Kg		
96	F	H	0.03	0.2	2.0	2.0	>100	10	23	18.91	6.1	3.64
108	H		0.046	0.079	1.2	5.7	>100	41	13.2	14.60	17.9	2.87
110	F		0.10	0.093	2.6	20	>100	>100	>100	15.27	-	3.02
112	H	CO_2Me	0.28	0.22	-	-	-	-	-	-	-	2.34
114	F	*=O	0.6	0.2	10.9	95	>100	>100	65	20.29	-	1.15
116	H		0.05	0.086	1.20	6.8	>100	82	30	-	2.7	3.03
118	H		0.072	0.079	1.10	1.40	>100	74	16	8.23	2.9	3.03
120	F	OH	0.2	0.25	3.30	>100	>100	>100	34	4.06	43.6	1.55

*Solubility data in μM: **120** (pH1=353; pH6.5=329; pH7.4=316); **118** (pH1=400; pH6.5=65; pH7.4=56); **116** (pH1=363; pH6.5=22; pH7.4=25).

Stereochemistry and IDO1 activity relationship. Since **120** is a mixture of 8 stereoisomers, we first determined the effect of C5 and C11 stereochemistry on isomers isolated from the active analogue **52**, which only contains 2 chiral centers. To this purpose, diastereomers of compound **52** were isolated by chiral SFC, giving rise to stereoisomers **121**-**124**. The absolute stereochemistry of each stereo isomer was determined by X-ray crystallography and ^{1}H-NMR. These *syn* and *anti* enantiomer pairs showed a typical splitting pattern by ^{1}H-NMR for C5-H, with a triplet δ 5.39 (*J* = 6.3 Hz, 1H) or a doublet of doublet δ 5.48 (*J* = 10.6, 3.2 Hz, 1H), respectively. The stereochemistry at the C5 ring junction plays an important role in the binding of imidazo[5,1-*a*]isoindole derivatives within the IDO1 active site. C5 *S*-substituted imidazoisoindole compounds were drastically more potent than the corresponding C5 *R*-stereoisomers, just as it had been predicted by *in silico* modeling. Stereoisomers **124** and **123** were 100-fold to 1000-fold more active than their corresponding C5 *R*-stereoisomers **121** and **122** in biochemical IDO1 assay. C11 *S*-

hydroxy stereoisomer **123** was over 5-fold more potent than C11 *R*-hydroxy stereoisomer **124** in a IDO1 biochemical inhibition assay (Figure 6).



121	**122**	**123**	**124**
IC_{50} = >25 μM	IC_{50} = 18 μM	IC_{50} = 0.021 μM	IC_{50} = 0.11 μM
C5-H: δ 5.48 (dd, 1H)	δ 5.39 (t, 1H)	δ 5.48 (dd, 1H)	d 5.39 (t, 1H)

Figure 6. Evaluation of **52** isomers towards IDO1 inhibition

We hypothesized that the cyclohexyl moiety occupies the hydrophobic region near Phe226 and the hydroxyl group forms a hydrogen bond interaction with the heme propionate side chain (Figure 7). Our modeling hypothesis was later confirmed by recently published X-ray co-crystal structure of various 5-*H* substituted imidazoisoindole analogues bound to IDO1.[19]

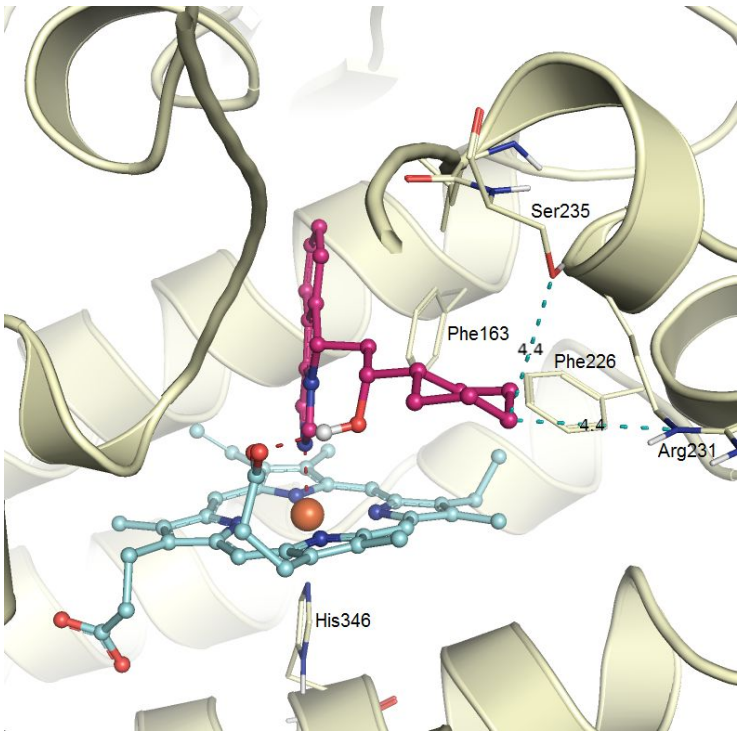
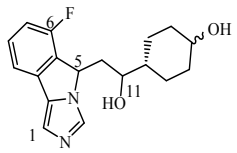


Figure 7. **123** minimized in the IDO1 (2D0T) pocket; ligand-receptor interactions including hydrogen bond shown by red dashes. The cyan dashes shows the distance between C15 of cyclohexyl and Ser235 oxygen (4.4 Å) and Arg231 nitrogen (4.4 Å).

Effect of Stereochemistry in 120 and selection of NLG-919. Encouraged by the overall profile of **120**, we synthesized both *cis* (**126**) and *trans*-cyclohexanol (**127**) derivatives. *Cis* and *trans*-cyclohexanol derivatives **126** and **127** respectively, were equipotent in biochemical assays as mixtures of diastereomers (Table 9). Diastereomeric mixtures **126** and **127** were separated by chiral SFC. As observed with the stereoisomers of compound **52**, the predominant effect on IDO1 activity was determined by the stereochemistry of C5.

Trans-cyclohexanol C5-*S* isomers **NLG-919** and **132** were 100-fold and 70-fold more potent than their corresponding C5-*R* isomers **133** and **134**, respectively. Similarly, *cis*-cyclohexanol isomers **130** and **128** were 35-fold and 3-fold more potent than their C5 *R*-isomers **129** and **131**, respectively. The stereochemistry of the C11-OH group also played an important secondary role in IDO1 inhibition, with the *R*-isomer being 2-fold to 5-fold more active than the corresponding *S*-isomer. Similarly, the stereo configuration of the cyclohexanol group played an important role, with the *trans* configuration being 3-fold to 10-fold more potent than the *cis* form. **NLG-919** exhibited superior biochemical and cellular potency, an acceptable CYP450 profile, low hERG inhibition (71 μM), a low protein binding (f_u 52%), low clearance in human liver microsomes (8.52 mL/min/Kg), and excellent solubility at wide pH range (>200 μM at pH 1-7.4).

Table 9. Effect of stereochemistry on C5 and C11

compound			IDO1 IC_{50} (μM)	EC_{50} (μM)	CYP 450 (IC_{50} (μM))				hERG EC_{50} (μM)	Human Liver μsome	f_u
					3A4	2D6	1A2	2B6		Ph1	%
	C5,C11	*cis/trans*								mL/min/Kg	
126	Mix of isomers	*cis*	0.26	-	-	-	-	-	-	-	-
128	(*S*, *S*)	*cis*	0.44	-	-	-	-	-	-	12.74	-
129	(*R*, *R*)	*cis*	3.4	-	-	-	-	-	-	-	-
130	(*S*, *R*)	*cis*	0.09	0.17	3.10	38	94	51	>100	18.51	46
131	(*R*, *S*)	*cis*	1.5	-	-	-	-	-	-	-	-
127	Mix of isomers	*trans*	0.2	-	-	-	-	-	-	-	-
132	(*S*, *S*)	*trans*	0.042	0.22	5.6	94	>100	43	-	6.20	35
133	(*R*, *S*)	*trans*	2.9	-	-	-	-	-	-	-	-
134	(*R*, *R*)	*trans*	3.7	-	-	-	-	-	-	-	-
NLG-919	(*S*, *R*)	*trans*	0.028	0.075	5.7	86	>100	>100	71	8.52	52

-: Not determined

The biochemical potency of **NLG-919** (IC_{50} 28 nM) was close to the concentration of enzyme being used in the enzymatic potency assay, indicating that **NLG-919** is a tight binding inhibitor of IDO1. Determination of the K_i was performed by measuring *vi/v0* at IDO1 enzyme concentrations of 63 to 1080 nM in the presence of varying concentrations of **NLG-919** (0-8100 nM) followed by non-linear fitting of the data to the Morrison equation.[20] This analysis indicated that **NLG-919** has a K_i of 5.8 nM. An analysis of the mode of inhibition by determination of the IC_{50} at different concentrations of *L*-Trp (10-400 μM) indicated that **NLG-919** inhibits IDO1 activity in an uncompetitive or non-competitive mode with respect to *L*-Trp substrate with $\alpha<1$. The non-competitive or uncompetitive mode of inhibition with respect to *L*-Trp was determined under conditions of pseudo-first order assuming a non-limiting concentration of O_2, the second substrate of the reaction, and is in agreement with the proposed mode of binding of **NLG-919** directly coordinated to the heme Fe^{+2}.

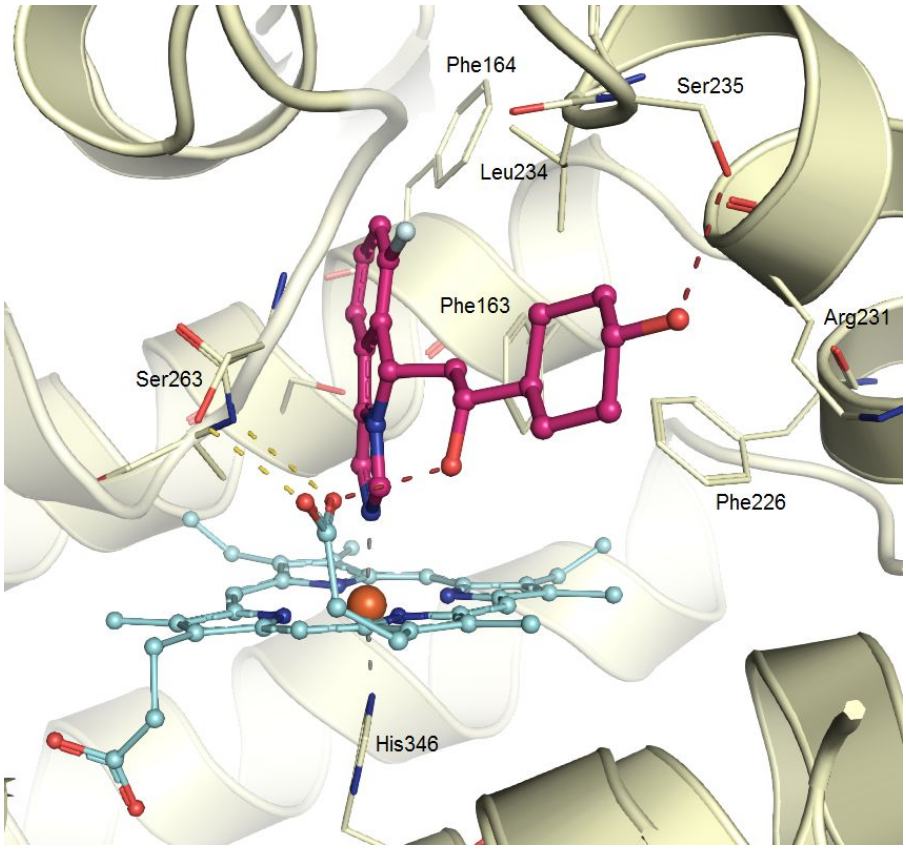


Figure 8. Crystal structure of **NLG-919** bound to human IDO1 (6O3I). The protein is colored in ivory. The heme molecule is displayed in light blue ball-and-stick, highlighting protein:heme interactions at the propionate (yellow dashes) and the iron atom (grey dashes). Hydrogen bonds from **NLG-919** to the protein are indicated in red dashes, and select residues in the immediate vicinity of the binding site are shown in stick and labeled.

The binding mode was corroborated by determination of the crystal structure of **NLG-919** bound to hIDO1. The core imidazoisoindole maintains its anticipated iron-coordinated position with the upper tricyclic scaffold forming tight Van der Waals contacts in the hydrophobic cavity created by IDO1 residues Val130, Phe163, Phe164, and Leu234. This positioning accounts for the dramatic negative impact of halogens at C-7 and C-8 in compounds **98** and **100**, while the design of the C-6 fluoro-substituent is well-braced to the C-α of Gly262 providing a positive contribution to

potency. The stereochemistry of the linker positions its hydroxyl group to achieve the designed hydrogen bond with the heme propionate, while the cyclohexanol moiety continues outward toward the solvent accessible opening of the heme-proximal cavity, its saturated bulk affording contacts in the widening channel. Additionally, the terminal hydroxyl is able to form a hydrogen bond interaction with Ser235, giving a specific orientation to the distal end of the ligand. Combined, the interplay of the stereochemistry and the observed interactions provides a consistent accounting for the potency and selectivity of **NLG-919** (Figure 8).

Pharmacological properties of NLG-919

NLG-919 showed favorable pharmacological properties that prompted us to select it as a clinical development candidate. First, **NLG-919** was not extensively metabolized by human or rat microsomes, with >85% remaining after 90 min incubation in the presence of Phase I+II cofactors. Second, **NLG-919** showed no CYP1A2, CYP2B6, CYP2C8, CYP2C9, and CYP2C19 (IC_{50} > 100 μM) inhibition; weak inhibitory effect for CYP2D6 (IC_{50} 86 μM) and moderate inhibitory effect on CYP3A4 with IC_{50} values of 5.7 μM (midazolam) or 15 μM (testosterone). In Caco-2 assay, **NLG-919** was highly permeable (22.7×10^{-6} cm/s from A to B, 35.0×10^{-6} cm/s from B to A) and demonstrated an efflux ratio <2 in the presence of verapamil at 100 μM indicating that **NLG-919** is not a potential substrate for P-glycoprotein (P- gp). **NLG-919** did not activate arylhydrocarbon receptor (AhR) at 10 μM and it exhibited low transactivation of PXR at 300 μM, suggesting low probability of CYP450 induction.

The pharmacokinetics of **NLG-919** was studied in rats, mice and dogs. Following single intravenous doses at 10-50 mg/kg in SD rats, **NLG-919** exhibited a half-life ($t_{1/2}$) of 1 h, and the clearance (CL) was about 23 – 39.1 mL/min/kg. The clearance decreased with increased dose level,

indicating inverse dose-dependent clearance and saturation of the elimination pathways. In C57B16 mice, the clearance was found to be ~26 mL/min/kg with a $t_{1/2}$ of 3-4 h. Following a single IV dose administration to dogs, **NLG-919** exhibited variable $t_{1/2}$, ranging from 1.6 – 6.9 h. **NLG-919** was rapidly eliminated in rats with a plasma clearance value ranging from 41.4 – 59.3 mL/min/kg. A similar trend of decreasing clearance with increased dose was observed in dogs, though to a lesser extent than in rats. The systemic exposure increased proportionally as the dose was increased from 1 to 25 mg/kg. Following single oral doses at 10, 25, and 50 mg/kg in SD rats, **NLG-919** was rapidly absorbed (T_{max} = 0.5 h) and exhibited moderate oral bioavailability of 41-60% with a $t_{1/2}$ of about 1 h. In C57B16 mice, **NLG-919** exhibited high oral bioavailability of 69% and 87% for the dose levels of 10 and 100 mg/kg, respectively and a $t_{1/2}$ of ~1 h for the lower doses and 3 h for the higher dose. In dogs, oral bioavailability increased from 14% at 5 mg/kg to 72% at 150 mg/kg, suggesting saturation of metabolic clearance at higher doses (Table 10).

Table 10. Single dose pharmacokinetic profile of **NLG-919** in different species after iv and oral dosing

Species	Dose (mg/kg)	CL (mL/min /kg)	$AUC_{0\text{-}inf}$ (ng·h/mL)		$t_{1/2}$ (h)		C_{max} (ng/mL)	%F
		IV	IV	PO	IV	PO	PO	
Rat	10	44.6	3930	1630	1.44	1.05	1560	41.5
	25	32.0	13400	7975	1.25	1.15	6420	60.4
	50	23.2	34950	15400	1.0	1.06	10050	44.1
Mice	10	27.5	6132	3777	3.9	1.2	2257	69.0
	50	25.0	34633	-	3.0	-	-	-
	100	-	-	60195	-	3.3	14165	87.0
Dog	1	59.3	282	-	2.2	-	-	-

	3	56.1	901	-	1.6	-	-	-
	5	-	-	207	-	-	132	13.7
	10	53.7	3127	-	4.8	-	-	-
	15	-	-	1045	-	-	1400	22.3
	25	41.4	10050	-	6.9	-	-	-
	50	-	-	8645	-	-	7855	43.0
	150	-	-	57750	-	-	40450	71.8

-: Not determined

NLG-919 was found to be moderately bound to plasma proteins (43% – 49% in SD rats, 48% – 52.0% in cynomolgus monkey and 52% – 55% in human plasma at 0.2, 2 and 20 μM concentration). After a single oral dose of 50 mg/kg administered to C57Bl/6 mouse, **NLG-919** was found to be present at higher concentration in plasma than in other organs, the maximum exposure occurred at 1 h post-dose.

The pharmacodynamic effect of **NLG-919** in vivo was measured in a mouse model where IDO1 activity is induced in the lungs by intranasal instillation of bacterial lipopolysaccharides.[21] In this mouse model, plasma Kyn was reduced by 50% and lung Kyn was reduced by 60%, 6 h after oral dosing of **NLG-919** at 50 mg/kg. Given that approximately 50% of systemic Kyn levels in plasma is contributed by IDO1 and the rest is contributed by the liver enzyme tryptophan 2,3-dioxygenase (TDO2),[22] the results suggest that an oral dose of 50 mg/kg produces almost complete inhibition of IDO1 activity in this model.

The antitumor activity of **NLG-919** was determined in the Pan02 tumor model in C57Bl/6 mice, where it demonstrated to reduce tumor growth and enhance survival as a single agent ($p=0.0005$; Figure 9A) as well as to enhance the antitumor effect of cyclophosphamide in a combination chemotherapy regimen ($p=0.0083$; Figure 9B).

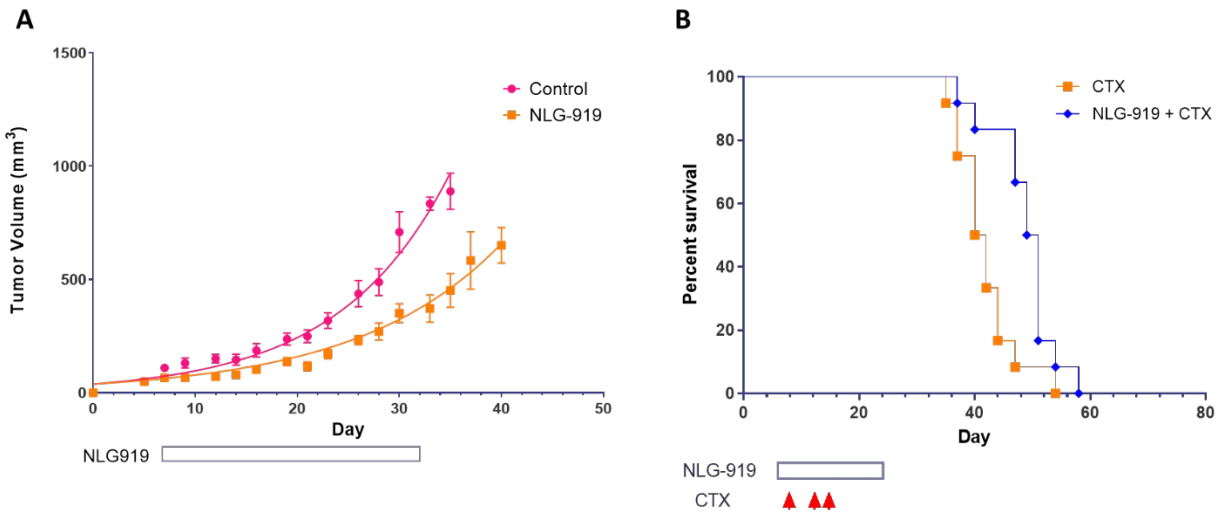


Figure 9. Antitumor activity of **NLG-919** in Pan02 tumor model. A) $5x10^6$ Pan02 cells were implanted s.c. into the flank of C57Bl/6 mice (n=7/group). **NLG-919** was dosed at 500 mg/kg/day, from Days 7-32. B) Survival of C57Bl/6 mice bearing Pan02 tumors (n=12/group), where mice were dosed with **NLG-919** at 400 mg/kg/day from days 7 to 25 post-tumor inoculation, with or without 3 doses of cyclophosphamide 100 mg/kg i.p. on Days 8, 13 and 15.

Toxicology studies were conducted in rats and dogs in 28-day repeat dose studies with 2-week recovery. It was determined that the non-observed adverse event level (NOAEL) was 250 mg/kg/dose BID for rats ($AUC_{0\text{-}inf}$ = 22500 h*ng/mL) and 200 mg/kg/dose BID for dogs ($AUC_{0\text{-}12h}$ of 22550 h*ng/mL).

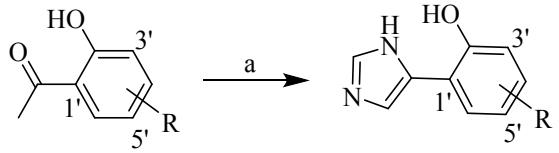
In summary, **NLG-919** had a favorable drug-like profile and was selected as a clinical candidate for further evaluation in Phase 1 clinical trials. Furthermore, this study describes for the first time, the use of imidazo[5,1-*a*]isoindoles as a new class of heme-binding chemical pharmacophore, which could become a structural scaffold for other heme-containing pharmacologic targets. In fact, further SAR studies focusing on different C5 side chain substituents have afforded potent specific

inhibitors of the related enzyme tryptophan 2,3-dioxygenase (TDO2) and dual inhibitors of both IDO1 and TDO2 enzymes.[23]

Chemistry

The synthesis of 4-phenylimidazole derivatives **4-8** was achieved by the reaction of α-bromoketone derivatives with formamide (Scheme 1).[24] **3** and **9** were synthesized according to the literature.[11]

Scheme 1. Synthesis of substituted 5-(phenyl)-1*H*-imidazole (**1**) derivatives[a]



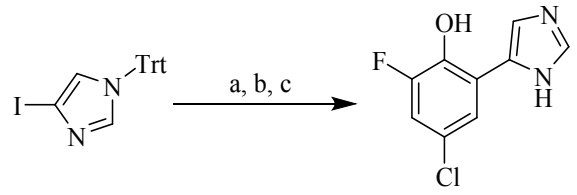
2a-f

3, R = H; **4**, R = 5'-Cl;
5, R = 5'-CH_3; **6**, R = 5'-Br;
7, R = 5'-F; **8**, R = 3', 5'-F.

Reagents and conditions[a]: (a) i) $CuBr_2$, $CHCl_3$, 60 °C; ii) H_2NCHO, 170-180 °C, 33-61%.

11 was prepared by palladium-catalyzed Negishi cross-coupling[25] of 4-iodo-1-trityl-1*H*-imidazole with 4-chloro-2-fluoro-6-iodophenol[26] followed by deprotection of the trityl group with acetic acid in methanol (Scheme 2).

Scheme 2. Synthesis of **11**[a]

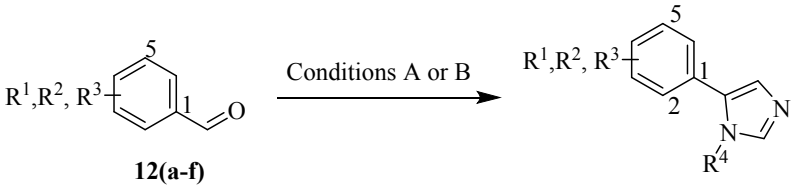


10 **11**

Reagents and conditions[a]: (a) i) EtMgBr, THF, rt, 1.5 h; ii) $ZnCl_2$, 1.5 h;
b) 4-Chloro-2-fluoro-6-iodophenol,10% $Pd(PPh_3)_4$, THF, 70 ^oC, 12 h;
c) AcOH, MeOH, 80 ^oC, 37%.

13-**20** and **36** were obtained via Scheme 3.[27] *N*-1 substituted arylimidazole derivatives were synthesized by the Van Leusen imidazole synthesis. TosMIC was allowed to react with the requisite benzaldehydes, followed by a dehydration and cyclization sequence (Scheme 3). The intermediate aldehyde (**12c**) for **36** was synthesized from 5-hydroxy-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one[28] via Scheme 4.

Scheme 3. Synthesis of **13-20** and **36** by Van Leusen imidazole reaction[a]



12(a-f)

Conditions A

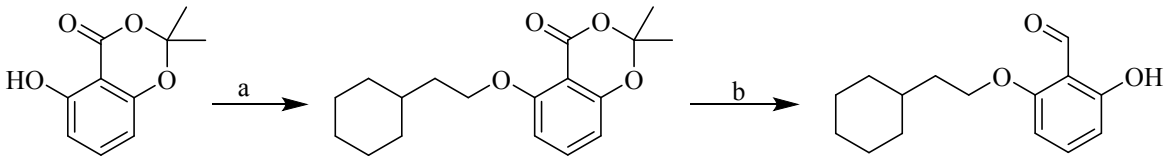
13, R^1= 2-OH, R^2 = 3-Cl, R^3 = R^4 = H; 41%
14, R^1= 2-OH, R^2 = 3-Cl, R^3 = 5-F, R^4 = H; 62%
36, R^1= R^4= H, R^2= 2-OH, R^3 = 6-Ethoxycyclohexyl; 46%

Conditions B

15, R^1 = R^2 = R^3= H, R^4 = -CH_2CH_2OH; 59%
16, R^1 = R^2 = R^3 = H, R^4= -CH_2CO_2Me; 36%
17, R^1 = R^2 = R^3 = H, R^4 = -$CH_2CH_2CO_2Et$: 30%
18, R^1 = R^2 = R^3 = H, R^4 = -$CH_2CH_2NHCOMe$: 26%
19, R^1 = 2-OMe, R^2 = R^3 = H, R^4 = -CH_2CH_2*t*-Bu; 45%
20, R^1 = 2-OBn, R^2 = 5-Cl, R^3 = H, R^4 = Cyclohexyl; 95%

Reagents and conditions[a]: Conditions A: (a) i) R^4NH_2, MeOH, 15-20 h; ii) TosMIC, piperidine, 36 h, 46-65%. Conditions B: (a) TosMIC, NaOt-Bu, THF, -40 ^oC, 50 min., b) Et_3N, $POCl_3$, -10 ^oC, 45 min., c) R^4NH_2, MeOH, 25 ^oC, 12 h, 26-95%.

Scheme 4. Synthesis of 2-(2-cyclohexylethoxy)-6-hydroxybenzaldehyde (**12c**)[a]



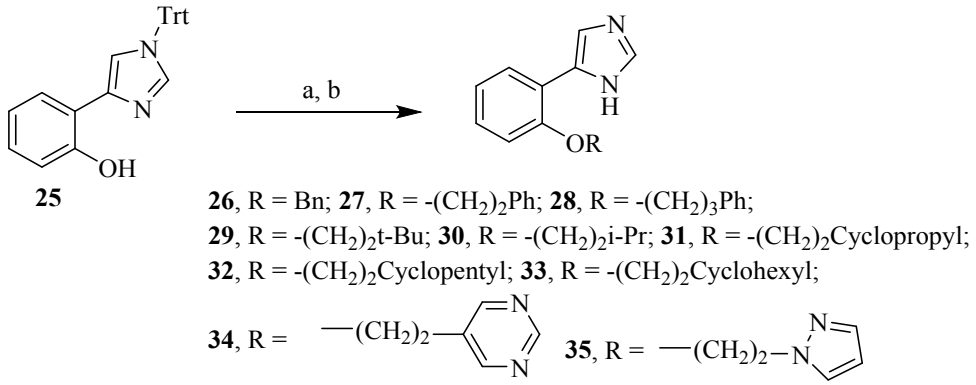
12c-SM1 **12c-SM2** **12c**

Reagents and conditions[a]: (a) 2-Cyclohexylethan-1-ol, DEAD, PPh_3, THF, overnight, 69%; (b) DIBAL-H, CH_2Cl_2, -78 ^oC, 1.5 h, 35%.

21 was prepared by demethylation of **19** in refluxing aqueous HBr. The benzyl group of **20** was removed by hydrogenation in the presence of Pd/C to afford **22**. Esters **16** and **17** were converted to *N*-methylamides **23** and **24** respectively by allowing them to react in the presence of methylamine.

The *O*-alkyl derivatives (**26-35**) were prepared by SN2 displacement reactions of 2-(1-trityl-1*H*-imidazol-4-yl)phenol (**25**) with their appropriate halide or tosylate followed by deprotection of the trityl group (Scheme 5).

Scheme 5. Synthesis of **26-35**[a]



25

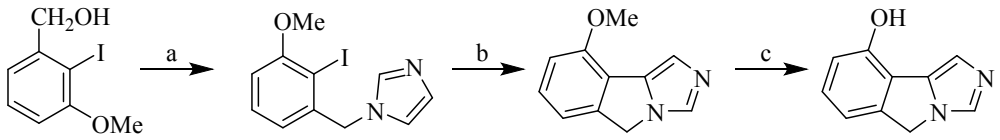
26, R = Bn; **27**, R = -$(CH_2)_2$Ph; **28**, R = -$(CH_2)_3$Ph;
29, R = -$(CH_2)_2$t-Bu; **30**, R = -$(CH_2)_2$i-Pr; **31**, R = -$(CH_2)_2$Cyclopropyl;
32, R = -$(CH_2)_2$Cyclopentyl; **33**, R = -$(CH_2)_2$Cyclohexyl;

34, R = **35**, R =

Reagents and conditions[a]: (a) i) NaH, THF, 0 °C, 1 h; ii) R-X, 0 °C-rt, 10-15 h; (b) AcOH, MeOH, 80 °C, 2 h, 15-73%.

41 was synthesized from 1-(2-iodo-3-methoxybenzyl)-1*H*-imidazole (**39**), by an intramolecular palladium-catalyzed Heck cross-coupling reaction followed by demethylation of **40**. 1-(2-Iodo-3-methoxybenzyl)-1*H*-imidazole (**39**) was prepared by a Mitsunobu reaction with (2-iodo-3-methoxyphenyl)methanol (**38**)[29] and imidazole (Scheme 6).

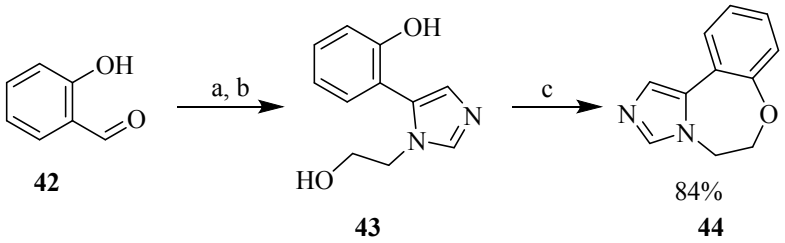
Scheme 6. Synthesis of **41**[a]



38 **39** **40** **41**

Reagents and conditions[a]: (a) Imidazole, DEAD, PPh_3, THF, 0-60 °C, 14 h, 43% ; (b) $Pd(OAc)_2$, PPh_3, K_2CO_3, DMSO, 110 °C, 36 h, 37%; (c) HBr (aq, 48 %), 100 °C, 14 h, 68%.

Scheme 7. Synthesis of **44**[a]

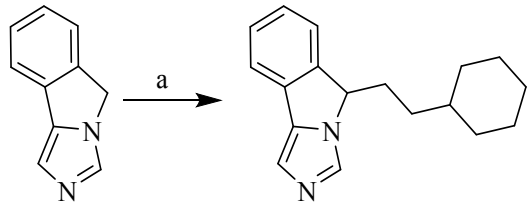


42 **43** **44**

Reagents and conditions[a]: (a) 2-Amino-1-ethanol, MeOH, 40 ^{o}C, 1 h; (b) TosMIC, DME/MeOH, rt, 72 h, 46%; (c) PPh_3, DEAD, THF, 0 ^{o}C-rt, 16 h, 84%.

Intramolecular Mitsunobu reaction of **43** afforded **44** (Scheme 7), **45** was synthesized from isoquinoline.[30] Alkylation of **37** with (2-iodoethyl)cyclohexane afforded **46** in 80% yield (Scheme 8).

Scheme 8. Synthesis of **46**[a]

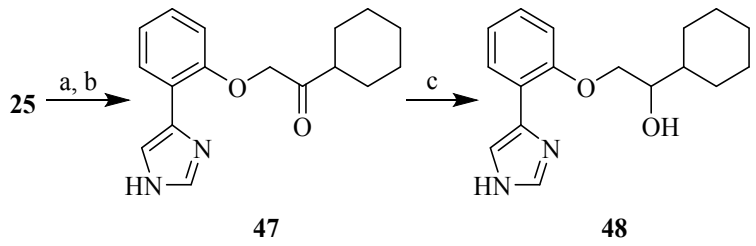


37 **46**

Reagents and conditions[a]: (a) i) *n*-BuLi, THF, -40 ^{o}C, 1 h; ii) (2-Iodoethyl)cyclohexane, -40 ^{o}C-rt, 14 h, 80%.

48 was prepared by an SN2 reaction of **25** with 2-bromo-1-cyclohexylethanone followed by deprotection of the trityl group with acetic acid and ketone reduction with $NaBH_4$ (Scheme 9).

Scheme 9. Synthesis of **48**[a]

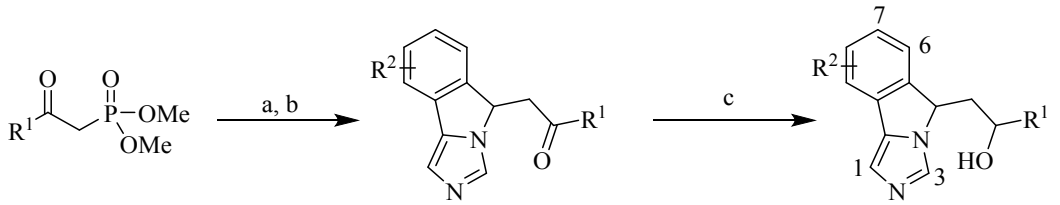


47 **48**

Reagents and conditions[a]: (a) i) NaH, THF, 0 ^{o}C, 1 h; ii) 2-Bromo-1-cyclohexylethan-1-one, 0 ^{o}C-rt, 14 h; (b) AcOH, MeOH, 80 ^{o}C, 2 h, 62%; (c) $NaBH_4$, MeOH, 0 ^{o}C-rt 1 h, 90%.

Imidazoisoindole **52**, **63**, **67**, **69**, **75**, **77**, **79**, **81**, **83**, **87**, **98**, **108**, **110**, **112**, **116**, **118** were synthesized by Horner-Wadsworth-Emmons reaction of dimethyl (2-oxo) phosphonates with substituted aldehydes followed by an acid-catalyzed in situ ring closure via a vinylogous intramolecular Michael addition and ketone reduction with sodium borohydride (Scheme 10).

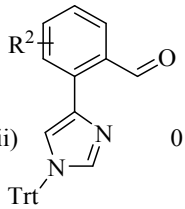
Scheme 10. Horner-Wadsworth-Emmons reaction of dimethyl (2-oxo) phosphonates with substituted aldehydes[a]



49(a-p)

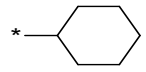
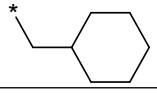
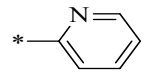
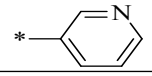
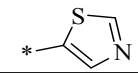
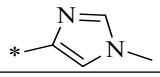
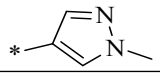
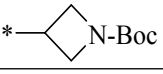
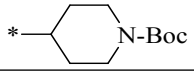
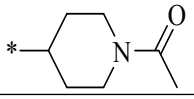
51, **62**, **66**, **68**, **74**, **76**, **78**, **80**, **82**, **86**, **97**, **107**, **109**, **111**, **113**, **115**, **117**, **125**

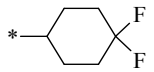
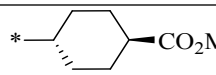
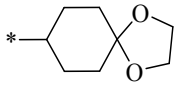
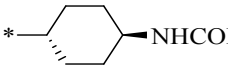
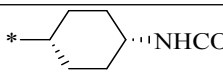
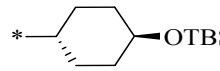
52, **63**, **67**, **69**, **75**, **77**, **79**, **81**, **83**, **87**, **98**, **108**, **110**, **112**, **116**, **118**



50a, **50b** or **50f**

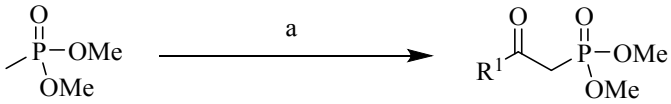
Reagents and conditions[a]: (a) i) NaH, THF, 0 ^{o}C, 40 min.; ii) 0 ^{o}C-rt, 16 h; (b) AcOH, MeOH, 90 ^{o}C, 2 h, 24-96%; (c), $NaBH_4$, MeOH, 0 ^{o}C-rt, 1 h, 43-95%.

R^1	R^2	Cmpd	Yield (%)	Cmpd	Yield (%)
	H	**51**	86	**52**	79
	7-F	**97**	94	**98**	93
	H	**62**	82	**63**	85
	H	**66**	75	**67**	82
	H	**68**	89	**69**	69
	H	**74**	30	**75**	75
	H	**76**	24	**77**	94
	H	**78**	88	**79**	77
	H	**80**	77	**81**	43
	H	**82**	89	**83**	83
	H	**86**	74	**87**	93
	H	**107**	96	**108**	61

	6-F	**109**	81	**110**	78
	H	**111**	90	**112**	87
	6-F	**113**	84	-	-
	H	**115**	53	**116**	63
	H	**117**	69	**118**	57
	6-F	**125**	79	-	-

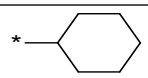
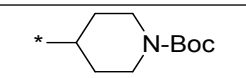
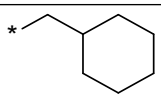
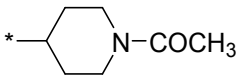
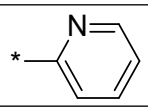
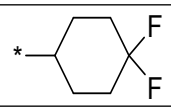
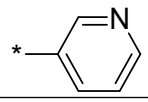
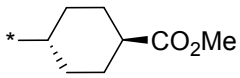
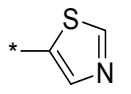
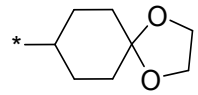
The dimethyl (2-oxo) phosphonate derivatives **49(a-p)** were synthesized according to Scheme 11, the ester intermediates **49(n-o)-SM** were synthesized by benzoylation of the corresponding methyl 4-aminocyclohexane carboxylates, **49p-SM** was synthesized according to the literature.[31]

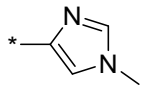
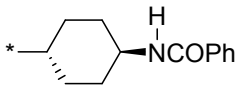
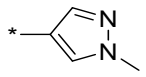
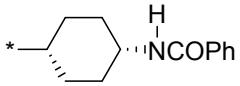
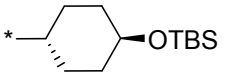
Scheme 11. Synthesis of dimethyl (2-oxo) phosphonates **49(a-p)**[a]



49(a-p)

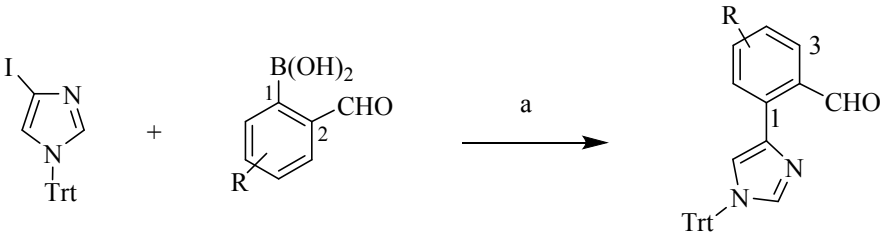
Reagents and conditions[a]: (a) i) *n*-BuLi, THF, -78 °C, 0.5 h; ii) $R^1CO_2R^2$ [**49(a-p)-SM**], -78-0 °C, 1.5 h.
[R^2 = Me for **49(a-o)-SM**; R^2 = Et for **49p-SM**]

Ester	Product		Ester	Product	
R^1	Cmpd	Yield (%)	R^1	Cmpd	Yield (%)
	**49a**	89		**49i**	68
	**49b**	70		**49j**	64
	**49c**	56		**49k**	82
	**49d**	65		**49l**	70
	**49e**	18		**49m**	72

	**49f**	29		**49n**	83
	**49g**	78.5		**49o**	54
	**49h**	99		**49p**	96

2-(1-Trityl-1*H*-imidazol-4-yl)benzaldehyde derivatives **50(a-f)** were synthesized by Suzuki cross-coupling of 4-iodo-1-trityl-1*H*-imidazole with the corresponding phenyl boronic acids (Scheme 12).

Scheme 12. Synthesis of **50** (**a-f**)[a]



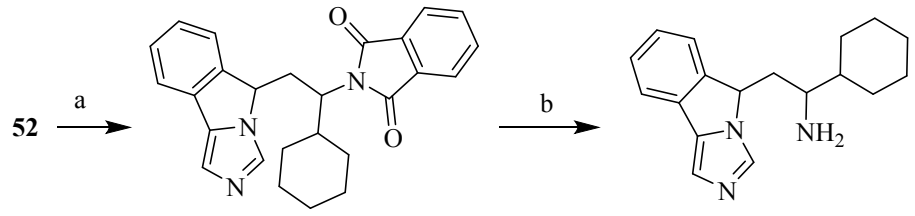
10 **50(a-f)-SM** **50(a-f)**

Reagents and conditions[a]: (a) K_3PO_4, $Pd(PPh_3)_4$, DMF/H_2O, 90 ^oC, 16 h, 46-87%.

R	Product	Yield (%)
H	**50a**	52
3-F	**50b**	46
5-F	**50c**	89
4-Cl	**50d**	48
5-Cl	**50e**	55
4-F	**50f**	87

Amine **54** was synthesized by converting **52** to phthalimide derivative **53**, followed by deprotection by hydrazine hydrate (Scheme 13).

Scheme 13. Synthesis of **54**[a]

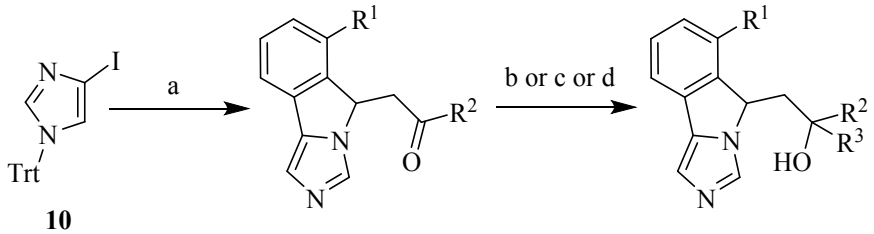


53 54

Reagents and conditions[a]: (a) PPh_3, DEAD, phthalimide, THF, 0 °C-rt , 16 h; (b) $NH_2NH_2.H_2O$, EtOH, 6 h, rt, 20%.

Imidazoisoindoles **57** and **102** were synthesized in three steps from **10**, by Negishi cross-coupling with **55a** or **55b** respectively, followed by an intramolecular aza-Michael cyclization assisted by acetic acid. Finally, reduction of the ketone with sodium borohydride afforded **57** and **102**. **58** was synthesized by Grignard addition of methylmagnesium bromide to ester **56** (Scheme 14).

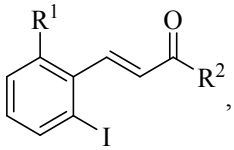
Scheme 14. Imidazoisoindoles **57**, **58**, **102** synthesis by Negishi cross-coupling[a]



10

56, R^1 = H, R^2 = OEt
101, R^1 = Cl, R^2 = Cy

57, $R^1 = R^2 = R^3$= H
58, R^1 = H, $R^2 = R^3$= CH_3
102, R^1 = Cl, R^2 = Cy, R^3 =H

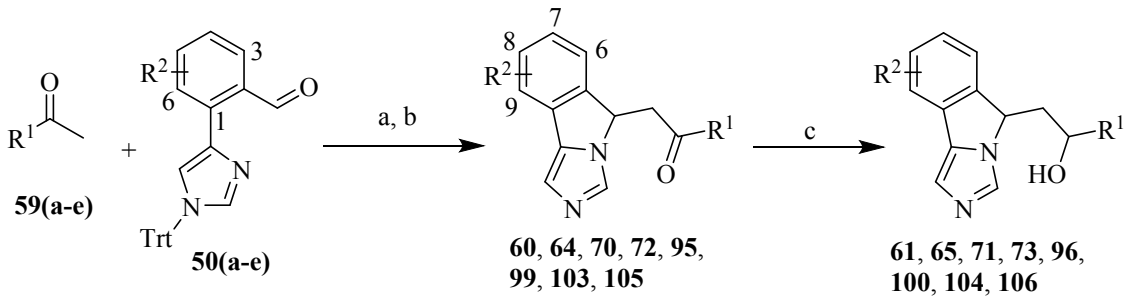
Reagents and conditions[a]: (a) i) EtMgBr, THF, rt, 1.5 h; ii) $ZnCl_2$,  , $Pd(PPh_3)_4$, rt-70 °C, 12.5 h;

55a or **55b**

iii) AcOH, MeOH, 80 °C, 3 h, 23-25%; b) $NaBH_4$, LiCl, THF, EtOH, rt, 16 h, 91%; c) $NaBH_4$, MeOH, 0 °C, 1 h, 98%; d) MeMgBr, THF, 0 °C-rt, 2 h, 52%.

Imidazoisoindoles **61**, **65**, **71**, **73**, **96**, **100**, **104**, **106** were synthesized by aldol condensation of substituted arylcarboxaldehydes with the corresponding ketones followed by intramolecular aza-Michael cyclization and ketone reduction with $NaBH_4$ (Scheme 15).

Scheme 15. Aldol condensation of 2-(1-trityl-1*H*-imidazol-4-yl)benzaldehydes with methyl ketones followed by intramolecular aza-Michael cyclization[a]

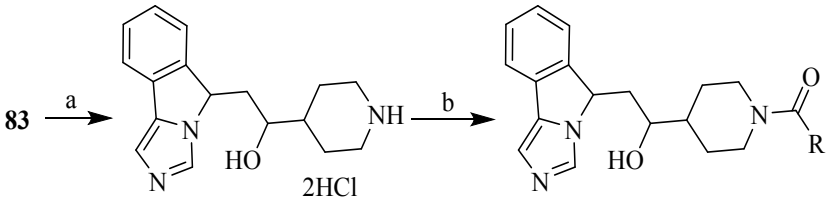


Reagents and conditions[a]: (a) i) NaOMe, THF/MeOH, rt, 3 h; ii) AcOH, MeOH, 80 ^{o}C, 3-10 h, 11-61%; (b) $NaBH_4$, MeOH, 0 ^{o}C-rt, 1 h, 15-98%.

Reagents		Products				
R^1	R^2	R^2	Cmpd	Yield (%)	Cmpd	Yield (%)
Cyclopentyl, **59a**	H, **50a**	H	**60**	11	**61**	91
Phenyl, **59b**	H, **50a**	H	**64**	45	**65**	73
4-Pyridyl, **59c**	H, **50a**	H	**70**	21	**71**	30
4-Tetrahydropyranyl, **59d**	H, **50a**	H	**72**	61	**73**	92
Cyclohexyl, **59e**	3-F, **50b**	6-F	**95**	50	**96**	96
Cyclohexyl, **59e**	5-F, **50c**	8-F	**99**	61	**100**	15
Cyclohexyl, **59e**	4-Cl, **50d**	7-Cl	**103**	43	**104**	98
Cyclohexyl, **59e**	5-Cl, **50e**	8-Cl	**105**	21	**106**	41

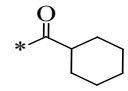
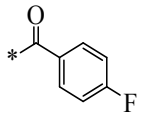
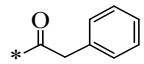
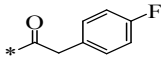
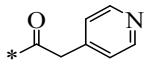
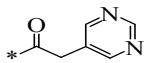
84 and **85** were synthesized by de-protecting **83** and **81** respectively. Piperidine amides (**88-93**) were synthesized by HATU coupling of **84** with substituted acids (Scheme 16).

Scheme 16. Amide synthesis by HATU coupling of **84** with substituted acids[a]



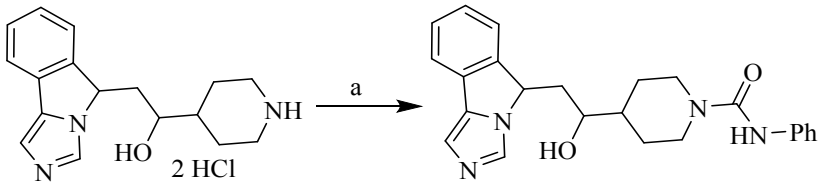
84 **88-93**

Reagents and conditions[a]: (a) TFA, HCl, CH_2Cl_2, rt, 2 h; (b) HATU, *i*-Pr_2NEt, DMF, rt, 18 h, 29-76%.

R	Cmpd	Yield (%)
	**88**	44
	**89**	29
	**90**	72
	**91**	80
	**92**	49
	**93**	76

Piperidinyl urea **94** was obtained by the reaction of **84** with phenyl isocyanate (Scheme 17).

Scheme 17. Synthesis of **94**[a]

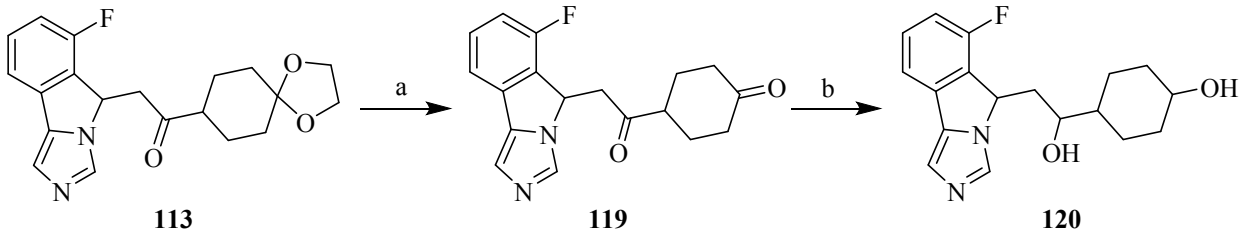


84 **94**

Reagents and conditions[a]: (a) PhNCO, *i*-Pr_2NEt, CH_2Cl_2, 0 ^oC, 0.5 h, 62%.

Compound **120** was prepared in two steps from **113**, involving ketal deprotection and ketone reduction (Scheme 18).

Scheme 18. Synthesis of **120**[a]



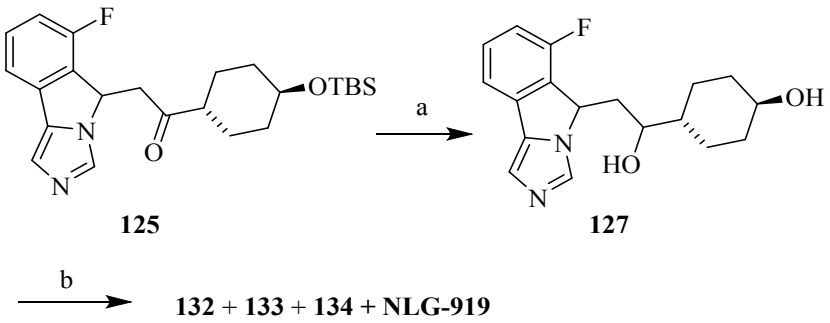
113 **119** **120**

Reagents and conditions[a]: (a) HCl, MeOH, 0-50 ^{o}C, 2.5 h, 99%; (b) $NaBH_4$, MeOH, 0 ^{o}C-rt, 1 h, 78%.

The diastereomeric mixture of **52** was separated by chiral SFC to provide isolated stereoisomers **121-124**. The absolute stereochemistry of **123** was assigned based on the single crystal X-ray data of the hydrobromide salt. Based on the ^{1}H-NMR resonance **121** was found to be the enantiomer of **123**. In both **121** and **123** the C5 proton exhibits a doublet of doublet δ 5.48 (dd, J = 10.6, 3.2 Hz, 1H). The stereochemistry of **122** and **124** (see supporting information for X-ray structure determination) was also assigned based on the X-ray crystallographic data of the hydrochloride and hydrobromide salts, respectively. Both **122** and **124** enantiomers exhibits a triplet δ 5.38 (t, J = 6.3 Hz, 1H) in the proton NMR for C5 proton (Figure 6).

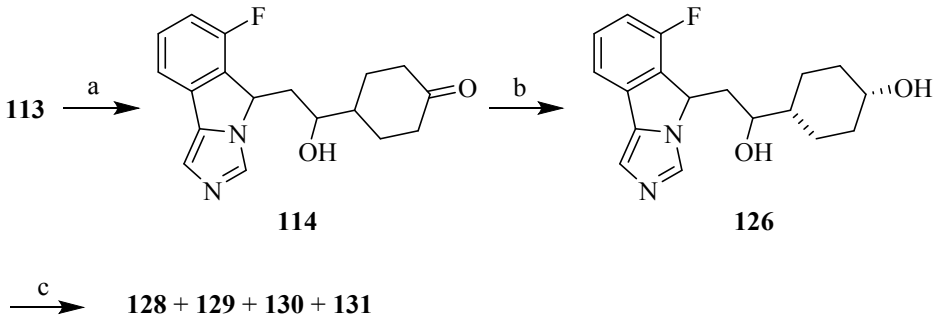
Sodium borohydride reduction of **125** and subsequent TBS deprotection yielded diastereomeric mixture **127**, which was separated by chiral SFC into 4-stereoisomers **132**, **133**, **134** and **NLG-919**. The absolute stereochemistry of **NLG-919** was determined based on the single crystal X-ray data. The C5 proton appears as a triplet in the case on **NLG-919** and **133**, δ 5.57 (t, J = 5.1 Hz, 1H) both having a relative *1,3-syn* conformation between the C5-H and C11-OH. Single enantiomers **132** and **134** exhibit a doublet of doublet splitting pattern at the C5-H by ^{1}H-NMR, δ 5.64 (dd, J = 10.8, 2.8 Hz, 1H). Based on the ^{1}H-NMR spectroscopic data and single crystal small molecule X-ray crystal data, **132** was assigned the C5 *S*-stereochemistry and **134** as C5 *R*-stereochemistry. **NLG-919** and **133** represents a pair of pseudoenantiomers, while **132** and **134** represents another pair (Scheme 19).

Scheme 19. Synthesis and chiral separation of **127**[a]



Reagents and conditions[a]: a) i) $NaBH_4$, MeOH, 0 ^oC-rt, 2 h; ii) 6N HCl, 50 ^oC, 45 min.; iii) $NaHCO_3$, 92%; b) Chiral SFC.

Scheme 20. Synthesis and chiral SFC separation of **126**[a]



Reagents and conditions[a]: (a) $NaBH_4$, HCl, MeOH, 0-50 ^oC, 2.5 h, 99%; (b) LS-Selectride, THF, -78 ^oC, 3 h, 86%; (c) Chiral SFC.

126 was synthesized by sodium borohydride reduction of C11 ketone **113** followed by deprotection of the C15 ketal to afford ketoalcohol **114**. Cyclohexanone **114** was subjected to LS-selectride reduction to afford *cis*-cyclohexanol **126** (Scheme 20). The diastereomeric mixture **126** was separated by chiral SFC to yield **128-131**. The stereochemistry of separated isomers of **126** was assigned based on their IDO1 inhibition activity and their ^1H NMR resonances. Compounds **130** and **131** are a pair of pseudoenantiomers, **128** and **129** are the other pair.

Conclusions

Structure-based drug design approach was used to evolve 5-phenyl-1*H*-imidazole into a novel class of heme coordinating imidazo[5,1-*a*]isoindole-based IDO1 inhibitors. Physicochemical properties were used as a guiding tool to optimize the lead molecule's ADME profile while minimizing CYP inhibition. Since IDO1 and CYP3A4 are heme-containing enzymes, a subtle balance was required to optimize the IDO1 potency while keeping CYP3A4 inhibition in check. Lowering the cLogP and placing a fluoro group at the 6-position of the imidazo[5,1-*a*]isoindole core improved CYP3A4 inhibition, metabolic clearance while maintaining IDO1 potency by maximizing H-bond interactions.

Experimental Section

All reagents and solvents were purchased from commercial sources. All commercial reagents and solvents were used as received without further purification. The reactions were monitored using analytical thin layer chromatography (TLC) with 0.25 mm EM Science silica gel plates (60F-254). The developed TLC plates were visualized by short wave UV light (254 nm) or immersion in potassium permanganate solution followed by heating on a hot plate. Flash chromatography was performed with Selecto Scientific silica gel, 32-63 μm particle sizes. All reactions were performed in flame or oven-dried glassware under a nitrogen atmosphere. All reactions were stirred magnetically at ambient temperature unless otherwise indicated. ^{1}H and ^{13}C NMR spectra were obtained with a Bruker DRX400, Varian VXR400 or VXR300. ^{1}H NMR spectra were reported in parts per million (δ) relative to TMS (0.0), DMSO-d_6 (2.50) or CD_3OD (4.80) as an internal reference. All ^{1}H NMR spectra were taken in $CDCl_3$ unless otherwise indicated. MS was conducted on Waters ACQUITY UPLC system with a QDa detector. High resolution mass spectrometry (HRMS) spectra for **NLG-919** was obtained using Agilent G6224A ESI-TOF mass

spectrometer. The purity of isolated stereoisomer compounds was determined on a Waters e2695 high performance liquid chromatography (HPLC) using a XBridge® 3.5 μM, 4.6 x 150 mm column (conditions provided in Supporting Information). Purity of all final target compounds was 95% or higher.

General procedure for the synthesis of 4-8 from the requisite acetophenone derivatives.

A solution of acetophenone derivative (6.03 mmol) in $CHCl_3$ (10 mL) was added to a refluxing suspension of (2.69 g, 12.06 mmol) of $CuBr_2$ in ethyl acetate (8 mL), the reaction was refluxed for 2h. After cooling to rt, the mixture was filtered through Celite bed and the solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (70 mL) and the organic layer was washed with 50% aq. $NaHCO_3$ (2 × 25 mL). The organic layer was washed with brine (25 mL), dried (Na_2SO_4), and evaporated in vacuo. The crude residue was passed through a short column and used as such for the next step. A solution of α-bromophenone derivative (1.34 mmol) was heated (170-180 °C) in formamide (10 mL) for 5-10 h. The solution was allowed to cool to rt and the mixture was diluted with saturated $NaHCO_3$ (20 mL) and the aqueous phase was extracted with EtOAc (3 x 50 mL). The combined organic layers were washed with water, brine, dried (Na_2SO_4) and concentrated in vacuo to afford the crude residue which was purified by flash column chromatography on silica gel to yield **4-8**.

General procedure for TosMIC chemistry

Conditions A: A solution of substituted benzaldehyde (3.19 mmol) in THF (3 mL) and ammonia solution (19.16 mmol, 2.0 M solution in EtOH) was stirred overnight at room temperature followed by the addition of TosMIC (3.19 mmol) and piperazine (4.79 mmol). After stirring for 36 h, the

solvent was evaporated and replaced with THF (10 mL) and continued stirring for another 24 h. The solvent was evaporated under reduced pressure and the crude was purified by column chromatography to afford **13**, **14** or **36**.

Conditions B:

To a stirred solution of NaO*t*-Bu (124.0 mg, 1.3 mmol) in THF (12 mL) at -40°C, was added a solution of 1-((isocyanomethyl)sulfonyl)-4-methylbenzene (390.0 mg, 2.0 mmol) in THF (6.0 mL). The solution was allowed to stir at -40 °C for 20 min and a solution of the substituted aldehyde (1.1 mmol) in THF (6.0 mL) was added while maintaining the temperature at -40 °C. The resulting mixture was allowed to stir for an additional 30 min and was poured into ice water (20 mL). The solution was neutralized with acetic acid (pH = 7) and the aqueous phase was extracted with dichloromethane (2 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford the crude product, which was filtered through a small plug of silica gel and used in next step.

To a stirred solution of the resulting crude formamide in THF (10 mL) at -5 °C was added Et_3N (1.39 mL, 10.0 mmol). The reaction mixture was cooled to -10 °C and $POCl_3$ (0.27 mL, 3.0 mmol) was added after 15 min. The solution was allowed to stir at -10 °C for an additional 30 min. The reaction mixture was poured into ice water (15 mL) and the aqueous layer was extracted with DCM (2 x 30 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was dissolved in MeOH (5 mL). The appropriate amine (2.0 mmol) was added and the reaction mixture was stirred for 12 h at 25 °C. The solvent was removed under reduced pressure and the resulting residue was purified by column chromatography on silica gel to afford **15**-**20**.

General procedure for the conversion of esters to amides (23-24): To the appropriate ester **16** or **17** (2.0 mmol) was added the amine (2.0 M in MeOH or EtOH, 10.0 mmol, 5.0 mL). The resulting solution was allowed to stir for 24 h at rt until completion of the reaction was observed (TLC). In some cases, complete conversion required heating at 50 °C. The solvent was removed under reduced pressure to afford the crude product, which was purified by column chromatography on silica gel using CH_2Cl_2/MeOH as the eluent to afford **23** or **24**.

General procedure for the alkylation of 2-(1*H*-Imidazol-4-yl)phenols (26-35, **47)**. To a stirred solution of 2-(1-trityl-*1H*-imidazol-4-yl)phenol (0.5 mmol) in anhydrous DMF (3 mL) at 0 °C was added NaH (36.0 mg, 0.75 mmol). The resulting suspension was allowed to stir for 10 min. To the resulting solution was added the appropriate alkylating reagent. After stirring overnight, the reaction mixture was carefully diluted with water and extracted with ethyl acetate (2 x 10 mL). The combined organic layers were washed with water, brine, and dried (Na_2SO_4). The solvent was removed under reduced pressure and the crude product was taken to next step without further purification. To a solution of the crude ether was added acetic acid (2.0 mL) and MeOH (4.0 mL). The solution was stirred at 80 °C for 2 h. The solution was allowed to cool to room temperature and the pH was adjusted to ~10 with 10% NaOH (aq). The aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic layer was washed with water, brine, and dried. The solvent was removed in vacuo to afford the crude residue, which was purified by flash column chromatography on silica gel to afford the desired products **26-35**, **47**.

General procedure for the synthesis of dimethyl (2-oxo)phosphonates 49(a-p). To a stirred solution of dimethyl methylphosphonate (3.14 g, 25.3 mmol) in 20 ml of anhydrous

tetrahydrofuran at -78 °C was added dropwise a solution of *n*-butyl lithium (10.13 mL, 25.3 mmol, 2.5 M in hexanes) under an atmosphere of N_2, and the mixture was stirred for 30 minutes. To this reaction mixture was added dropwise a solution of the appropriate commercially available methyl or ethyl ester or compound **49(n-o)-SM** or **49p-SM** (12.7 mmol) as a solution in THF (5 mL). After being stirred for 30 minutes, the reaction mixture was allowed to warm to 0 °C and stirred for 1 h. The solvent was distilled-off and the crude was diluted with saturated NH_4Cl (10 mL) and 10 ml of water. The mixture was extracted with ethyl acetate (2 x 40 mL). The combined ethyl acetate layers were washed with water (1 x 20 mL), brine (1 x 20 mL) and dried over anhydrous sodium sulfate. The solution was filtered and concentrated under reduced pressure to afford the crude product. The crude was purified by column chromatography to afford **49**(**a**-**p**).

General procedure for Suzuki Cross-Coupling for the synthesis of 50(a-f). A suspension of 4-iodo-1-trityl-1*H*-imidazole (6.88 mmol), the appropriate 2-formyl boronic acid derivative (10.31 mmol) and K_3PO_4 (20.63 mmol) in *N*,*N*-dimethylformamide (30 mL) and water (6 mL) was purged with nitrogen for 5 minutes, followed by the addition of $Pd(PPh_3)_4$ and the mixture was purged with nitrogen for another 5 minutes. The reaction mixture was stirred at 90 °C for 16 h under an atmosphere of N_2. The solution was allowed to cool and was filtered through a plug of Celite. The mixture was diluted with water (50 mL) and EtOAc (25 mL). The organic layer was collected and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic extracts were washed with water (2 x 25 mL), brine and dried (Na_2SO_4). The solution was filtered and the solvent was removed under reduced pressure to afford the crude product, chromatographic purification on silica gel afforded **50(a-f)**.

General procedure for the synthesis of 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanones by Horner-Wadsworth-Emmons reaction followed by cyclization. To a suspension of 95% NaH (17.4 mg, 0.7 mmol) in THF (3 mL) at 0 °C was added the appropriate phosphonate reagent **49**(**a**-**p**) (0.75 mmol) as a solution in THF (2 mL) and the mixture was stirred for 40 min. The appropriate benzaldehyde (**50a**, **50b** or **50f**) was added as a solution in THF (3 mL) drop wise over a period of 3 min. The reaction was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure and the crude was diluted with saturated NH_4Cl (10 mL) and water (10 mL). The aqueous layer was extracted with CH_2Cl_2 (2 x 20 mL) and the combined organic extracts were washed with brine (15 mL), dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude product. To the crude residue was added AcOH (1 mL), MeOH (3 mL) and the solution was stirred at 90 °C for 2 h. After cooling to rt, the solvent was distilled-off and the crude was stirred in a mixture of saturated K_2CO_3 (5 mL) and EtOAc (25 mL). The organic layer was separated and the aqueous layer was extracted with EtOAc (2 x 25 mL). The combined organic layers were washed with water, brine and dried (Na_2SO_4) and the solvent evaporated under reduced pressure. The crude residue was purified by flash column chromatography on silica gel to afford **51**, **62**, **66**, **68**, **74**, **76**, **78**, **80**, **82**, **86**, **97**, **107**, **109**, **111**, **113**, **115**, **117** or **125**.

General procedure for palladium-catalyzed Negishi cross-coupling of aryl iodides with 4-iodo-1-trityl-1*H*-imidazole. To a stirred solution of 4-iodo-1-trityl-1*H*-imidazole (218 mg, 0.5 mmol) in anhydrous THF (4 mL) at rt was added EtMgBr (1.0 M in THF, 0.5 mmol, 0.5 mL) dropwise, under an atmosphere of N_2. The resulting solution was allowed to stir for 90 min and anhydrous $ZnCl_2$ (0.5 mmol, 68.2 mg) was added. The resulting white suspension was allowed to

stir for 90 min and a solution of the appropriate aryl iodide **55a** or **55b** (0.5 mmol) in THF (1 mL) was added followed by the immediate addition of $Pd(PPh_3)_4$ (56 mg, 0.05 mmol). The reaction mixture was allowed to stir at 70 °C for 12 h under an atmosphere of N_2. After cooling to room temperature, the solution was diluted with CH_2Cl_2 (20 mL) and the organic layer was washed with an EDTA (aq) buffer (pH = 9) (2 x 5 mL) and brine. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. The crude residue was used in next step without further purification. To a solution of the crude imidazole from the previous step was added acetic acid (1.0 mL) and MeOH (4.0 mL). The solution was stirred at 80-90 °C for 3 h. The reaction mixture was allowed to cool to room temperature and the pH was adjusted to ~10 with saturated. K_2CO_3 (aq). The aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic extract was washed with water, brine and dried (Na_2SO_4). The solvent was removed in vacuo to afford the crude residue, which was purified by flash column chromatography on silica gel to afford **56** or **101**.

General procedure for the synthesis of 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanones by Aldol condensation of 2-(1-trityl-1*H*-imidazol-4-yl)benzaldehydes with methyl ketones followed by cyclization. To a solution of the appropriate aldehyde **50(a-e)** (0.97 mmol) and ketone **59(a-e)** (0.97 mmol) in anhydrous THF (5 mL) at rt was added NaOEt (1.25 mmol, 21 wt % solution in EtOH) and the yellow solution was allowed to stir 3 h at rt. The solvent was distilled-off and the crude was diluted with saturated NH_4Cl (10 mL) and the aqueous layer was extracted with dichloromethane (3 x 20 mL). The combined organic extracts were washed with brine, dried over Na_2SO_4 and the solvent evaporated under reduced pressure to afford the crude product. To the crude imidazole from the previous step was added acetic acid (1.0 mL) and MeOH (4.0 mL). The

solution was stirred at 90 °C for 3-10 h. The reaction mixture was allowed to cool to room temperature and the pH was adjusted to ~10 with saturated K_2CO_3 (aq). The aqueous phase was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with water, brine, and dried (Na_2SO_4). The solvent was removed in vacuo to afford the crude residue, which was purified by flash column chromatography on silica gel to afford **60**, **64**, **70**, **72**, **95**, **99**, **103** or **105**.

General procedure for the reduction of 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanones to 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanols. To a solution of the appropriate ketone (0.25 mmol) in MeOH (2 mL) at 0 °C, was added $NaBH_4$ (0.75 mmol) and the solution was allowed to stir for 1 h. The solvent was removed under reduced pressure and 2M HCl (2 mL) was added to the crude. The solution was allowed to stir for 10 min and was made basic by saturated K_2CO_3 solution. The aqueous layer was extracted with CH_2Cl_2 (3 x 5 mL). The combined organic layers were washed with brine, dried ($MgSO_4$) and concentrated under reduced pressure to afford the crude residue. The crude was purified by column chromatography using 1-10% MeOH:DCM gradient to afford **52**, **61**, **63**, **65**, **67**, **69**, **71**, **73**, **75**, **77**, **79**, **81**, **83**, **87**, **96**, **98**, **100**, **102**, **104**, **106**, **108**, **110**, **112**, **116**, **118** or **120**.

General procedure for the synthesis of 88-93 using HATU Coupling. To a vial containing 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-(piperidin-4-yl)ethan-1-ol dihydrochloride (0.25 mmol) in DMF (4 mL) was added the corresponding carboxylic acid (0.26 mmol), DIPEA (1.5 mmol) and HATU (0.28 mmol). The reaction mixture was stirred at rt for 18 h and poured into water (10 mL) and the aqueous layer was extracted with dichloromethane (2 x 20 mL). The combined organic

layers were washed with water (2 x 10 mL), dried over Na_2SO_4, and concentrated. The crude product was purified by flash column chromatography to afford **88-93**.

Chiral separation of 52. Column: RegisPack #783104, 250 mm x 4.6 mm, 5 µm, hexane/ethanol (90/10) + 0.1% DEA, 1.5 mL/min, RT = 4.30 (**121**), RT = 5.4 (**122**), RT= 6.52 (**123**), RT = 8.19 (**124**). The SFC separation was carried using RegisPack5 column. Co-solvent: IPA + 0.2% DEA, CO_2 flow rate: 3.0, CoSolvent flow rate: 1.0, 254 nm.

Chiral separation of 126. Column: RegisPack #783104, 250 mm x 4.6 mm, 5 µm, hexane/ethanol (90/10) + 0.1% DEA, 1.5 mL/min, RT = 11.68 (**128**), RT = 14.53 (**129**), RT = 15.75 (**130**), RT = 19.56 (**131**). The SFC separation was carried using RegisPack5 column (250 mm x 50 mm, 5 µm), Isopropanol/CO2 (27/73).

Chiral separation of 127. Column: RegisPack #783104, 250 mm x 4.6 mm, 5 µm, hexane/2-propanol (90/10) + 0.1% DEA, 1.5 mL/min, RT = 12.0 (**132**), RT = 13.88 (**133**), RT = 16.56 (**NLG-919**), RT = 22.37 (**134**). The SFC separation was carried in two step process, first separation was done using AD-H prep column, 250 x 50 mm, 5 µm, Isopropanol/CO_2 26/74 to separate **132** and **134**. For the second separation (*S,S*)-Whelk-O1 column (25 cm x 50 mm, 5 µm, Isopropanol + 0.5% DEA/CO_2 (27/73), 280 nm) was used to separate **NLG-919** and **133**.

4-Chloro-2-(1*H*-imidazol-5-yl)phenol (4). Yield: 48%. LCMS (ESI, *m/z*): 195.3 $[M+H]^+$.
^{1}H NMR (DMSO-d_6): δ 6.80 (d, *J* = 8.8 Hz, 1H), 7.02-7.04 (m, 1H), 7.72 (s, 1H), 7.81 (s, 1H), 7.89 (s, 1H).

2-(1*H*-Imidazol-5-yl)-4-methylphenol (5). Yield: 51%. LCMS (ESI, *m/z*): 175.3 $[M+H]^+$. ^1H NMR: δ 2.29 (s, 3H), 6.89 (d, *J* = 8.2 Hz, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.28 (s, 1H), 7.33 (s, 1H), 7.68 (s, 1H).

4-Bromo-2-(1*H*-imidazol-5-yl)phenol (6). Yield: 59%. LCMS (ESI, *m/z*): 239.2 $[M]^+$.^1H NMR: δ 6.85 (d, *J* = 8.8 Hz, 1H), 7.22 (dd, *J* = 8.8, 2.4 Hz, 1H), 7.34 (s, 1H), 7.56 (s, 1H), 7.71 (s, 1H), 8.87 (br s, 1H), 9.8 (br s, 1H).

4-Fluoro-2-(1*H*-imidazol-5-yl)phenol (7). Yield: 33%. LCMS (ESI, *m/z*): 179.3 $[M+H]^+$. ^1H NMR: δ 6.84-6.95 (m, 2H), 7.13-7.17 (m, 1H), 7.36 (s, 1H), 7.76 (s, 1H).

2,4-Difluoro-6-(1*H*-imidazol-5-yl)phenol (8). Yield: 61%. LCMS (ESI, *m/z*): 197.3 $[M+H]^+$. ^1H NMR: δ 6.98-7.06 (m, 1H), 7.38-7.42 (m, 1H), 7.87 (s, 1H), 7.97 (s, 1H).

4-Chloro-2-fluoro-6-(1*H*-imidazol-5-yl)phenol (11). To a stirred solution of 4-iodo-1-trityl-1*H*-imidazole (704.6 mg, 1.62 mmol) in anhydrous THF (8 mL) at rt was added EtMgBr (1.0 M in THF, 1.76 mL, 1.76 mmol) dropwise, under an atmosphere of N_2. The resulting solution was allowed to stir for 90 min and anhydrous $ZnCl_2$ (240.2 mg, 1.76 mmol) was added. The resulting white suspension was allowed to stir for 90 minutes and a solution of the aryl iodide (200 mg, 0.734 mmol) in THF (2 mL) was added followed by the immediate addition of $Pd(PPh_3)_4$ (84.8 mg, 0.073 mmol). The reaction mixture was allowed to stir at 70 °C for 12 h under an atmosphere of N_2. After cooling to room temperature, EDTA (aq) buffer (pH = 9), 10 mL was added and the product was extracted with CH_2Cl_2 (2x25 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to afford the crude product. To a solution of the crude product

was added acetic acid (2.0 mL) and MeOH (4.0 mL). The solution was stirred at 80 °C for 2 h. The reaction mixture was allowed to cool to room temperature and the pH was adjusted to 7-8 with saturated $NaHCO_3$ solution. The aqueous phase was extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with water, brine, and dried. The solvent was removed in vacuo to afford the crude residue, which was purified by flash column chromatography on silica gel to afford the desired product as off-white solid (57 mg, 37%). LCMS (ESI, *m/z*): 213.7 $[M+H]^+$. ^1H NMR (DMSO-d*6*): δ 7.21 (d, *J* = 8.0 Hz, 1H), 7.63 (s, 1H), 7.94 (s, 1H), 8.01 (s, 1H), 12.78 (br s, 1H), 12.88 (br s, 1H).

5-(2-Cyclohexylethoxy)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (12c-SM2). To a stirred solution of 5-hydroxy-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (3.89 mmol), 2-cyclohexylethan-1-ol (3.89 mmol) and triphenyl phosphine (4.28 mmol) in anhydrous THF (15 mL) at 0˚C was added DEAD (40% in toluene, 4.28 mmol, 1.95 mL) dropwise. The yellow solution was allowed to warm to room temperature and stirring was continued overnight. After evaporating the solvent under reduced pressure, the crude residue was dissolved in DCM (15 mL). The organic layer was washed with 10% NaOH (2 x 10 mL), water and brine. The organic phase was dried (Na_2SO_4), filtered and evaporated under reduced pressure. The crude residue was purified by column chromatography on silica gel (69%). ^1H NMR: δ 1.10-2.20 (m, 13H), 1.70 (s, 6H), 4.06 (t, *J* = 6.8 Hz, 2H), 6.47 (dd, *J* = 8.4, 0.8 Hz, 1H), 6.56 (d, *J* = 7.6 Hz, 1H), 6.47 (t, *J* = 7.6 Hz, 1H).

2-(2-Cyclohexylethoxy)-6-hydroxybenzaldehyde (12c). To a solution of 5-(2-cyclohexylethoxy)-2,2-dimethyl-4*H*-benzo[*d*][1,3]dioxin-4-one (290 mg, 0.952 mmol) in CH_2Cl_2 (6 mL) at -78 °C was added DIBAL-H (1.91 mmol, 1M in CH_2Cl_2). After stirring for 1.5 h at -78

°C the reaction was quenched by adding 1M HCl (2 mL) and MeOH (2 mL) and the reaction was allowed to warm to room temperature. Water (10 mL) was added and the aqueous phase was extracted with CH_2Cl_2 (3 x 30 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude residue which was purified by flash column chromatography on silica gel to afford the desired product (83 mg, 35%). ^{1}H NMR: δ 1.09 – 0.91 (m, 1H), 1.17-1.32 (m, 3H), 1.45-1.54 (m, 1H), 1.65-1.80 (m, 7H), 4.09 (t, *J* = 6.5 Hz, 1H), 6.37 (d, *J* = 8.3 Hz, 1H), 6.51 (d, *J* = 8.5 Hz, 1H), 10.37 (s, 1H), 11.98 (s, 1H).

2-Chloro-6-(1*H*-imidazol-5-yl)phenol (13). Yield: 41%. LCMS (ESI, *m/z*): 195.3 $[M+H]^+$. ^{1}H NMR: δ 6.79 (t, *J* = 7.8 Hz, 1H), 7.24 (doublet merged with $CHCl_3$, 1H), 7.40 (s, 2H), 7.76 (s, 1H), 9.36 (br s, 1H), 12.96 (br s, 1H).

2-Chloro-4-fluoro-6-(1*H*-imidazol-5-yl)phenol (14). Yield: 62%. LCMS (ESI, *m/z*): 213.3 $[M+H]^+$. ^{1}H NMR (MeOH-d_4): δ 6.95-6.99 (m, 1 H), 7.29-7.33 (m, 1H), 7.61 (s, 1H), 7.84 (s, 1H).

2-(5-Phenyl-1*H*-imidazole-1-yl)ethanol (15). Yield: 59%. LCMS (ESI, *m/z*): 189.2 $[M+H]^+$. ^{1}H NMR: δ 3.72 (t, *J* = 5.2 Hz, 2H), 4.01 (t, *J* = 5.2 Hz, 2H), 5.09 (br s, 1H), 6.83 (d, *J* = 0.8 Hz, 1H), 7.29-7.42 (m, 5H), 7.51 (d, *J* = 0.8 Hz, 1H).

Methyl 2-(5-phenyl-1*H*-imidazol-1-yl)acetate (16): Yield 36%. ^{1}H NMR: δ 3.7 (s, 3H), 4.66 (s, 2H), 7.07 (d, *J* = 1.2 Hz, 1H), 7.26-7.3 (m, 2H), 7.34-7.44 (m, 3H), 7.63 (d, *J* = 0.8 Hz, 1H).

Ethyl 3-(5-phenyl-1*H*-imidazol-1-yl)propanoate (17). Yield: 30%. ^{1}H NMR: δ 1.21 (t, *J* = 7.1 Hz, 3H), 2.57 (t, *J* = 6.9 Hz, 2H), 4.10 (q, *J* = 7.2 Hz, 2H), 4.30 (t, *J* = 6.8 Hz, 2H), 7.06 (s, 1H), 7.37-7.47 (m, 5H), 7.61 (s, 1H).

***N*-(2-(5-Phenyl-1*H*-imidazol-1-yl)ethyl)acetamide (18)**. Yield: 26%. LCMS (ESI, *m/z*): 230.3 $[M+H]^+$. ^{1}H NMR: δ 1.85 (s, 3H), 3.32-3.40 (m, 2H), 4.17 (t, *J* = 7.6 Hz, 2H), 7.02 (s, 1H), 7.33-7.45 (m, 5H), 7.50 (s, 1H).

1-(3,3-Dimethylbutyl)-5-(2-methoxyphenyl)-1*H*-imidazole (19). Yield: 45%. LCMS (ESI, *m/z*): 259.3 $[M+H]^+$. ^{1}H NMR: δ 0.78 (s, 9H), 1.42-1.54 (m, 2H), 3.75-3.85 (m, 5H), 6.92-7.50 (m, 3H), 7.20-7.29 (m, 1H), 7.38 (t, *J* = 7.5 Hz, 1H), 7.54 (s, 1H).

5-(2-(Benzyloxy)-5-chlorophenyl)-1-cyclohexyl-1*H*-imidazole (20). Yield: 95%. LCMS (ESI, *m/z*): 366.4 $[M+H]^+$. ^{1}H NMR: δ 1.13-1.19 (m, 3H), 1.44-1.52 (m, 2H), 1.63-1.85 (m, 5H), 3.64-3.70 (m, 1H), 5.01 (s, 2H), 6.96 (s, 1H), 7.21 (d, *J* = 6.0 Hz, 2H), 7.25 (d, *J* = 2.5 Hz, 1H), 7.29-7.33 (m, 4H), 7.61 (s, 1H).

2-(1-(3,3-Dimethylbutyl)-1*H*-imidazol-5-yl)phenol (21). A solution of 1-(3,3-dimethylbutyl)-5-(2-methoxyphenyl)-1*H*-imidazole 20 (150 mg, 0.580 mmol) in 48% HBr (3 mL) was stirred at 110 °C for 16 h. The solution was allowed to cool to rt and was poured into saturated $NaHCO_3$ (10 mL). The aqueous phase was extracted with ethyl acetate (2 x 30 mL). The combined organic layers were dried over sodium sulfate and concentrated under reduced pressure to afford the crude residue, which was purified by column chromatography on silica gel to afford the desired product

(110 mg, 77%). LCMS (ESI, *m/z*): 245.4 $[M+H]^+$. ^1H NMR: δ 1.40-1.53 (m, 2H), 3.93-4.10 (m 2H), 6.91 (t, *J* = 7.2 Hz, 1H), 7.02-7.15 (m, 2H), 7.27-7.45 (m 2H), 8.70 (s, 1H).

4-Chloro-2-(1-cyclohexyl-1*H*-imidazol-5-yl)phenol (22). To a solution of 5-(2-(benzyloxy)-5-chlorophenyl)-1-cyclohexyl-1*H*-imidazole **20** (161 mg, 0.438 mmol) in MeOH (5 mL) at rt, was added HCl (0.351 mL, 1.25 M in MeOH) followed by 10% Pd/C (0.043 mmol) and the mixture was evacuated and purged with H_2 balloon. The solution was stirred under a positive pressure of H_2 balloon overnight. After purging the reaction mixture with nitrogen, the reaction mixture was filtered through a celite plug and the solvent was evaporated under reduced pressure to afford the crude residue. The crude residue was basified with saturated K_2CO_3 solution and the product was extracted with EtOAc (3 x 30 mL). The combined organic extract was dried (Na_2SO_4) and concentrated under reduced pressure to afford crude. Chromatographic purification afforded the desired product as off-white solid (92 mg, 75.7%). LCMS (ESI, *m/z*): 277.3 $[M+H]^+$. ^1H NMR (DMSO-d*6*): δ 1.13 -1.20 (m, 3H), 1.29-1.90 (m, 7H), 3.61-3.68 (m, 1H), 6.79 (s, 1H), 6.95 (d, *J* = 8.6 Hz, 1H), 7.15 (d, *J* = 1.8 Hz, 1H), 7.29 (d, *J* = 8.5 Hz, 1H), 7.85 (s, 1H), 10.1 (br s, 1H).

***N*-Methyl-2-(5-phenyl-1*H*-imidazol-1-yl)acetamide (23)**. Yield: 54%. LCMS (ESI, *m/z*): 216.3 $[M+H]^+$. ^1H NMR: δ 2.66 (s, 3H), 4.67 (s, 2H), 6.99 (s, 1H), 7.3-7.45 (m, 5H), 7.73 (s, 1H).

***N*-Methyl-3-(5-phenyl-1*H*-imidazol-1-yl)propanamide (24)**. Yield: 59%. LCMS (ESI, *m/z*): 230.3 $[M+H]^+$. ^1H NMR: δ 2.39-2.44 (t, *J* = 6.7 Hz, 2H), 2.73-2.74 (d, *J* = 4.8 Hz, 3H), 4.36 (t, *J* = 6.7 Hz, 2H), 5.96 (br s, 1H), 7.09 (br s, 1H), 7.27-7.47 (m, 5H), 7.61 (br s, 1H).

2-(1-Trityl-1*H*-imidazol-4-yl)phenol (25). To a solution of **3** (2.0 g, 12.49 mmol) in anhydrous DMF (20 mL) at rt was added trimethylamine (1.58 g, 15.61 mmol). After stirring for 10 minutes, a solution of chlorotriphenylmethane (3.48 g, 12.49 mmol) in DMF (15 mL) was added dropwise over a period of 2-3 minutes and continued stirring for 5 h. The reaction mixture was poured into water (80 mL), the solid was filtered-off and washed with water (2 x 50 mL). The crude solid was dissolved in CH_2Cl_2 (50 mL), the organic layer was washed with brine, the solvent was evaporated under reduced pressure to afford **25** as white solid (4.82 g, 95%). ^{1}H NMR: δ 6.79 (t, *J* = 7.5 Hz, 1H), 6.98–7.00 (m, 1H), 7.08 –7.40 (m, 18H), 7.54 (s, 1H), 12.22 (br s, 1H).

5-(2-(Benzyloxy)phenyl)-1*H*-imidazole (26). Yield: 65%. LCMS (ESI, *m/z*): 251.3 $[M+H]^+$. ^{1}H NMR: δ 5.14 (s, 2H), 7.00-7.03 (m, 2H), 7.19 (dt, *J* = 8.2, 2.3 Hz, 1H), 7.33-7.44 (m, 5H), 7.51 (s, 1H), 7.56 (s, 1H), 7.90 (d, *J* = 7.2 Hz, 1H).

5-(2-Phenethoxyphenyl)-1*H*-imidazole (27). Yield: 32%. LCMS (ESI, *m/z*): 265.3 $[M+H]^+$. ^{1}H NMR: δ 3.20 (t, *J* = 6.4 Hz, 2H), 4.40 (t, *J* = 6.4 Hz, 2H), 6.97-7.01 (m, 2H), 7.18-7.22 (m, 1H), 7.28-7.39 (m, 7H), 7.73 (dd, *J* = 8.4, 1.6 Hz, 1H).

5-(2-(3-Phenylpropoxy)phenyl)-1*H*-imidazole (28). Yield: 59%. LCMS (ESI, *m/z*): 279.3 $[M+H]^+$. ^{1}H NMR: δ 2.17-2.24 (m, 2H), 2.82 (t, *J* = 7.6 Hz, 2H), 4.09 (t, *J* = 6.4 Hz, 2H), 6.90 (d, *J* = 8.0 Hz, 1H), 6.97-7.01 (m, 1H), 7.16-7.19 (m, 4H), 7.24-7.29 (m, 2H), 7.59 (s, 1H), 7.65 (s, 1H), 7.89 (s, 1H).

5-(2-(3,3-Dimethylbutoxy)phenyl)-1*H*-imidazole (29). Yield: 55%. LCMS (ESI, *m/z*): 245.4 $[M+H]^+$. ^1H NMR: δ 1.01 (s, 9H), 1.85 (t, *J* = 7.6 Hz, 2H), 4.15 (t, *J* = 7.6 Hz, 2H), 6.96-7.02 (m, 2H), 7.19-7.23 (m, 1H), 7.58 (s, 1H), 7.69 (s, 1H), 7.87 (d, *J* = 7.2 Hz, 1H).

4-(2-(Isopentyloxy)phenyl)-1*H*-imidazole (30). Yield: 21%. LCMS (ESI, *m/z*): 231.3 $[M+H]^+$. ^1H NMR: δ 0.99 (d, *J* = 6.2 Hz, 6H), 1.79-1.84 (m, 2H), 4.13 (t, *J* = 6.6 Hz, 2H), 6.96-7.04 (m, 2H), 7.19 (d, *J* = 1.6 Hz, 1H), 7.55 (s, 1H), 7.69 (s, 1H), 7.84 (d, *J* = 6.4 Hz, 1H).

4-(2-(2-Cyclopropylethoxy)phenyl)-1*H*-imidazole (31). Yield: 72%. LCMS (ESI, *m/z*): 229.3 $[M+H]^+$. ^1H NMR: δ 0.13-0.19 (m, 2H), 0.50-0.57 (m, 2H), 0.84-0.89 (m, 1H), 1.77-1.84 (m, 2H), 4.16-4.20 (t, *J* = 6.6 Hz, 2H), 6.98-7.04 (m, 2H), 7.19-7.26 (m, 1H), 7.58 (s, 1H), 7.71 (s, 1H), 7.86 (d, *J* = 7.8 Hz, 2H).

4-(2-(2-Cyclopentylethoxy)phenyl)-1*H*-imidazole (32).Yield: 69%. LCMS (ESI, *m/z*): 257.3 $[M+H]^+$. ^1H NMR: δ 1.10-1.25 (m, 2H),1.47-1.70 (m, 4H), 1.75-2.00 (m, 5H), 4.09 (t, *J* = 6.4 Hz, 2H), 6.90-7.10 (m, 2H), 7.19 (td, *J* = 7.6, 1.6 Hz, 1H), 7.53 (s, 1H), 7.69 (s, 1H), 8.11 (dd, *J* = 8.0, 1.6 Hz, 1H), 9.54 (s, 1H).

4-(2-(2-Cyclohexylethoxy)phenyl)-1*H*-imidazole (33). Yield: 73%. LCMS (ESI, *m/z*): 271.3 $[M+H]^+$. ^1H NMR: δ 1.02-1.15 (m, 2H), 1.20-1.27 (m, 3H), 1.47-1.49 (m, 1H), 1.50-1.84 (m, 7H), 4.15 (t, *J* = 6.8 Hz, 2H), 6.97 (d, *J* = 8.3 Hz, 1H), 7.02 (d, *J* = 7.6 Hz, 1H), 7.20 (d, *J* = 7.2 Hz, 1H), 7.54 (s, 1H), 7.68 (s, 1H), 7.82 (s, 1H).

5-(2-(2-(1*H*-Imidazol-4-yl)phenoxy)ethyl)pyrimidine (34). Yield 42%. LCMS (ESI, *m/z*): 267.3 $[M+H]^+$. ^1H NMR (MeOH-d_4): δ 3.24 (t, *J* = 6.0 Hz, 2H), 4.44 (t, *J* = 6.0 Hz, 2H), 7.02 (t, *J* = 7.5 Hz, 1H), 7.09 (t, *J* = 8.4 Hz, 1H), 7.21-7.26 (m, 1H), 7.30 (s, 2H), 7.71 (s, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 8.74 (s, 1H), 8.99 (s, 1H).

1-(2-(2-(1*H*-Imidazol-4-yl)phenoxy)ethyl)-1H-pyrazole (35). Yield: 15%. LCMS (ESI, *m/z*): 255.3 $[M+H]^+$. ^1H NMR: δ 4.35 (t, *J* = 4.5 Hz, 2H), 4.64 (t, *J* = 4.5 Hz, 2H), 6.33 (s, 1H), 6.85 (d, *J* = 8.2 Hz, 1H), 7.01 (t, *J* = 7.5 Hz, 1H), 7.45 (s, 1H), 7.49 (d, *J* = 1.6 Hz, 1H), 7.62 (s, 1H), 7.70 (d, *J* = 7.6 Hz, 1H), 7.77 (s, 1H).

3-(2-Cyclohexylethoxy)-2-(1*H*-imidazol-4-yl)phenol (36). Yield: 46%. LCMS (ESI, *m/z*): 287.3 $[M+H]^+$. ^1H NMR: δ 0.90-1.05 (m, 2H), 1.10-1.30 (m, 3H), 1.42-1.58 (m, 1H), 1.66-1.82 (m, 7H), 4.09 (t, *J* = 6.8 Hz, 2H), 6.45 (d, *J* = 8.4 Hz, 1H), 7.06 (t, *J* = 8.4 Hz, 1H), 7.66 (s, 1H), 7.69 (s, 1H), 9.69 (br s, 1H).

5*H*-Imidazo[5,1-*a*]isoindole (37). Synthesized as per the literature.[32] LCMS (ESI, *m/z*): 157 $[M+H]^+$. ^1H NMR: δ 7.13 (s, 1H), 7.22-7.26 (m, 1H), 7.34-7.39 (m, 2H), 7.53 (d, *J* = 7.4 Hz, 1H), 7.71 (s, 1H).

1-(2-Iodo-3-methoxybenzyl)-1*H*-imidazole (39). To a solution of (2-iodo-3-methoxyphenyl)methanol[29] (0.350 g, 1.33 mmol), imidazole (0.181 g, 2.65 mmol) and triphenyl phosphine (0.382 g, 1.46 mmol) in tetrahydrofuran (5 mL) at 0 °C was added diethyl azodicarboxylate (40% solution in toluene; 0.664 mL). The yellow solution was allowed to warm

to room temperature and stirred at 60 °C overnight. After evaporating the solvent under reduced pressure, the crude was purified by column chromatography to yield **39** (179 mg, 43%). ^{1}H NMR: δ 3.90 (s, 3H), 5.19 (s, 2H), 6.43 (d, *J* = 5.7 Hz, 1H), 6.77 (d, *J* = 6.1 Hz, 1H), 6.93 (s, l H), 7.10 (s, 1H), 7.24 (t, *J* = 6.0 Hz, 1H).

9-Methoxy-5*H*-imidazo[5,1-*a*]isoindole (40). A mixture of l-(2-iodo-3-methoxybenzyl)-1*H*-imidazole (**39)** (0.180 g, 0.573 mmol), potassium carbonate (1.146 mmol) in dimethyl sulfoxide was purged with nitrogen for 5 min to which triphenylphosphine (0.057 mmol) and $Pd(OAC)_2$ (0.0286 mmol) were added. The mixture was stirred under nitrogen atmosphere at 110 °C for 36 h. After cooling to room temperature, the reaction mixture was diluted with water (15 mL) and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford crude product. Chromatographic purification afforded **40** (39.8 mg, 37%). ^{1}H NMR: δ 3.96 (s, 3H), 5.00 (s, 2H), 6.88 (d, *J* = 8.3 Hz, 1H), 6.99 (d, *J* = 7.5 Hz, 1H), 7.17 (s, 1 H), 7.22 (t, *J* = 8.0 Hz, 1H), 7.69 (s, 1 H).

5*H*-Imidazo[5,1-*a*]isoindol-9-ol (**41**). A solution of 9-methoxy-5H-imidazo[5,1-a]isoindole (**40)** (37 mg, 0.198 mmol) in hydrobromic acid (2 mL, 48% aq.) was stirred at 100 °C overnight (14 h). After cooling to room temperature, the excess of hydrobromic acid was distilled off and the crude was diluted with 10% aqueous NaOH solution (15 mL) and washed with toluene (10 mL) to remove unreacted methyl ether. The aqueous layer was acidified with HCl and then basified with saturated K_2CO_3 solution. The product was extracted with EtOAc (3 x 15 mL). The combined organic extract was dried over sodium sulfate and concentrated under reduced pressure to afford **41** (14 mg, 68% brsm). LCMS (ESI, *m/z*): 173.2 $[M+H]^+$. ^{1}H NMR (MeOH-d_4): δ 5.07 (s, 2H),

6.80 (d, *J* = 8.1 Hz, 1H), 6.94 (dd, *J* = 6.8, 0.72 Hz, 1H), 7.0 (s, 1H), 7.12 (t, *J* = 7.8 Hz, 1H), 7.80 (s, 1H).

2-(1-(2-Hydroxyethyl)-1*H*-imidazol-5-yl)phenol (43): To a stirred solution of 2-amino-1-ethanol (282.6 mg, 4.63 mmol) in MeOH (5 mL) was added a solution of salicylaldehyde (565 mg, 4.63 mmol) in MeOH (5 mL). The reaction mixture was heated at 40 °C for 1 h and was concentrated to a give the crude amine as a yellow liquid, which was used in the next step immediately. To a solution of the crude imine in DME/MeOH (15 mL, 4:1) was added TosMIC (1.08 g, 5.55 mmol) and K_2CO_3 (1.41 g, 10.17 mmol). The solution was allowed to stir at room temperature for 3 days. The solvent was evaporated under reduced pressure and the crude product was purified by flash column chromatography on silica gel to afford **43** (438 mg, 46% yield. ^{1}H NMR: δ 3.32 (s, 1H), 3.57 (t, *J* = 5.6 Hz, 2H), 4.01 (t, *J* = 5.6 Hz, 2H), 6.86 (d, *J* = 5.6 Hz, 2H), 6.89 (s, 1H), 7.16 (dd, *J* = 8.0, 1.6 Hz, 1H), 7.24 (td, *J* = 8.0, 2.0 Hz, 1H), 7.75 (1H).

5,6-Dihydrobenzo[*f*]imidazo[1,5-*d*][1,4]oxazepine (44). To a stirred solution of 2-(1-(2-hydroxyethyl)-1*H*-imidazol-5-yl)phenol (**43**) (100.0 mg, 0.490 mmol) and PPh_3 (154.1 mg, 0.587 mmol) in THF (4 mL) at 0 °C, was added DEAD (0.22 mL, 40% solution in toluene, 0.75 mmol). The resulting yellow solution was allowed to warm to rt and stirred overnight. The solvent was removed under reduced pressure and the crude residue was purified by flash column chromatography on silica gel to afford **44** (76 mg, 83%). ^{1}H NMR: δ 4.35-4.45 (m, 4H), 6.94-7.05 (m, 2H), 7.11-7.19 (m, 1H), 7.43 (d, *J* = 0.8 Hz, 1H), 7.49 (s, 1H), 7.67 (dd, *J* = 8.0, 1.6 Hz, 1H).

Imidazo[5,1-*a*]isoquinoline (45). Synthesized as per the literature in 31% yield.[30] LCMS (ESI, *m/z*): 169 $[M+H]^+$. ^1H NMR: δ 6.78 (d, *J* = 7.4 Hz, 1H), 7.41 (t, *J* = 7.6 Hz, 1H), 7.50 (t, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 7.8 Hz, 1H), 7.73 (d, *J* = 7.3 Hz, 1H), 7.81 (s, 1H), 8.01 (d, *J* = 7.9 Hz, 1H), 8.07 (s, 1H).

5-(2-Cyclohexylethyl)-5*H*-imidazo[5,1-*a*]isoindole (46). To a solution of **37** (858 mg, 5.49 mmol) in anhydrous THF (10 mL) at -40°C was added *n*BuLi (2.20 mL, 5.49 mmol, 2.5 M solution hexanes). After stirring for 1.0 h at -40 °C, (2-bromoethyl)cyclohexane (700 mg, 3.66 mmol) was added and the reaction was allowed to warm to -30 °C and stirred overnight. The reaction was quenched by adding saturated NH_4Cl (10 mL) and water (20 mL), the product was extracted with CH_2Cl_2 (3 x 35 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure to afford crude mixture, the crude mixture was purified by Combi-flash chromatography to yield **46** (0.780 g, 80%). LCMS (ESI, *m/z*): 267.4 $[M+H]^+$. ^1H NMR: δ 0.65–0.81 (m, 2H), 0.92–1.16 (m, 6H), 1.46–1.63 (m, 5H), 1.80 (ddt, *J* = 11.3, 8.9, 6.2 Hz, 1H), 1.95–2.09 (m, 1H), 4.98 (t, *J* = 5.7 Hz, 1H), 7.08 (s, 1H), 7.09–7.15 (m, 1H), 7.18–7.25 (m, 2H), 7.39 (d, *J* = 7.5 Hz, 1H), 7.58 (s, 1H).

2-(2-(1*H*-Imidazol-4-yl)phenoxy)-1-cyclohexylethan-1-one (47). Yield: 62%. LCMS (ESI, *m/z*): 385.3 $[M+H]^+$. ^1H NMR: δ 1.28-1.39 (m, 3H), 1.46-1.55 (m, 2H), 1.74 (d, *J* = 9.6 Hz, 1H), 1.84-1.93 (m, 4H), 2.49-2.54 (m, 1H), 4.91 (s, 2H), 6.87 (d, *J* = 8.0 Hz, 1H), 7.05 (dt, *J* = 7.6, 0.8 Hz, 1H), 7.20 (dt, *J* = 7.2, 1.6 Hz, 1H), 7.58 (s, 1H), 7.77 (d, *J* = 7.2 Hz, 1H), 7.80 (s, 1H).

2-(2-(1*H*-Imidazol-4-yl)phenoxy)-1-cyclohexylethan-1-ol (48). To a mixture 2-(2-(1H-imidazol-4-yl)phenoxy)-l -cyclohexylethanone **47** (130 mg, 0.457 mmol) in MeOH (4 mL) at 0 °C, was added $NaBH_4$ (52 mg, 1.37 mmol) and the solution was allowed to stir at room temperature for 1 h. The solvent was distilled-off and the crude was acidified with dil HCl (2 N) and again basified by saturated aqueous $NaHCO_3$ solution, the product was extracted with EtOAc (3 x 15 mL). The combined organic extracts were washed with brine, dried, and concentrated under reduced pressure to afford **48** (118 mg, 90%). LCMS (ESI, *m/z*): 287.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 1.04-1.21 (m, 5H), 1.49-1.50 (m, 1H), 1.61-1.68 (m, 4H), 1.85 (d, *J* = 12.4 Hz, 1H), 3.62-3.65 (m, 1H), 3.96 (dd, *J* = 9.6, 6.4 Hz, 1H), 4.07 (dd, *J* = 10, 3.2 Hz, 1H), 4.97 (br s, 1H), 6.93 (t, *J* = 7.2 Hz, 1H), 7.03 (d, *J* = 7.6 Hz, 1H), 7.12 (d, *J* = 7.2 Hz, 1H), 7.67 (s, 1H), 7.70 (s, 1 H), 8.03 (s, 1H), 12.01 (br s, 1H).

Methyl (1*r*,4*r*)-4-benzamidocyclohexane-1-carboxylate (49n-SM). To a suspension of (1*r*, 4*r*)-methyl 4-aminocyclohexanecarboxylate hydrochloride (0.63 g, 3.26 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added diisopropylethylamine (1.71 mL, 9.79 mmol) and the suspension was stirred for 10 minutes. Benzoyl chloride (0.45 mL, 3.92 mmol) was added dropwise and the clear solution was allowed to warm to rt and stirred overnight. The reaction was diluted with water (15 mL) and CH_2Cl_2 (15 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 25 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduce pressure to afford the crude product. Chromatographic purification afforded **49n-SM** as a white solid (200 mg, 23%). ^{1}H NMR (MeOH-d_4) δ 1.46 (q, *J* = 11.5 Hz, 2H), 1.60 (q, *J* = 12.0 Hz, 2H), 2.09 (d, *J* = 11.2 Hz, 4H), 2.37 (t, *J* = 12.0 Hz, 1H), 3.71 (s, 3H), 3.90 (t, *J* = 11.4 Hz, 1H), 7.46–7.57 (m, 3H), 7.83 (d, *J* = 7.1 Hz, 2H).

Methyl (1*s*,4*s*)-4-benzamidocyclohexane-1-carboxylate (49o-SM). To a suspension of (1*s*, 4*s*)-methyl 4-aminocyclohexanecarboxylate hydrochloride (0.63 g, 3.26 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added diisopropylethylamine (1.71 mL, 9.79 mmol) and the suspension was stirred for 10 minutes. Benzoyl chloride (0.45 mL, 3.92 mmol) was added dropwise and the clear solution was allowed to warm to rt and stirred overnight. The reaction was diluted with water (15 mL) and CH_2Cl_2 (15 mL), the organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (2 x 25 mL). The combined organic extract was dried over Na_2SO_4 and concentrated under reduced pressure to afford the crude. Chromatographic purification afforded **49o-SM** (745 mg, 87%). ^{1}H NMR δ 1.70–1.73 (m, 2H), 1.76–1.90 (m, 4H), 1.95–2.06 (m, 2H), 2.55–2.61 (m, 1H), 3.72 (s, 3H), 4.14–4.20 (m, 1H), 6.14 (d, J = 6.0 Hz, 1H), 7.43–7.47 (m, 2H), 7.49–7.51 (m, 1H), 7.76–7.78 (m, 2H).

Dimethyl (2-cyclohexyl-2-oxoethyl)phosphonate (49a). Yield: 89%. ^{1}H NMR: δ 1.14–1.38 (m, 5H), 1.62–1.94 (m, 5H), 2.52–2.58 (m, 1H), 3.12 (d, J = 22.5 Hz, 2H), 3.76 (s, 3H), 3.79 (s, 3H).

Dimethyl 3-cyclohexyl-2-oxopropylphosphonate (49b). Yield: 70%. ^{1}H NMR: δ 0.60–1.15 (m, 5H), 1.35–1.71 (m, 6H), 2.28 (d, J = 8.8 Hz, 2H), 2.86 (d, J = 22.8 Hz, 2H), 3.55 (s, 3H), 3.59 (s, 3H).

Dimethyl (2-oxo-2-(pyridin-2-yl)ethyl)phosphonate (49c). Yield: 56%. ^{1}H NMR: δ 3.69–3.77 (m, 6H), 3.96–4.04 (m, 2H), 7.45–7.48 (m, 1H), 7.80–7.85 (m, 1H), 8.03–8.09 (m, 1H), 8.66–8.69 (m, 1H).

Dimethyl (2-oxo-2-(pyridin-3-yl)ethyl)phosphonate (49d). Yield: 65%. ^{1}H NMR δ 3.60–3.80 (m, 8H), 7.41–7.45 (m, 1H), 8.26–8.29 (m, 1H), 8.78–8.79 (m, 1H), 9.18 (m, 1H).

Dimethyl (2-oxo-2-(thiazol-5-yl)ethyl)phosphonate (49e). Yield: 18%. ^{1}H NMR δ 3.58 (d, J = 22.9 Hz, 2H), 3.74 (s, 3H), 3.79 (s, 3H), 8.52 (s, 1H), 9.04 (s, 1H).

Dimethyl (2-(1-methyl-1H-imidazol-4-yl)-2-oxoethyl)phosphonate (49f). Yield: 29%. ^{1}H NMR δ 7.63 (s, 1H), 7.44 (s, 1H), 3.79 (s, 3H), 3.75 (s, 3H), 3.71 (s, 3H), 3.68 (d, J = 22.5 Hz, 2H).

Dimethyl (2-(1-methyl-1H-pyrazol-4-yl)-2-oxoethyl)phosphonate (49g). Yield: 78.5%. ^{1}H NMR: δ 2.41 (s, 3H), 3.46 (d, J = 22.5 Hz, 2H), 3.78 (s, 3H), 3.81 (s, 3H), 6.21 (dq, J = 3.5, 0.9 Hz, 1H), 7.24 (dd, J = 3.6, 0.7 Hz, 1H).

***tert*-Butyl 3-(2-(dimethoxyphosphoryl)acetyl)azetidine-1-carboxylate (49h)**. Yield: 99%. ^{1}H NMR: δ 1.43 (s, 9H), 3.11 (d, J = 22.9 Hz, 2H), 3.68-3.82 (m, 7H), 3.81– 4.12 (m, 4H).

***tert*-Butyl 4-(2-(dimethoxyphosphoryl)acetyl)piperidine-1-carboxylate (49i)**. Yield: 61%. ^{1}H NMR: δ 1.44 (s, 9H), 1.47–1.57 (m, 2H), 1.65 (s, 2H), 1.85 (d, J = 13.2 Hz, 2H), 2.68–2.84 (m, 3H), 3.13 (d, J = 22.7 Hz, 2H), 3.77 (s, 3H), 3.80 (s, 3H).

Dimethyl (2-(1-acetylpiperidin-4-yl)-2-oxoethyl)phosphonate (**49j**). Yield: 64%. ^{1}H NMR: δ 1.44–1.69 (m, 2H), 1.83–1.89 (m, 2H), 2.07 (s, 3H), 2.66–2.73 (m, 1H), 2.79–2.85 (m, 1H), 3.03–3.22 (m, 3H), 3.75 (s, 3H), 3.78 (s, 3H), 3.78–3.83 (m, 1H), 4.50-4.55 (m, 1H).

Dimethyl 2-(4,4-difluorocyclohexyl)-2-oxoethylphosphonate (49k). Yield: 82%. ^{1}H NMR δ 1.72–1.81 (m, 4H), 1.96–1.98 (m, 2H), 2.11–2.13 (m, 2H), 2.68–2.70 (m, 1H), 3.14 (d, J = 22.4 Hz, 2H), 3.77 (s, 3H), 3.80 (s, 3H).

Methyl (1*r*,4*r*)-4-(2-(dimethoxyphosphoryl)acetyl)cyclohexane-1-carboxylate (49l). Yield: 70%. ^{1}H NMR: δ 1.31–1.53 (m, 4H), 2.00–2.20 (m, 4H), 2.23–2.31 (m, 1H), 2.53–2.61 (m, 1H), 3.13 (d, J = 22.6 Hz, 2H), 3.67 (s, 3H), 3.77 (s, 3H), 3.80 (s, 3H).

Dimethyl 2-oxo-2-(1,4-dioxaspiro[4.5]decan-8-yl)ethylphosphonate (49m). Yield: 72%. ^{1}H NMR δ 1.42–1.94 (m, 8H), 2.53–2.62 (m, 1H), 3.11 (d, J = 22.6 Hz, 2H), 3.73-3.75 (m, 3H), 3.76-3.78 (m, 3H), 3.90–3.92 (m, 4H).

Dimethyl (2-((1*r*,4*r*)-4-benzamidocyclohexyl)-2-oxoethyl)phosphonate (49n). Yield: 54%. ^{1}H NMR**:** δ 1.29 (dq, J = 3.1, 12.1 Hz, 2H), 1.54 (dq, J = 11.5, 3.0 Hz, 2H), 2.04 (d, J = 12.9 Hz, 2H), 2.12 (dd, J = 12.6, 3.0 Hz, 2H), 2.60 (tt, J = 12.0, 3.4 Hz, 1H), 3.15 (d, J = 22.6 Hz, 2H), 3.78 (s, 3H), 3.80 (s, 3H), 3.93-3.99 (m, 1H), 5.98 (d, J = 7.7 Hz, 1H), 7.41-7.45 (m, 2H), 7.48-7.52 (m, 2H), 7.74 (d, J = 7.1 Hz, 2H).

Dimethyl (2-((1*s*,4*s*)-4-benzamidocyclohexyl)-2-oxoethyl)phosphonate (49o). Yield: 83%. ^{1}H NMR: δ 1.76–1.85 (m, 8H), 2.78–2.79 (m, 1H), 3.18 (d, J = 22.8 Hz, 2H), 3.80 (s, 3H), 3.83 (s, 3H), 4.21–4.25 (m, 1H), 7.40–7.52 (m, 3H), 7.76–7.78 (m, 2H).

Dimethyl (2-((1*r*,4*r*)-4-((*tert*-butyldimethylsilyl)oxy)cyclohexyl)-2-oxoethyl)phosphonate (49p). Yield: 96%. ^{1}H NMR: δ 0.01 (s, 6H), 0.82 (s, 9H), 1.19–1.32 (m, 4H), 1.85–1.88 (m, 4H), 2.41–2.47 (m, 1H), 3.08 (d, J = 22.8 Hz, 2H), 3.49–3.51 (m, 1H), 3.72 (s, 3H), 3.74 (s, 3H).

2-(1-Trityl-1*H*-imidazol-4-yl)benzaldehyde (50a). Yield: 52%. ^{1}H NMR: δ 7.03 (s, 1H), 7.18–7.20 (m, 6H), 7.36–7.39 (m, 10H), 7.53–7.58 (m, 3H), 7.64 (d, J = 7.8 Hz, 1H), 7.93 (d, J = 7.9 Hz, 1H).

2-Fluoro-6-(1-trityl-1*H*-imidazol-4-yl)benzaldehyde (50b). Yield: 46%. ^{1}H NMR: δ 7.02–7.07 (m, 1H), 7.10 (d, J = 1.6 Hz, 1H), 7.16–7.18 (m, 6H), 7.36–7.39 (m, 9H), 7.46–7.52 (m, 2H), 7.57 (s, 1H), 10.27 (s, 1H).

4-Fluoro-2-(1-trityl-1*H*-imidazol-4-yl)benzaldehyde (50c). Yield: 89%. ^{1}H NMR (MeOH-d_4): δ 7.16–7.27 (m, 6H), 7.29–7.47 (m, 3H), 7.60–7.70 (m, 9H), 7.85–7.90 (m, 2H), 10.26 (s, 1H).

5-Chloro-2-(1-trityl-1*H*-imidazol-4-yl)benzaldehyde (50d). Yield: 48%. ^{1}H NMR: δ 7.04 (d, J = 1.2 Hz, 1H), 7.10–7.19 (m, 5H), 7.32–7.38 (m, 12H), 7.58 (dd, J = 8.4, 2.4 Hz, 1H), 7.57–7.59 (m, 2H), 7.89 (d, J = 2.0 Hz, 1H), 10.34 (s, 1H).

4-Chloro-2-(1-trityl-1*H*-imidazol-4-yl)benzaldehyde (50e). Yield: 55%. ^{1}H NMR: δ 7.08–7.38 (m, 18 H), 7.60 (s, 1H), 7.88 (d, J = 8.4 Hz, 1H), 10.41 (s, 1H).

5-Fluoro-2-(1-trityl-1*H*-imidazol-4-yl)benzaldehyde (50f). Yield 87%. ^{1}H NMR: δ 7.02 (d, *J* = 1.4 Hz, 1H), 7.07–7.15 (m, 1H), 7.14–7.23 (m, 4H), 7.22–7.33 (m, 4H), 7.32–7.43 (m, 8H), 7.54 – 7.73 (m, 2H), 10.34 (d, *J* = 3.0 Hz, 1H).

1-Cyclohexyl-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (51). Yield: 86%. ^{1}H NMR: δ 1.21–1.50 (m, 5H), 1.62–1.91 (m, 5H), 2.28–2.45 (m, 1H), 2.89 (dd, *J* = 18.1, 9.0 Hz, 1H), 3.18 (dd, *J* = 18.1, 3.2 Hz, 1H), 5.61-5.64 (m, 1H), 7.16 (s, 1H), 7.21–7.28 (m, 2H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.53 (d, 1H, *J* = 6.2 Hz, 1H), 7.60 (s, 1H).

1-Cyclohexyl-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanol (52). Yield: 79%. LCMS (ESI, *m/z*): 283.3 $[M+H]^+$.^{1}H NMR (a mixture of diastereomers): δ 1.01–1.29 (m, 5H), 1.35–1.43 (m, 1H), 1.64–1.92 (m, 5H), 2.08 (ddd, *J* = 14.3, 7.0, 3.0 Hz, 1H), 2.26 – 2.14 (m, 1H), 2.30 (br s, 1H), 3.72–3.83 (two m, 1H), 5.39 (t, *J* = 6.2 Hz, 0.8H), 5.52 (dd, *J* = 10.8, 3.2 Hz, 0.2H), 7.19 and 7.20 (two s, 1H), 7.24–7.29 (m, 1H), 7.35–7.40 (m, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.85 and 7.90 (two s, 1H).

2-(1-Cyclohexyl-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)isoindoline-1,3-dione (53). To a solution of PPh_3 (255.4 mg, 0.973 mmol) in anhydrous THF (10 mL) at 0 °C was added phthalimide (143.3 mg, 0.974 mmol) and **52** (250 mg, 0.885 mmol). After stirring for 5 minutes, diethyl azodicarboxylate (0.444 mL) was added dropwise. The reaction mixture was allowed to warm to rt and stirred for 16 h. The solvent was distilled-off and the crude was dissolved in CH_2Cl_2 (50 mL), washed with 10% aq. NaOH solution (2 x 20 mL), water and brine. The organic layer

was dried (Na_2SO_4) and the solvent was removed under reduced pressure to afford crude product. The crude was taken without purification to the next step. LCMS (ESI, *m/z*): 412.33 $[M+H]^+$.

1-Cyclohexyl-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanamine (54). To a solution of crude **53** in ethanol (10 mL) was added hydrazine monohydrate (177 mg, 3.54 mmol). After stirring the mixture for 6 h at 80 °C, the solution was cooled to rt and the solvent was distilled-off. The crude product was dissolved in CH_2Cl_2 (30 mL) and the organic phase was washed with water (2 x 10 mL). The crude was purified by column chromatography to afford **54** (50 mg, 20%). LCMS (ESI, *m/z*): 282.3 $[M+H]^+$. ^1H NMR (mixture of diastereomers): δ 0.94–1.27 (m, 7H), 1.62–2.06 (two m, 6H), 2.76–2.96 (two m, 1H), 5.36–5.58 (two m, 1H), 7.16–7.25 (m, 2H), 7.31-7.45 (m, 2H), 7.50–7.54 (m, 1H), 7.78 and 7.81 (two s, 1H).

Ethyl (*E*)-3-(2-iodophenyl)acrylate (55a). Synthesized in 92% yield according to the literature.[33]

(*E*)-3-(2-Chloro-6-iodophenyl)-1-cyclohexylprop-2-en-1-one (55b). To a solution of 2-chloro-6-iodobenzaldehyde (1.16 g, 4.36 mmol) in anhydrous MeOH (15 mL) at rt was added NaOMe (8.72 mL, 4.36 mmol, 0.5 M in MeOH) and the yellow solution was allowed to stir for 5 min. 1-Cyclohexylethan-1-one (0.550 g, 4.36 mmol) was added dropwise as a solution in MeOH (3 mL). After stirring overnight, the solvent was removed under reduced pressure and the crude was diluted with saturated NH_4Cl (20 mL). The aqueous layer was extracted with CH_2Cl_2 (3 x 20 mL) and the combined organic extracts were dried ($MgSO_4$) and the solvent distilled off under reduced pressure to afford a crude residue. The crude product was purified by silica flash chromatography to afford **55b** (1.03 g, 63%).^1H NMR: δ 1.22–1.45 (m, 5 H), 1.70–174 (m, 1H), 1.79–1.85 (m, 2H), 1.93–

1.99 (m, 2H), 2.61–2.65 (m, 1H), 6.67 (d, *J* = 16 Hz, 1H), 6.93 (t, *J* = 8.0 Hz, 1H), 7.42 (d, *J* = 8.0 Hz, 1H), 7.48 (d, *J* = 16.0 Hz, 1H), 7.82 (d, *J* = 8.0 Hz, 1H).

Ethyl 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)acetate (56). Yield: 23%. ^{1}H NMR: δ 1.31 (t, *J* = 7.5 Hz, 3H), 2.67 (dd, *J* = 20.0, 12.0 Hz, 1H), 3.07 (dd, *J* = 20.0, 4.0 Hz, 1H), 4.25 (q, *J* = 6.0 Hz, 2H), 5.53 (dd, *J* = 12.0 Hz, 4.0 Hz, 1H), 7.16 (s, 1H), 7.21–7.37 (m, 3H), 7.51 (d, *J* = 6.0 Hz, 1H), 7.75 (s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)ethan-1-ol (57). To a solution of **56** (3.51 mmol) in a 1:2 mixture of THF:EtOH (24 mL) at rt, was added $NaBH_4$ (12.28 mmol) and LiCl (12.28 mmol). After stirring overnight, the solvents were distilled off and the crude was diluted with saturated NH_4C1 (20 mL). The aqueous layer was extracted with CH_2CI_2 (3 x 40 mL). The combined organic extracts were dried over $MgS0_4$ and the solvent distilled off under reduced pressure to afford the crude residue. The crude product was purified by silica flash chromatography to afford **57** (638 mg, 91 %). LCMS (ESI, *m/z*): 201.3 $[M+H]^+$.^{1}H NMR: δ 2.04-2.08 (m, 1H), 2.36-2.40 (m, 1H), 3.84 (t, *J* = 6.3 Hz, 2H), 5.37-5.41 (m, 1H), 7.17 (s, 1H), 7.25-7.28 (m, 1H), 7.35 (d, *J* = 6.9 Hz, 1H), 7.38 (d, *J* = 7.2 Hz, 1H), 7.54 (d, *J* = 7.5 Hz, 1H), 7.76 (s, 1H).

1-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-2-methylpropan-2-ol (58). To a stirred solution of **56** (48 mg, 0.20 mmol) in THF at 0 °C was added MeMgBr 1.0 M in THF (0.4 mL) dropwise. The resulting solution was allowed to stir at rt for 2 h. The reaction was quenched by cautious addition of methanol to the reaction mixture. The crude mixture was concentrated and purified by column chromatography to afford **58** (24 mg, 52%). LCMS (ESI, *m/z*): 229.3 $[M+H]^+$. ^{1}H NMR: δ 1.43 (s,

3H), 1.49 (s, 3H), 2.05–2.30 (m, 2H), 5.30–5.35 (m, 1H), 7.14 (s, 1H), 7.20–7.40 (m, 3H), 7.52 (d, J = 9.6 Hz, 1H), 8.02 (s, 1H).

1-Cyclopentyl-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (60). Yield: 11%. The compound was taken as such to the next step without analysis.

1-Cyclopentyl-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanol (61). Yield: 91%. LCMS (ESI, *m/z*): 269.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.11–1.41 (m, 2H), 1.55–1.70 (m, 4H), 1.83–2.17 (m, 4H), 3.74–3.79 (m, 1H), 5.38, 5.49 (one t and one d, J_1 = 6.0 Hz, J_2 = 6.0 Hz, 1H), 7.18 (s, 1H), 7.25 (d merged with $CHCl_3$, 1H), 7.38 (t, J = 7.2 Hz, 1H), 7.46 (d, J = 7.6 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H), 7.84 (s, 1H).

1-Cyclohexyl-3-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)propan-2-one (62). Yield: 82%. ^{1}H NMR: δ 0.85–1.35 (m, 5H), 1.55–1.18 (m, 5H), 1.80–1.95 (m, 1H), 2.25–2.38 (m, 2H), 2.70–2.80 (m, 1H), 3.16 (dd, J = 14.8, 2.4 Hz, 1H), 5.50–5.60 (m, 1H), 7.16 (s, 1H), 7.20–7.30 (m, 3H), 7.35 (t, J = 5.4 Hz, 1H), 7.41 (d, J = 5.4 Hz, 1H), 7.73 (s, 1H).

1-Cyclohexyl-3-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)propan-2-ol (63). Yield: 85%. LCMS (ESI, *m/z*): 297.4 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 0.80–1-85 (m, 14H), 2.00–2.20 (m, 1H), 4.20–4.50 (m, 1H), 5.30–5.60 (m, 1H), 7.14 (s, 1H), 7.20–7.39 (m, 2H), 7.43 (d, J = 7.2 Hz, 1H), 7.43 (d, J = 7.2 Hz, 1H), 7.91 (s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-phenylethan-1-one (64). Yield: 45%. ^{1}H NMR: δ 3.44 (dd, *J* = 20.0, 8.0 Hz, 1H), 3.72 (dd, *J* = 20.0, 4.0 Hz, 1H), 5.83 (d, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.25-7.29 (m, 1H), 7.40 (t, *J* = 10.0 Hz, 2H), 7.47 (t, *J* = 8.0 Hz, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 7.61 (t, *J* = 6.0 Hz, 1H), 7.74 (s, 1H), 7.97 (d, *J* = 8.0 Hz, 2H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-phenylethanol (65). Yield: 73%. LCMS (ESI, *m/z*): 277.3 $[M+H]^+$.^{1}H NMR (a mixture of diastereomers): δ 1.87–1.94 and 2.27–2.35 (two m, 1H), 2.40–2.57 (m, 1H), 5.06–5.11 (m, 1H), 5.33 (t, *J* = 6.1 Hz, 0.8H), 5.54 (dd, *J* = 10.6, 3.4 Hz, 0.2H), 7.08 and 7.12 (two s, 1H), 7.18–7.54 (m, 9H), 7.63 and 7.86 (two s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(pyridin-2-yl)ethanone (66). Yield 75%. ^{1}H NMR: δ 3.60 (dd, *J* = 19.2, 10.0 Hz, 1H), 3.91 (dd, *J* = 19.2, 3.2 Hz, 1H), 5.65 (dd, *J* = 10, 3.2 Hz, 1H), 7.08 (s, 1H), 7.13–7.17 (m, 1H), 7.25–7.30 (m, 2H), 7.38–7.45 (m, 2H), 7.66 (s, 1H), 7.78–7.80 (m, 1H), 8.05 (d, *J* = 8.0 Hz, 1H), 8.51–8.53 (m, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(pyridin-2-yl)ethanol (67). Yield: 82%. LCMS (ESI, *m/z*): 278.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 2.25–2.33 (m, 2H), 5.06–5.07 (m, 1H), 5.09 (br s, 1H), 5.35–5.38 and 5.46-5.49 (two m, 1H), 7.02 (s, 1H), 7.13-7.24 (m, 4H), 7.44–7.48 (m, 2H), 7.57–7.62 (m, 2H), 8.46–8.47 (m, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(pyridin-3-yl)ethanone (68). Yield: 89%. ^{1}H NMR: δ 3.57–3.67 (m, 1H), 3.80–3.95 (m, 1H), 6.01–6.05 (m, 1H), 7.27–7.73 (m, 6H), 8.29–8.36 (m, 1H), 8.61 (s, 1H), 8.86 (d, *J* = 3.0 Hz, 1H), 9.18 (s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(pyridin-3-yl)ethanol (69). Yield: 69%. LCMS (ESI, *m/z*): 278.3 $[M+H]^+$. ^1H NMR (a mixture of diastereomers): δ 1.75–2.43 (m, 2H), 5.07–5.12 (m, 1H), 5.38–5.40 and 5.56–5.58 (two m, 2H), 6.98 and 7.11 (two s, 1H), 7.19–7.33 (m, 3H), 7.43–7.49 (m, 2H), 7.43–7.79 (m, 2H), 8.3–8.51 (m, 2H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(pyridin-4-yl)ethan-1-one (70). Yield: 21%. ^1H NMR: δ 3.42–3.49 (dd, *J* = 18.8, 9.2 Hz, 1H), 3.76 (dd, *J* = 18.8, 3.6 Hz, 1H), 5.83 (dd, *J* = 9.2, 3.2 Hz, 1H), 7.20 (s, 1H), 7.28–7.32 (m, 1H), 7.38–7.45 (m, 2H), 7.58–7.60 (m, 1H), 7.74–7.75 (m, 2H), 7.81 (s, 1H), 8.84–8.86 (m, 2H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(pyridin-4-yl)ethan-1-ol (71). Yield: 30%. LCMS (ESI, *m/z*): 278.3 $[M+H]^+$.^1H NMR (mixture of diastereomers): δ 1.98–2.32 (m, 2H), 3.59 (br, 1H), 5.03–5.06 (m, 1H), 5.42–5.45 and 5.56–5.58 (two m, 1H), 7.20–7.23 (m, 1H), 7.24–7.25 (m, 4H), 7.34 (t, *J* = 7.0 Hz, 1H), 7.41 (d, *J* = 7.2 Hz, 1H), 7.49 (d, *J* = 7.6 Hz, 1H), 8.44–8.46 (m, 2H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(tetrahydro-2*H*-pyran-4-yl)ethanone (72). Yield: 61%. ^1H NMR: δ 1.75–1.84 (m, 4H), 2.58–2.62 (m, 1H), 2.90 (dd, *J* = 18.4, 9.6 Hz, 1H), 3.21 (dd, *J* = 18.4, 3.6 Hz, 1H), 3.38–3.45 (m, 2H), 3.99–4.01 (m, 2H), 5.65 (dd, *J* = 9.6, 3.6 Hz, 1H), 7.17 (s, 1H), 7.22–7.30 (m, 2H), 7.38 (dt, *J* = 7.2, 0.8 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.61 (s, 1H).

2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-(tetrahydro-2*H*-pyran-4-yl)etanol (73). Yield: 92%. LCMS (ESI, *m/z*): 285.3 $[M+H]^+$.^1H NMR (a mixture of diastereomers): δ 1.39–1.51 (m, 2H), 1.59–1.65 (m, 1H), 1.71–1.75 (m, 1H), 2.11–2.17 (m, 1H), 3.32–3.39 (m, 3H), 3.69–3.73 (m, 1H),

3.96–4.05 (m, 3H), 5.39 and 5.49 (two m, 1H), 7.17 (s, 1H), 7.23–7.28 (m, 1H), 7.33–7.44 (m, 2H), 7.55 (d, *J* = 8.0 Hz, 1H), 7.84 (s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(thiazol-5-yl)ethanone (74). Yield: 30%. ^{1}H NMR: δ 3.40 (dd, *J* = 9.6, 18.4 Hz, 1H), 3.71 (dd, *J* = 18.0, 3.2 Hz, 1H), 5.80 (dd, *J* = 2.8, 9.2 Hz, 1H), 7.21 (s, 1H), 7.29 (d, *J* = 7.2 Hz, 1H), 7.36–7.41 (m, 2H), 7.57 (d, *J* = 8.0 Hz, 1H), 8.41 (s, 1H), 7.73 (s, 1H), 9.0 (s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(thiazol-5-yl)ethanol (75). Yield: 75%. LCMS (ESI, *m/z*): 284.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.83–1.86, 1.99–2.06, 2.40–2.56 and 2.64–2.70 (m, 2H), 5.33–5.36 and 5.55–5.57 (two m, 1H), 5.39–5.44 (m, 1H), 7.07 (s, 1H), 7.22-7.34 (m, 1H, merged with chloroform), 7.37 (t, *J* = 7.6 Hz, 1H), 7.44 (d, *J* = 7. 6 Hz, 1H), 7.52 (d, *J* = 7.5 Hz, 1H), 7.71 and 7.73 (two s, 1H), 7.84 (s, 1H), 8.71 and 8.72 (two s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(1-methyl-1*H*-imidazol-4-yl)ethanone (76). Yield 24%. ^{1}H NMR: δ 3.42 (d, *J* = 9.9 Hz, 1H), 3.48 (d, *J* = 8.7 Hz, 1H), 3.75 (s, 3H), 5.75 (dd, *J* = 9.8, 3.4 Hz, 1H), 7.26–7.19 (m, 1H), 7.14 (s, 1H), 7.35 (m, 2H), 7.41 (d, *J* = 1.0 Hz, 1H), 7.52 (dd, *J* = 7.35, 1.2 Hz, 1H), 7.71 (s, 1H), 7.67 (d, *J* = 1.2 Hz, 1H).

2-(5*H*-Imidazo[5,1-a]isoindol-5-yl)-1-(1-methyl-1H-imidazol-4-yl)ethan-1-ol (77). Yield 94%. LCMS (ESI, *m/z*): 281.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.96–2.04, 2.48–2.53 and 2.66–2.71 (three m, 2H), 3.61 (s, 3H), 4.98–5.10 (m, 1H), 5.29–5.32 and 5.51–5.55 (two m,

1H), 6.70 and 6.75 (two s, 1H), 7.11 (s, 1H), 7.16–7.25 (m, 2H), 7.32 (m, 1H), 7.37 (s, 1H), 7.49 (t, J = 7.80 Hz, 1H), 7.67 and 7.88 (two s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(1-methyl-1*H*-pyrazol-4-yl)ethan-1-one (78). Yield: 88%. ^{1}H NMR: δ 2.39 (s, 3H), 3.27 (dd, J = 17.9, 9.7 Hz, 1H), 3.52 (dd, J = 17.9, 3.7 Hz, 1H), 5.80 (dd, J = 9.7, 3.6 Hz, 1H), 6.20 (dd, J = 3.4, 1.0 Hz, 1H), 7.17 (d, J = 3.5 Hz, 1H), 7.18 (s, 1H), 7.24–7.31 (m, 1H), 7.36–7.44 (m, 2H), 7.56 (d, J = 7.6 Hz, 1H) 7.72 (s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(1-methyl-1*H*-pyrazol-4-yl)ethan-1-ol (79). Yield: 77%. LCMS (ESI, *m/z*): 281.3 $[M+H]^+$. ^{1}H NMR: δ 2.27 and 2.28 (two s , 3H), 2.42–2.55 and 2.70–2.77 (two m, 2H), 3.53 (br s, 1H), 5.04 (dd, J = 8.2, 6.0 Hz, 1H), 5.30 (t, J = 6.3 Hz, 1H), 5.88–5.98 and 5.90–5.92 (two m, 1H), 6.13 and 6.15 (two d, J = 3.2 Hz, 1H), 7.12 and 7.14 (two s, 1H), 7.24–7.28 (m, 1H), 7.37 (t, J = 7.6 Hz, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.74 and 7.90 (two s, 1H).

***tert*-Butyl 3-(2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)acetyl)azetidine-1-carboxylate (80)**. Yield 77%. ^{1}H NMR: δ 1.38 (s, 9H), 2.85–2.92 (m, 1H), 3.20–3.25 (m, 1H), 3.44–3.48 (m, 1H), 3.65–3.70 (m, 2H), 4.01–4.28 (m, 2H), 5.63–5.66 (m, 1H), 7.16 (s, 1H), 7.21–7.31 (m, 2H), 7.36–7.40 (m, 1H), 7.53–7.55 (m, 1H), 7.66 (s, 1H).

***tert*-Butyl 3-(1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)azetidine-1-carboxylate (81)**. Yield: 43%. LCMS (ESI, *m/z*): 356.4 $[M+H]^+$.^{1}H NMR (a mixture of diastereomers): δ 1.42 (s, 9H), 1.92–2.23 (m, 2H), 2.50–2.63 (m, 1H), 3.61–3.80 (m, 2H), 3.90–4.02 (m, 3H), 4.20–4.58

(br s, 1H), 5.33–5.41 and 5.52–5.58 (two m, 1H), 7.13 (s, 1H), 7.21–7.28 (m, 2H), 7.30–7.39 (m, 1H), 7.41–7.48 (m, 1H), 7.58 (d, J = 14.3 Hz, 1H), 7.93 and 7.99 (two s, 1H).

***tert*-Butyl 4-(2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)acetyl)piperidine-1-carboxylate (82)**. Yield 89.1%. ^{1}H NMR: δ 1.44 (s, 9H), 1.50–1.82 (m, 4H), 2.72–2.76 (m, 2H), 2.90 (dd, J = 18.5, 9.4 Hz, 1H), 3.21 (dd, J = 18.57, 3.6 Hz, 1H), 5.63 (dd, J = 9.6, 3.6 Hz, 1H), 7.16 (s, 1H), 7.23 (m, 2H), 7.35–7.39 (m, 1H), 7.53 (d, J = 7.5 Hz, 1H), 7.59 (s, 1H).

***tert*-Butyl 4-(1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidine-1-carboxylate (83)**. Yield: 83%. LCMS (ESI, *m/z*): 384.5 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.23–1.31 (m, 2H), 1.46 (s, 9H), 1.50-1.61 (m, 2H), 1.79–1.89 (m, 1H), 2.08 (ddd, J = 14.3, 6.7, 2.8 Hz, 1H), 2.15–2.21 (m, 1H), 2.57–2.74 (m, 2H), 3.74–3.84 (m, 1H), 4.05–4.28 (m, 2H), 5.39 (t, J = 6.1 Hz, 0.8H), 5.51–5.56 (m, 0.2H), 7.15 (s, 1H), 7.25 (t, J = 7.0 Hz, 1H), 7.35–7.40 (m, 1H), 7.43 (d, J = 6.4 Hz, 1H), 7.55 (d, J = 7.6 Hz, 1H),7.79 and 7.81 (two s, 1H).

2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)-1-(piperidin-4-yl)ethan-1-ol dihydrochloride (84). To a solution of *tert*-butyl 4-(1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidine-1-carboxylate **83** (1.0 g, 2.61 mmol) in dichloromethane (20 mL) at rt was added trifluoroacetic acid (8.92 g, 78.23 mmol). The resulting solution was stirred at rt for 2 h and concentrated. The crude was dissolved in methanol (8 mL) and hydrogen chloride (4M in dioxane, 7.82 mmol) was added. The mixture was concentrated and dried under high vacuum to give the desired product as a dihydrochloride salt in quantitative yield, which was used directly in the next step without further purification. LCMS (ESI, *m/z*): 284.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, a mixture of diastereomers):

δ 1.51-1.58 (m, 3H), 1.68–1.71 (m, 1H), 1.84–1.97 (m, 1H), 2.02–2.16 (m, 2H), 2.68–2.83 (m, 2H), 3.15–3.29 (m, 2H), 3.78 (d, *J* = 10.1 Hz, 1H), 5.77–5.88 (m, 1H), 7.44–7.58 (m, 2H), 7.68 and 7.78 (two d, *J* = 7.2 Hz, 1H), 7.87 (d, *J* = 7.0 Hz, 1H), 7.95 and 7.96 (two s, 1H), 8.98–9.13 (m, 1H), 9.38 (d, *J* = 10.9 Hz, 1H), 9.43 and 9.67 (two s, 1H).

5-(2-(Azetidin-1-ium-3-yl)-2-hydroxyethyl)-5*H*-imidazo[5,1-*a*]isoindol-2-ium chloride (85). Synthesized according to the same procedure as compound **84**. Yield: 82%. LCMS (ESI, *m/z*): 256.3 $[M+H]^+$. ^{1}H NMR free base (a mixture of diastereomers): δ 1.56–1.78 (m, 1H), 1.80–2.11 (m, 2H), 3.56 (d, *J* = 12 Hz, 2H), 3.71–3.77 (m, 2H), 3.82–4.20 (m, 2H), 5.40–5.49 (m, 1H), 7.12–7.47 (m, 3H), 7.55–7.63 (m, 2H), 7.99 (s, 1H).

1-(1-Acetylpiperidin-4-yl)-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (86). Yield: 74%. ^{1}H NMR: δ 1.52–1.72 (m, 2H), 1.83–1.96 (m, 2H), 2.09 (s, 3H), 2.54–2.72 (m, 2H), 2.83–2.98 (m, 1H), 3.03–3.28 (two m, 2H), 3.80–3.92 (m, 1H), 4.56–4.61 (m, 1H), 5.60–5.66 (m, 1H), 7.16 (s, 1H), 7.22–7.30 (m, 2H), 7.38 (td, *J* = 7.2, 1.8 Hz, 1H), 7.54 (d, *J* = 7.7 Hz, 1H), 7.59 (d, *J* = 3.8 Hz, 1H).

1-(4-(1-Hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidin-1-yl)ethanone (87). Yield: 93%. LCMS (ESI, *m/z*): 326.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.21–1.34 (m, 2H), 1.57–1.71 (m, 2H), 1.83–1.93 (m, 1H), 2.04–2.13 (m, 4H), 2.16–2.24 (m, 1H), 2.44–2.50 (m, 1H), 2.97–3.03 (m, 1H), 3.70–3.90 (two m, 2H), 4.64–4.72 (m, 1H), 5.36–5.41 and 5.47–5.56 (two m, 1H), 7.16 (s, 1H), 7.23–7.28 (m, 2H), 7.35–7.43 (m, 2H), 7.55 (d, *J* = 7.5 Hz, 1H), 7.81 and 7.82 (two s, 1H).

Cyclohexyl(4-(1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidin-1-yl)methanone (88). Yield: 44%. LCMS (ESI, *m/z*): 394.5 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.20–1.34 (m, 6H), 1.49–1.77 (m, 10H), 1.87–1.97 (m, 1H), 2.03–2.08 (m, 1H), 2.16–2.20 (m, 1H), 2.46 (t, *J* = 12.0 Hz, 2H), 3.76–3.79 (m, 1H), 3.97 (t, *J* = 16.2 Hz, 1H), 4.70 (t, *J* = 14.2 Hz, 1H), 5.38–5.41 and 5.51–5.56 (two m, 1H), 7.15 (s, 1H), 7.23–7.27 (m, 1H, merged with chloroform), 7.37 (t, *J* = 7.8 Hz, 1H), 7.44 (d, *J* = 7.6 Hz, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.79 and 7.82 (two s, 1H).

(4-Fluorophenyl)(4-(1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidin-1-yl)methanone (89). Yield: 29%. LCMS (ESI, *m/z*): 406.4 $[M+H]^+$.^{1}H NMR: δ 1.29–1.37 (m, 3H), 1.57–1.87 (m, 3H), 2.18–2.36 (m, 2H), 3.73–3.86 (m, 4H), 4.74 (br s, 1H), 5.44–5.49 and 5.58–5.63 (two m, 1H), 7.08 (t, *J* = 7.2 Hz, 2H), 7.23 (s, 1H), 7.31–7.48 (m, 5H), 7.59 (d, *J* = 7.2 Hz, 1H), 8.20 an d 8.27 (two s, 1H).

1-(4-(1-Hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidin-1-yl)-2-phenylethan-1-one (90). Yield: 72%. LCMS (ESI, *m/z*): 402.4 $[M+H]^+$. ^{1}H NMR: δ 1.04–1.12 (m, 1H) 1.21–1.31 (m, 1H), 1.53–1.70 (m, 2H), 1.80–1.88 (m, 1H), 2.07–2.16 (m, 1H), 2.38–2.64 (m, 1H), 2.93 (td, *J* = 13.2, 2.7 Hz, 1H), 3.72–3.96 (m, 6H), 4.68–4.75 (m, 1H), 5.45–5.48 and 5.64–5.70 (two m, 1H), 7.21–7.44 (m, 10H), 7.53 (d, *J* = 7.8 Hz, 1H).

2-(4-Fluorophenyl)-1-(4-(1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidin-1-yl)ethanone (91). Yield: 80%. LCMS (ESI, *m/z*): 420.4 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.08–1.13 (m, 1H), 1.23–1.30 (m, 1H), 1.54–1.63 (m, 2H), 1.78 and 1.86 (two d, *J* = 13.0 Hz, 1H), 1.99–2.12 (m, 2H), 2.49 (dt, *J* = 12.8, 2.4 Hz, 1H), 2.93 (dt, *J* = 12.8, 3.0 Hz,

1H), 3.66 (d, *J* = 4.4 Hz, 2H), 3.71–3.73 (m, 2H, merged with broad singlet of OH), 3.90 (t, *J* = 15.2 Hz, 1H), 4.68 (t, *J* = 13.6 Hz, 1H), 5.30–5.37 and 5.47–5.50 (two m, 1H), 6.94–7.00 (m, 2H), 7.13 (s, 1H), 7.15–7.20 (m, 2H), 7.22–7.31 (m, 1H, merged with chloroform), 7.35–7.41 (m, 2H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.84 (d, *J* = 5.2 Hz, 1H).

1-(4-(1-Hydroxy-2-(5H-imidazo[5,1-a]isoindol-5-yl)ethyl)piperidin-1-yl)-2-(pyridin-4-yl)ethan-1-one (92). Yield: 49%. LCMS (ESI, *m/z*): 403.4 $[M+H]^+$. ^1H NMR (a mixture of diastereomers): δ 1.08–1.16 (m, 1H), 1.23–1.33 (m, 1H), 1.57–1.65 (m, 2H), 1.86 (t, *J* = 14.0 Hz, 1H), 1.99–2.17 (two m, 1H), 2.52 (dt, *J* = 12.8, 2.4 Hz, 1H), 2.97 (dt, *J* = 12.8, 4.0 Hz, 1H), 3.70 (d, *J* = 7.2 Hz, 2H), 3.70–3.76 (m, 1H, merged with doublet at 3.70), 3.83 (t, *J* = 13.8 Hz, 1H), 4.30 (br s, 1H), 4.69 (t, *J* = 14.0 Hz, 1H), 5.32–5.36 and 5.51–5.53 (two m, 1H), 7.10 and 7.12 (two s, 1H), 7.16–7.25 (m, 3H), 7.35–7.41 (m, 2H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.76 (d, *J* = 4.4 Hz, 1H), 8.49–8.52 (m, 2H).

1-(4-(1-Hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)piperidin-1-yl)-2-(pyrimidin-5-yl)ethanone (93). Yield: 76%. LCMS (ESI, *m/z*): 404.5 $[M+H]^+$.^1H NMR (a mixture of diastereomers): δ 1.30–1.41 (m, 3H), 1.63–1.72 (m, 2H), 1.87–2.22 (m, 2H), 2.56 (t, *J* = 12.4 Hz, 1H), 3.01–3.15 (m, 2H), 3.67 (d, *J* = 6.0 Hz, 1H), 3.83–3.85 (m, 1H), 3.96 (t, *J* = 14.6 Hz, 1H), 4.66 (t, *J* = 14.6 Hz, 1H), 5.44–5.46 and 5.62–5.65 (two m, 1H), 7.17 and 7.19 (two s, 1H), 7.26–7.30 (m, 1H, merged with chloroform), 7.39 (t, *J* = 7.4 Hz, 1H), 7.46 (d, *J* = 7.6 Hz, 1H), 7.56 (d, *J* = 7.2 Hz, 1H), 8.14 (d, *J* = 13.2 Hz, 1H), 8.63 (d, *J* = 4.4 Hz, 2H), 9.08–9.10 (m, 1H).

4-(1-Hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)-*N*-phenylpiperidine-1-carboxamide (94). To a vial containing 2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-(piperidin-4-yl)ethan-1-ol dihydrochloride **84** (75 mg, 0.265 mmol) in dichloromethane (5 mL) was added DIPEA (1.06 mmol) and phenylisocyanate (0.265 mmol). The reaction mixture was stirred at rt for 30 min and concentrated. The residue was dissolved in dichloromethane (30 mL) and washed with water (3 x 10 mL). The organic layer was dried over Na_2SO_4 and concentrated. The crude product was purified by flash column chromatography to afford **94** (66.30 mg, 62%). LCMS (ESI, *m/z*): 403.5 $[M+H]^+$. ^{1}H NMR (MeOH-d_4): δ 1.33–1.43 (m, 2H), 1.59–1.71 (m, 2H), 1.92–1.95 (m, 1H), 2.10–2.22 (m, 2H), 2.87 (t, *J* = 11.8 Hz, 2H), 3.79–3.83 (m, 1H), 4.25 (t, *J* = 15.2 Hz, 2H), 5.48 and 5.52-5.55 (t, *J* = 6.0 Hz and m, 1H), 7.03 (t, *J* = 7.4 Hz, 1H), 7.16 and 7.19 (two s, 1H), 7.28 (t, *J* = 8.0 Hz, 2H), 7.33–7.37 (m, 3H), 7.43 (t, *J* = 7.4 Hz, 1H), 7.62 (dd, *J* = 21.6, 7.6, Hz, 2H), 7.94 and 7.97 (two s, 1H), 8.01 (s, 1H).

1-Cyclohexyl-2-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (95). Yield: 50%. ^{1}H NMR: δ 1.20–1.48 (m, 5H), 1.66–1.69 (m, 1H), 1.78–1.92 (m, 4H), 2.36–2.44 (m, 1H), 2.79 (dd, *J* = 20.0, 12.0 Hz, 1H), 3.50 (dd, *J* = 20.0, 4.0 Hz, 1H), 5.77 (d, *J* = 8.0 Hz, 1H), 6.94 (t, *J* = 8.0 Hz, 1H), 7.18 (s, 1H), 7.31–7.37 (m, 2H), 7.62 (s, 1H).

1-Cyclohexyl-2-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)etanol (96). Yield: 96%. LCMS (ESI, *m/z*): 301.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 0.98–1.39 (m, 6H), 1.65–1.79 (m, 5H), 2.03–2.07 (m, 1H) 2.34–2.50 (m, 2H), 3.54–3.73 (m, 1H), 5.46 and 5.67 (two dd, J_1 = 8.0, 3.0 Hz, J_2 = 10.4, 3.0 Hz, 1H), 6.93 (t, *J* = 8.0 Hz, 1H), 7.17 (d, *J* = 7.2 Hz, 1H), 7.30–7.37 (m, 2H), 7.82 and 7.88 (two s, 1H).

1-Cyclohexyl-2-(7-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethan-1-one (97). Yield: 94%. ^{1}H NMR: δ 1.07–1.50 (m, 4H), 1.58–1.73 (m, 2H), 1.75–1.90 (m, 4H), 2.40 (tt, *J* = 11.5, 3.4 Hz, 1H), 2.96 (dd, *J* = 18.5, 9.3 Hz, 1H), 3.19 (dd, *J* = 18.5, 3.8 Hz, 1H), 5.65 (dd, *J* = 9.3, 3.7 Hz, 1H), 7.02–7.07 (m, 1H), 7.10 (td, *J* = 8.8, 2.3 Hz, 1H), 7.16 (s, 1H), 7.51 (dd, *J* = 8.4, 4.8 Hz, 1H), 7.79 (s, 1H).

1-Cyclohexyl-2-(7-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethan-1-ol (98). Yield: 93%. LCMS (ESI, *m/z*): 301.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 0.91–1.30 (m, 5H), 1.33–1.42 (m, 1H), 1.55–1.95 (m, 5H), 1.95–2.05 (m, 1H), 2.17 (tdd, *J* = 14.2, 10.8, 6.8 Hz, 1H), 3.12 (br s, 1H), 3.67–3.84 (m, 1H), 5.34–5.37 and 5.47–5.5 (two m, 1H), 6.99–7.12 (m, 2H), 7.16 (dd, *J* = 8.7, 2.3 Hz, 1H), 7.45 (dd, *J* = 8.4, 4.9 Hz, 1H), 7.85 and 7.90 (two s, 1H).

1-Cyclohexyl-2-(8-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (99). Yield: 58%. The compound was taken as such to the next step without analysis.

1-Cyclohexyl-2-(8-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)etanol (100). Yield: 15%. LCMS (ESI, *m/z*): 301.3 $[M+H]^+$. ^{1}H NMR (MeOH-d_4, a mixture of diastereomers): δ 1.00–2.30 (m, 13H), 3.50–3.57 (m, 1H), 5.35 and 5.50 (m, 1H), 7.18–7.50 (m, 3H), 7.60–7.65 (m, 1H), 7.92 and 7.98 (two s, 1H).

2-(6-Chloro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-cyclohexylethanone (101). Yield: 25%. ^{1}H NMR: δ 1.18–1.36 (m, 5H), 1.68–1.88 (m, 5H), 2.37–2.40 (m, 1H), 2.64 (dd, *J* = 20.0, 10.0 Hz,

1H), 3.79 (dd, *J* = 16.0, 4.0 Hz, 1H), 5.70 (d, *J* = 8.0 Hz, 1H), 7.17–7.20 (m, 2H), 7.32 (t, *J* = 8.0 Hz, 1H), 7.43 (d, *J* = 8.0 Hz, 1H), 7.61 (s, 1H).

2-(6-Chloro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-cyclohexylethanol (102). Yield: 98%. LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 0.96–1.35 (m, 6H), 1.60–1.86 (m, 5H), 1.97–2.10 (m, 1H), 2.52–2.69 (m, 1H), 3.58–3.69 (two m, 1H), 5.31 and 5.59 (two dd, J_1= 6.0, 2.80 Hz, J_2 = 10.4, 2.80 Hz, 1H), 7.16–7.19 (m, 2H), 7.26–7.31 (m, 1H), 7.41 (t, *J* = 5.4 Hz, 1H), 7.82 and 7.94 (two s, 1H).

2-(7-Chloro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-cyclohexylethanone (103). Yield: 43%. ^{1}H NMR: δ 1.19–1.46 (m, 5H), 1.68–1.70 (m, 1H), 1.78–1.91 (m, 5H), 2.35–2.43 (m, 1H), 2.91 (dd, *J* = 20.0, 10.0 Hz, 1H), 3.18 (dd, *J* = 20.0, 4.0 Hz, 1H), 5.61 (dd, *J* = 8.0, 4.0 Hz, 1H), 7.15 (s, 1H), 7.28 (s, 1H), 7.35 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.60 (s, 1H).

2-(7-Chloro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-cyclohexylethanol (104). Yield: 98%. LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.00–1.28 (m, 5H), 1.37–1.40 (m, 1H), 1.66–2.01 (m, 5H), 1.91–2.0 (m, 1H), 2.12–2.23 (m, 1H), 3.71–3.75 (m, 1H), 7.15 (s, 1H), 7.33 (d, *J* = 8.0 Hz, 1H), 7.45 (d, *J* = 8.0 Hz, 1H), 7.79, 7.82 (two s, 1H).

2-(8-Chloro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-cyclohexylethanone (105). Yield: 21%. ^{1}H NMR (MeOH-d_4): δ 1.10–1.90 (m, 10H), 2.42–2.48 (m, 1H), 2.99 (dd, *J* = 18.9, 9.0 Hz, 1H), 3.40 (dd, *J* = 18.9, 3.6 Hz, 1H), 5.58–5.62 (m, 1H), 6.95–7.08 (m, 1H), 7.16–7.88 (m, 4H).

2-(8-Chloro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-cyclohexylethanol (106). Yield: 41%. LCMS (ESI, *m/z*): 318.3 $[M+H]^+$.^{1}H NMR (MeO*H*-d_4, a mixture of diastereomers): δ 1.00–2.30 (m, 13H), 3.60–3.70 (m, 1H), 5.35–5.50 (two m, 1H), 6.95–7.08 (m, 1H), 7.16–7.88 (m, 4H).

1-(4,4-Difluorocyclohexyl)-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (107). Yield: 96%. ^{1}H NMR: δ 1.73–1.80 (m, 4H), 1.91–1.95 (m, 2H), 2.10–2.14 (m, 2H), 2.41–2.47 (m, 1H), 2.90 (dd, *J* = 18.8, 9.4 Hz, 1H), 3.21 (dd, *J* = 3.6, 18.4 Hz, 1H), 5.60 (dd, *J* = 3.4, 9.4 Hz, 1H), 7.13 (s, 1H), 7.22–7.28 (m, 2H), 7.36 (t, *J* = 7.2 Hz, 1H), 7.51 (d, *J* = 7.6 Hz, 1H), 7.57 (s, 1H).

1-(4,4-Difluorocyclohexyl)-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanol (108). Yield: 61%. LCMS (ESI, *m/z*): 319.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.26–1.36 (m, 3H), 1.63–1.97 (m, 5H), 2.02–2.08 (m, 3H), 3.69–3.72 (m, 1H), 5.02 and 5.12 (two d, *J* = 6.0 Hz, 1H), 5.34–5.53 and 5.41–5.43 (two m, 1H), 7.10 and 7.12 (two s, 1H), 7.25 (t, *J* = 7.4 Hz, 1H), 7.36 (t, *J* = 7.4 Hz, 1H), 7.54–7.58 (m, 2H), 7.91 and 7.93 (two s, 1H).

1-(4,4-Difluorocyclohexyl)-2-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (109). Yield: 81%. ^{1}H NMR: δ 1.65–1.82 (m, 4H), 1.90–2.01 (m, 2H), 2.11–2.16 (m, 2H), 2.44–2.48 (m, 1H), 2.79 (dd, *J* = 18.4, 10.4 Hz, 1H), 3.52 (dd, *J* = 18.4, 2.0 Hz, 1H), 5.72 (d, *J* = 10.4 Hz, 1H), 6.92 (t, *J* = 8.8 Hz, 1H), 7.15 (s, 1H), 7.28-7.35 (m, 2H), 7.58 (s, 1H).

1-(4,4-Difluorocyclohexyl)-2-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanol (110). Yield: 78%. LCMS (ESI, *m/z*): 337.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6, a mixture of diastereomers): δ 1.21–1.29 (m, 3H), 1.56–1.72 (m, 4H), 1.88–1.96 (m, 3H), 2.28 and 2.32 (two t, *J* = 5.0 Hz, 1H), 3.41–

3.44 and 3.62-3.65 (two m, 1H), 4.73 and 5.17 (two d, J = 8.2 Hz, 1H), 5.56–5.59 and 5.61–5.64 (two m, 1H), 7.03–7.17 (m, 2H), 7.39–7.44 (m, 2H), 7.91 and 7.95 (two s, 1H).

(*trans*)-Methyl 4-(2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)acetyl)cyclohexanecarboxylate (111). Yield: 90%. ^{1}H NMR: δ 1.38–1.49 (m, 4H), 1.95–2.11 (m, 4H), 2.27–2.32 (m, 1H), 2.27–2.42 (m, 1H), 2.91 (dd, J = 18.5, 9.5 Hz, 1H), 3.21 (dd, J = 18.5, 3.5 Hz, 1H), 3.67 (s, 3H), 5.63 (dd, J = 9.5, 3.3 Hz, 1H), 7.17 (s, 1H), 7.22-7.29 (m, 2H), 7.38 (t, J = 7.5 Hz, 1H), 7.54 (d, J = 7.6 Hz, 1H), 7.60 (s, 1H).

(*trans*)-Methyl-4-((1*R*)-1-hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)cyclohexanecarboxylate (112). Yield: 87%. LCMS (ESI, *m/z*): 341.4 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.05–1.20 (m, 2H), 1.42 (qt, J = 12.7, 4.0 Hz, 3H), 1.63–1.82 (m, 1H), 1.92–2.10 (m, 4H), 2.11–2.31 (m, 2H), 3.65 (s, 3H), 3.72–3.83 (m, 1H), 5.36 (t, J = 6.2 Hz, 0.7H), 5.52 (dd, J = 10.8, 3.1 Hz, 0.3H), 7.14 (s, 1H), 7.23 (t, J = 7.4 Hz, 1H), 7.31–7.40 (m, 1H), 7.42 (d, J = 7.7 Hz, 1H), 7.53 (d, J = 7.6 Hz, 1H), 7.83 (s, 1H).

2-(6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-(1,4-dioxaspiro[4.5]decan-8-yl)ethanone (113). Yield: 84%. ^{1}H NMR (MeOH-d_4): δ 1.48–1.91 (m, 6H), 2.35–2.65 (m, 2H), 3.58–3.65 (m, 1H), 3.91 (s, 4H), 5.79–5.82 (m, 1H), 7.01–7.07 (m, 1H), 7.16 (m, 1H), 7.42–7.45 (m, 2H), 7.70 (s, 1H).

4-(2-(6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-one (114). To a solution of the ketone **113** (3.77g, 10.56 mmol) in MeOH (50 mL) at 0 °C, was added $NaBH_4$ (1.20

g, 31.69 mmol) portion wise and the solution was allowed to warm to rt and stirred for 1 h. 3N HCl was added cautiously until the pH = 3-4. The mixture was stirred at 50 °C for 1.5 h. After cooling to rt, the solution was basified with saturated $NaHCO_3$ solution and the aqueous layer was extracted with CH_2Cl_2 (3 x 40 mL). The combined organic layers were washed with brine, dried (Na_2SO_4), and concentrated. The residue was purified by flash column chromatography to afford **114** (3.31 g, 99.7%). ^{1}H NMR (a mixture of diastereomers): δ 1.56–2.46 (m, 11H), 3.73–3.82 (m, 1H), 5.48–5.50 and 5.62–5.65 (two m, 1H), 6.93–6.97 (m, 1H), 7.17 (s, 1H), 7.26–7.37 (m, 2H), 7.79 and 7.86 (two s, 1H).

***N*-((*cis*)-4-(2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)acetyl)cyclohexyl)benzamide (115)**. Yield: 53%. ^{1}H NMR: δ 1.25–1.34 (m, 2H), 1.53–1.63 (m, 2H), 1.98–2.08 (m, 2H), 2.20 (t, *J* = 11.6 Hz, 2H), 2.36 (t, *J* = 12.2 Hz, 1H), 2.90 (dd, *J* = 18.6, 9.4 Hz, 1H), 3.25 (dd, *J* = 18.4, 3.2 Hz, 1H), 3.93–4.00 (m, 1H), 5.63 (dd, *J* = 9.2, 3.2 Hz, 1H), 6.32 (d, *J* = 6.8 Hz, 1H), 7.19 (s, 1H), 7.26–7.35 (m, 2H, merged with chloroform), 7.38–7.43 (m, 3H), 7.48 (d, *J* = 7.2 Hz, 1H), 7.55 (d, *J* = 7.6 Hz, 1H), 7.71 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H).

***N*-((*trans*)-4-(1-Hydroxy-2-(5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethyl)cyclohexyl)benzamide (116)**. Yield: 57%. LCMS (ESI, *m/z*): 402.4 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.14–1.45 (m, 4H), 1.74 (d, *J* = 10.6 Hz, 1H), 1.97 (d, *J* = 10.6 Hz, 1H), 2.09–2.20 (m, 4H), 3.71–3.82 (m, 1H), 3.85–3.95 (m, 1H), 5.3–5.40 and 5.48–5.59 (two m, 1H), 6.03 (d, *J* = 7.6 Hz, 1H), 7.17 (s, 1H), 7.21–7.30 (m, 1H, merged with chloroform), 7.31–7.51 (m, 5H), 7.55 (d, *J* = 7.4 Hz, 1H), 7.74 (d, *J* = 7.6 Hz, 2H), 7.83 (s, 1H).

***N*-((*trans*)-4-(2-(5*H*-Imidazo[5,1-*a*]isoindol-5-yl)acetyl)cyclohexyl)benzamide (117)**. Yield: 69%. ^{1}H NMR: δ 1.19–1.36 (m, 2H), 1.48–1.65 (m, 2H), 1.93–2.07 (m, 2H), 2.18–2.23 (m, 2H), 2.35 (tt, *J* = 12.2, 3.2 Hz, 1H), 2.90 (dd, *J* = 18.4, 9.5 Hz, 1H), 3.22 (dd, *J* = 18.4, 3.6 Hz, 1H), 3.92-3.99 (m, 1H), 5.62 (dd, *J* = 9.4, 3.4 Hz, 1H), 6.23 (d, *J* = 7.6 Hz, 1H), 7.23-7.32 (m, 3H, merged with chloroform), 7.34–7.42 (m, 3H), 7.46–7.50 (m, 1H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.63 (s, 1H), 7.76 (d, *J* = 7.6 Hz, 2H).

***N*-((*cis*)-4-(1-Hydroxy-2-(5H-imidazo[5,1-*a*]isoindol-5-yl)ethyl)cyclohexyl)benzamide (118)**. Yield: 63%. LCMS (ESI, *m/z*): 402.5 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.27–1.46 (m, 5H), 1.79 (d, *J* = 12.0 Hz, 1H), 2.04–2.22 (m, 5H), 3.78–3.88 (m, 2H), 5.47–5.49 and 5.53–5.54 (two m, 1H), 7.16 and 7.19 (two s, 1H), 7.35 (t, *J* = 7.4 Hz, 1H), 7.42–7.49 (m, 3H), 7.53 (d, *J* = 7.2 Hz, 1H), 7.59 (d, *J* = 7.6 Hz, 1H), 7.64 (d, *J* = 7.6 Hz, 1H), 7.82 (d, *J* = 7.6 Hz, 2H), 7.97 and 8.01 (two s, 1H).

4-(2-(6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)acetyl)cyclohexan-1-one (119). To a solution of the ketone **113** (270 mg, 0.757 mmol) in dioxane (10 mL) at 0 °C, was added 3N HCl (2 mL) and the mixture was stirred at 40 °C for 2 h. The mixture was basified by adding Na_2CO_3 and the product was extracted with DCM (3 x 30 mL). The combined organic extract was dried (Na_2SO_4) and concentrated under reduced pressure to afford the crude, chromatographic purification afforded **119 as** pale yellow oil (216 mg, 91%). ^{1}H NMR: δ 1.85–2.06 (m, 2H), 2.13–2.60 (m, 6H), 2.73–2.99 (m, 2H), 3.62 (dd, *J* = 18.6, 2.2 Hz, 1H), 5.78 (dd, *J* = 10.7, 2.1 Hz, 1H), 6.94–6.99 (m, 1H), 7.20 (s, 1H), 7.32–7.44 (m, 2H), 7.64 (s, 1H).

4-(2-(6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (120). Yield: 78%. LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^1H NMR (a mixture of diastereomers): δ 0.80–1.90 (m, 10H), 2.23–2.30 (m, 1H), 3.25–3.29 (m, 1H), 3.67–3.49 and 3.72–3.77 (two m, 1H), 4.23, 4.48 and 4.61 (three br s, 1H), 5.06 (br s, 1H), 5.55–5.59 and 5.62–5.67 (two m, 1H), 7.00–7.14 (m, 1H), 7.16, 7.19 and 7.20 (three s, 1H), 7.38–7.46 (m, 2H), 7.91, 7.92 and 7.95 (three s, 1H).

(*R*)-1-Cyclohexyl-2-((*R*)-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethan-1-ol (121). LCMS (ESI, *m/z*): 283.3 $[M+H]^+$.^1H NMR: δ 0.95–1.30 (m, 5H), 1.35–1.43 (m, 1H), 1.61–1.83 (m, 5H), 1.88 (d, 1H, *J* = 12.7 Hz), 2.23 (ddd, *J* = 14.3, 11.0, 3.2 Hz, 1H), 2.86 (br s, 1H), 3.78 (ddd, *J* = 10.9, 5.9, 2.7 Hz, 1H), 5.49 (dd, *J* = 10.5, 2.8 Hz, 1H), 7.18 (s, 1H), 7.21–7.29 (m, 1H), 7.31–7.40 (m, 2H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.80 (s, 1H).

(*S*)-1-Cyclohexyl-2-((*R*)-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethan-1-ol (122). LCMS (ESI, *m/z*): 283.3 $[M+H]^+$. ^1H NMR: δ 1.01–1.29 (m, 5H), 1.35–1.43 (m, 1H), 1.64–1.88 (m, 5H), 2.07 (ddd, *J* = 14.3, 7.1, 3.0 Hz, 1H), 2.18 (ddd, *J* = 14.2, 10.3, 5.4 Hz, 1H), 3.74 (ddd, *J* = 10.2, 5.6, 3.2 Hz, 1H), 5.39 (t, 1H, *J* = 6.3 Hz), 7.18 (s, 1H), 7.32–7.21 (m, 1H), 7.38 (t, *J* = 7.6 Hz, 1H), 7.46 (d, *J* = 7.7 Hz, 1H), 7.56 (d, *J* = 7.6 Hz, 1H), 7.82 (s, 1H).

(*S*)-1-Cyclohexyl-2-((*S*)-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethan-1-ol (123). LCMS (ESI, *m/z*): 283.3 $[M+H]^+$.^1H NMR: δ 0.91–1.33 (m, 5H), 1.35–1.41 (m, 1H), 1.58–1.98 (m, 6H), 2.23 (ddd, *J* = 14.3, 11.0, 3.2 Hz, 1H), 2.45 (br s, 1H), 3.78 (ddd, *J* = 11.0, 5.9, 2.6 Hz, 1H), 5.48 (dd, *J* = 10.6, 3.2 Hz, 1H), 7.19 (s, 1H), 7.21–7.29 (m, 1H), 7.32–7.42 (m, 2H), 7.54 (d, *J* = 7.6 Hz, 1H), 7.80 (s, 1H).

(*R*)-1-Cyclohexyl-2-((*S*)-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethan-1-ol (124). LCMS (ESI, *m/z*): 283.3 $[M+H]^+$. ^{1}H NMR: δ 0.82–1.49 (m, 6H), 1.57–1.98 (m, 5H), 2.05 (ddd, *J* = 14.2, 7.1, 2.9 Hz, 1H), 2.17 (ddd, *J* = 14.1, 10.4, 5.5 Hz, 1H), 2.67 (br s, 1H), 3.76 (ddd, *J* = 10.2, 5.5, 2.9 Hz, 1H), 5.38 (t, *J* = 6.3 Hz, 1H), 7.16 (s, 1H), 7.21–7.27 (m, 1H), 7.37 (t, *J* = 7.5 Hz, 1H), 7.45 (d, 1H, *J* = 7.6 Hz), 7.55 (d, 1H, *J* = 7.6 Hz), 7.82 (s, 1H).

1-((*trans*)-4-((*tert*-Butyldimethylsilyl)oxy)cyclohexyl)-2-(6-fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)ethanone (125). Yield: 79%. ^{1}H NMR: δ 0.028 (s, 6H), 0.88 (s, 9H), 11.27–1.96 (m, 8H), 2.32–2.38 (m, 1H), 2.80 (dd, *J* = 18.8, 10.6 Hz, 1H), 3.48–3.57 (m, 2H), 5.75 (d, *J* = 9.3 Hz, 1H), 6.91–6.95 (m, 1H), 7.17 (s, 1H), 7.23–7.39 (m, 2H), 7.59 and 7.64 (two s, 1H).

(1*s*,4*s*)-4-(2-(6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (126). To a solution of **114** (3.31 g, 10.53 mmol) in dry THF (75 mL) at -78 °C under nitrogen atmosphere was added *LS*-selectride (21.59 mL, 21.59 mmol) solution (1.0 M in THF). The resulting mixture was stirred vigorously for 3 h at -78°C and then allowed to equilibrate to room temperature (1 h). The reaction mixture was hydrolyzed with 2 ml of water and 2 ml of ethanol. The reaction was acidified with 6N HCl followed by basification with saturated potassium carbonate solution. The product was extracted with 5% trifluoroethanol/DCM (3 x 40 mL). The combined organic extract was dried over $MgSO_4$ and concentrated under reduced pressure to afford the crude. Chromatographic purification afforded **126** (2.63 g, 79 %). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (a mixture of diastereomers): δ 1.45–2.15 (m, 10H), 2.35–2.51 (m, 1H), 3.66–3.79 (two m, 1H), 4.03 (br s, 1H), 5.45–5.48 and 5.68–5.72 (two m), 5.67 (dd, *J* = 10.6, 2.8 Hz, 1H), 6.91–6.95 (m, 1H), 7.19 (d, *J* = 5.4 Hz, 1H), 7.25–7.39 (m, 2H), 7.88 (two, s, 1H).

(*trans*)-4-(2-(6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexanol (127). To a solution of **125** (1.50 g, 3.50 mmol) in MeOH (15 mL) at 0 °C, was added $NaBH_4$ (397.2 mg, 10.50 mmol) and the solution was allowed to stir for 1 h at room temperature. After cooling to 0 °C, 6N HCl (5 mL) was added cautiously to the reaction mixture and the mixture was stirred for 45 minutes at 50 °C. After cooling to room temperature, the reaction mixture was made basic by saturated $NaHCO_3$ solution and the reaction was diluted with water (25 mL). The product was extracted with 5% trifluoroethanol/dichloromethane mixture CH_2Cl_2 (4 x 50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure to afford the crude residue. The crude was purified by column chromatography to afford **127** as off-white solid (1.02 g, 92%). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^1H NMR (a mixture of diastereomers): δ 1.07–2.52 (m, 11H), 3.48–3.68 (two m, 2H), 5.45 (t, *J* = 6.0 Hz, 1H), 5.65 (dd, *J* = 9.0, 3.0 Hz, 1H), 6.89–6.96 (m, 1H), 7.16 (s, 1H), 7.29–7.38 (m, 2H), 7.80 and 7.88 (two s, 1H).

(1*R*,4*s*)-4-((*S*)-2-((*S*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (128). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^1H NMR (DMSO-d*6*): δ 1.05–1.80 (m, 10H), 2.26 (ddd, *J* = 14.0, 10.8, 3.1 Hz, 1H), 3.57–3.65 (m, 1H), 3.74 (br s, 1H), 4.21 (d, *J* = 3.4 Hz, 1H), 5.01 (d, *J* = 6.1 Hz, 1H), 5.66 (dd, *J* = 10.9, 2.9 Hz, 1H), 6.97–7.17 (m, 1H), 7.20 (s, 1H), 7.35–7.60 (m, 2H), 7.92 (s, 1H).

(1*S*,4*s*)-4-((*R*)-2-((*R*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (129). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^1H NMR (DMSO-d_6): δ 1.14–1.74 (m, 10H), 2.26 (ddd, *J* = 14.2, 10.8, 3.0 Hz, 1H), 3.56–3.65 (m, 1H), 3.75 (br s, 1H), 4.21 (d, *J* = 3.4 Hz, 1H), 5.02

(d, *J* = 6.2 Hz, 1H), 5.66 (dd, *J* = 10.9, 2.9 Hz, 1H), 7.10 (ddd, *J* = 9.4, 6.9, 2.2 Hz, 1H), 7.20 (s, 1H), 7.31–7.59 (m, 2H), 7.92 (s, 1H).

(1*S*,4*s*)-4-((*R*)-2-((*S*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (130). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 1.10–1.48 (m, 7H), 1.51–1.71 (m, 2H), 1.83–1.91 (m, 1H), 2.33 (dt, *J* = 14.7, 3.8 Hz, 1H), 3.34–3.44 (m, 1H), 3.71–3.79 (m, 1H), 4.21 (d, *J* = 3.1 Hz, 1H), 4.59 (d, *J* = 5.9 Hz, 1H), 5.58 (t, *J* = 5.0 Hz, 1H), 7.03–7.10 (m, 1H), 7.16 (s, 1H), 7.37–7.46 (m, 2H), 7.95 (s, 1H).

(1*R*,4*s*)-4-((*S*)-2-((*R*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (131). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 1.12–1.46 (m, 7H), 1.56–1.63 (m, 2H), 1.83–1.91 (m, 1H), 2.33 (dt, *J* = 14.5, 3.7 Hz, 1H), 3.37–3.41 (m, 1H), 3.66–3.92 (m, 1H), 4.19 (d, *J* = 3.2 Hz, 1H), 4.58 (d, *J* = 5.8 Hz, 1H), 5.58 (t, *J* = 5.0 Hz, 1H), 7.0–7.10 (m, 1H), 7.16 (s, 1H), 7.40–7.46 (m, 2H), 7.95 (s, 1H).

(1*S*,4*r*)-4-((*S*)-2-((*S*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (132). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 0.89–1.13 (m, 4H), 1.15–1.27 (m, 1H), 1.47–1.60 (m, 2H), 1.71–1.93 (m, 3H), 2.27 (ddd, *J* = 14.1, 10.8, 3.1 Hz, 1H), 3.22–3.31 (m, 1H), 3.52–3.61 (m, 1H), 4.47 (d, *J* = 4.4 Hz, 1H), 5.05 (d, *J* = 6.1 Hz, 1H), 5.64 (dd, *J* = 10.8, 2.9 Hz, 1H), 7.06–7.13 (m, 1H), 7.19 (s, 1H), 7.40–7.46 (m, 2H), 7.91 (s, 1H).

(1*S*,4*r*)-4-((*S*)-2-((*R*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (133). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 0.92–1.16 (m, 5H), 1.50–1.69 (m, 2H), 1.75–1.93 (m, 3H), 2.29 (dt, *J* = 14.6, 4.1 Hz, 1H), 3.20–3.30 (m, 1H), 3.34–3.41

(m, 1H), 4.45 (d, *J* = 4.4 Hz, 1H), 4.59 (d, *J* = 5.7 Hz, 1H), 5.57 (t, *J* = 5.2 Hz, 1H), 7.03–7.17 (m, 1H), 7.16 (s, 1H), 7.38–7.47 (m, 2H), 7.94 (s, 1H).

(1*R*,4*r*)-4-((*R*)-2-((*R*)-6-Fluoro-5H-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (134). LCMS (ESI, *m/z*): 317.3 $[M+H]^+$. ^{1}H NMR (DMSO-d_6): δ 0.93–1.24 (m, 5H), 1.38–1.64 (m, 2H), 1.78–1.84 (m, 3H), 2.23–2.30 (m, 1H), 3.22–3.31 (m, 1H), 3.53–3.61 (m, 1H), 4.45 (d, *J* = 4.4 Hz, 1H), 5.04 (d, *J* = 6.2 Hz, 1H), 5.64 (dd, *J* = 10.8, 2.8 Hz, 1H), 7.07–7.13 (m, 1H), 7.19 (s, 1H), 7.37–7.48 (m, 2H), 7.91 (s, 1H).

(1*R*,4*r*)-4-((*R*)-2-((*S*)-6-Fluoro-5*H*-imidazo[5,1-*a*]isoindol-5-yl)-1-hydroxyethyl)cyclohexan-1-ol (NLG-919). HRMS (ESI, *m/z*): calcd for $(C_{18}H_{21}FN_2O_2 + H)^+$, 317.1665; found, 317.1669. ^{1}H NMR (DMSO-d_6): δ 0.86–1.21 (m, 5H), 1.52– 1.55 (m, 1H), 1.63– 1.67 (m, 1H), 1.75–1.93 (m, 3H), 2.29 (dt, *J* = 14.6, 4.0 Hz, 1H), 3.22–3.29 (m, 1H), 3.35–3.40 (m, 1H), 4.45 (d, *J* = 4.4 Hz, 1H), 4.59 (d, *J* = 5.7 Hz, 1H), 5.57 (t, *J* = 5.1 Hz, 1H), 7.05–7.10 (m, 1H), 7.16 (s, 1H), 7.40–7.44 (m, 2H), 7.95 (s, 1H). ^{13}C NMR (101 MHz, DMSO-d_6): δ 25.92, 27.45, 35.33, 35.49, 43.33, 58.43, 70.15, 71.75, 114.43, 114.63, 117.01, 117.04, 118.54, 131.33, 131.49, 131.81, 131.89, 132.46, 132.52, 133.95, 137.27, 157.07, 159.52.

hIDO1 Enzymatic Assay Determination

The IC_{50} values for the IDO1 inhibitors were determined by testing the activity of IDO1 in a mixture containing 50 mM potassium phosphate buffer at pH 6.5; 70 nM purified human IDO1 protein, 200 μM L-tryptophan, 20 mM ascorbate, 20 μM methylene blue, 0.1% DMSO. The IDO1 inhibitors were initially diluted in DMSO at 100 mM and subsequently diluted in potassium

phosphate 50 mM, added to the reaction mixture at final concentrations ranging from 100 μM to 200 pM and pre-incubated with the enzyme for 5 min at 25°C. The reaction was started by addition of tryptophan in the reaction mix and incubated 15 min at 37°C. The reaction was stopped by addition of 0.5 vol of 30% trichloroacetic acid and incubated 30 min at 60°C to hydrolyze *N*-formylkynurenine to kynurenine. The reaction was centrifuged at 3400 g for 5 min to remove precipitated protein and the supernatant was reacted with 2% (w/v) of *p*-dimethylaminobenzaldehyde in acetic acid. The reaction was incubated 10 min at 25°C and read at 480 nm in a spectrophotometer. Control samples with no IDO1 inhibitor, or with no IDO1 enzyme or with the reference inhibitors 1-methyl-tryptophan (200 μM) and menadione (1.2 μM) were used as controls to set the parameters for the non-linear regressions necessary for determination of the IC_{50} for each compound. At least 8-10 test compound concentrations were tested in duplicate for determination of the IC_{50} curves. Blank samples were prepared for each concentration of the IDO inhibitor tested. Nonlinear regressions and determination of the IC_{50} values were performed using the GraphPad Prism 4 software.

hIDO1 Cellular Potency Assay – Determination of EC_{50}

293-T-Rex™ cells (Invitrogen) constitutively expressing a Tet-Off operator binding repressor protein were transfected with plasmid pcDNA-tetO-IDO1 expressing human IDO1 under the control of the doxycycline-inducible CMV-tet promoter, and selected in DBZ medium (DMEM, 10 % FBS, 1X Penicillin + Streptomycin, 2mM L-glutamine, 5 μg/mL blasticidin and 25 μg/ml Zeocin) at 37°C with a 5% CO2 in air atmosphere. Individual clones were isolated by limiting dilution cloning from these populations. These clones were assayed for IDO1 activity and the clones that showed the highest levels of IDO1 activity inducible by doxycycline and low

expression under non-induced conditions were used for subsequent cell based IDO1 assays. To setup an IDO1 cell based activity assay, IDO1-293-T-Rex cells were harvested and resuspended in DBZ media at 10^6 cells/mL, and split into poly-D-lysine coated 96-well plates at 100,000 cells per well. 100 μL of Neutral medium (DBZ medium, 200 μM L-tryptophan) or Induction media (Neutral medium supplemented with 5 μM doxycycline) were added to the cells and incubated 28 h at 37 °C. After the IDO1 induction period, medium was removed and replaced with Induction or Neutral medium containing different concentrations of each IDO1 inhibitor (3 μM to 50 pM). At least 8-10 concentrations were tested in triplicate for determination of EC_{50} curves. The cells incubated in Neutral medium serve as negative control of the assay. The cells incubated in Induction medium and without inhibitor serve as the positive control of the assay. The incubation was carried out for 16 h at 37°C in a cell culture incubator. Supernatant was precipitated with 30% TCA and the cleared supernatant was treated with of 4% (w/v) of p-dimethylaminobenzaldehyde in acetic acid, incubated for 10 min. Kynurenine concentration is determined by measuring the absorbance at 480 nm.

Computational Methods

Small molecules were minimized with molecular operating environment (MOE) software. The protein was prepared using standard parameters, the charge of Fe was adjusted to Fe++. Minimization of ligands was done by fixing the nitrogen atom to the heme Fe++ at 2.1-2.2 Å. The Amber 10 EHT force field was used for energy minimizations. The molecular figures were rendered with PyMOL Version 2.3.0 (http://pymol.org), Schrodinger LLC.

Crystallography methods

IDO1 M1-G403 with a N terminal His tag was transformed into E.coli BL21 (DE3) cells. The cells were cultured in LB medium supplied with 0.5 mM 5-Aminolevulinic acid hydrochloride (5-ALA) at 37°C until OD 600 reached 0.6 and then induced with 0.3 mM Isopropyl β-D-1-thiogalactopyranoside (IPTG). The cultures were then incubated at 16°C for 20 hours and the cells were harvested by centrifugation at 9000 rpm for 10 min. The cells were lysed in buffer of 25 mM Tris pH 7.5, 150 mM sodium chloride, 10 mM imidazole, 1 mM phenylmethane sulfonyl fluoride (PMSF) and ethylenediaminetetraacetic acid (EDTA)-free protease inhibitor tablets (Roche). The protein was purified using equilibrated NiNTA (Roche) beads by gravity flow. The protein was eluted by 300 mM imidazole, treated with Tev protease, and placed into a 3500 MWCO dialysis cassette while dialyzing against a buffer of 25 mM Tris pH 7.5, 150 mM sodium chloride and 1 mM Tris (2-carboxyethy1) phosphine (TCEP). A reverse nickel column purification was done to capture the His-tagged protein while the cleaved protein was collected in the flow-through. The His-cleaved IDO1 protein was then concentrated with 10000 MWCO concentrators (Amicon) and then injected into an equilibrated S75 26/60 gel filtration column (GE) for the final purification. Fractions containing the protein were pooled, concentrated to 10 mg/mL and flash frozen for storage at -80°C. The protein concentration was determined by measuring A_{280} absorbance via a NanoDrop 2000c Spectrophotometer (Thermo Fisher Scientific). Heme incorporation was determined by measuring the ratio of A_{406}/A_{280}. Based on a reference value of 2.2 for 100% heme incorporation[34], IDO1 samples purified for crystallography were measured to have 70% heme.

Single crystals of IDO1-heme were grown in a 1:1 ratio of protein:well solution of 0.1M Bis Tris Propane pH 6.5, 20% Peg 3350 and 0.2M Potassium Thiocyanate. The crystals were then dehydrated by increasing the Peg 3350 concentration from 20% to 40% over a period of 4 days. Crystals were soaked in a cryobuffer of 10% Xylitol then flash frozen in liquid nitrogen.

Diffraction data were collected at the Stanford Synchrotron Radiation Lightsource beamline 12-2, reduced with autoPROC[35], and the structure was determined by molecular replacement methods as implemented in Phaser.[36] The final model was created using iterative rounds of manual rebuilding in COOT[37] and refinement with BUSTER[38]. Coordinates and structure factors are deposited with the Protein Data Bank (PDB) under accession code 6O3I.

Pharmacokinetics

All pharmacokinetic experiments in animals were carried out in conformance with AAALAC International and NIH guidelines as reported in the "Guide for the Care and Use of Laboratory Animals," National Research Council- ILAR, 8th Edition 2011. All animal procedures were approved by Iowa State University IACUC.

In vivo pharmacodynamics

See supporting information.

Supporting Information. Calculation of K_i, mode of inhibition of **NLG-919**, in vivo pharmacodynamics, single crystal X-ray data for **122-124**, **132** and **NLG-919**, co-crystal data for **NLG-919** bound in hIDO, chiral and achiral HPLC of selected final compounds, ^{1}H NMR spectra of **121- 124**, ^{1}H and ^{13}C NMR spectra of **NLG-919**.

The following files are available free of charge.

Kumar et al - Supporting Information.pdf

Molecular formula strings.csv.

PDB ID Codes: 6O3I (**NLG-919** co-crystal with hIDO). Authors will release the atomic coordinates and experimental data upon article publication.

CCDC accession code: **NLG-919**, 1905358; **132**, 1905087; **124.HCl**, 1905094; **123.HBr**, 1905089; **122.HBr**, 1905093. Authors will release the atomic coordinates and experimental data upon article publication.

AUTHOR INFORMATION

Corresponding Author

* Phone: 515-598-2927. Fax: 515-296-3520. Email: mmautino@linkp.com.

Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

▽These authors contributed equally. All medicinal chemistry performed at NewLink Genetics, Inc. and all protein crystallography performed at Genentech.

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ABBREVIATIONS

GCN2, general control nonderepressible 2; FoxP3, forkhead box P3; hIDO1, human indoleamine 2,3-dioxygenase 1; HLM, human liver microsomes; f_u, fraction unbound; CTX, Cyclophospamide; TosMIC, toluenesulfonylmethyl isocyanide; brsm, based on recovered starting material; DIPEA, *N, N*-diisopropylethylamine; Ph1, phase 1; SD, Sprague Dawley.

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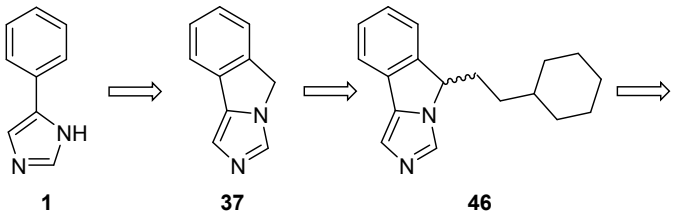
Table of Contents



1

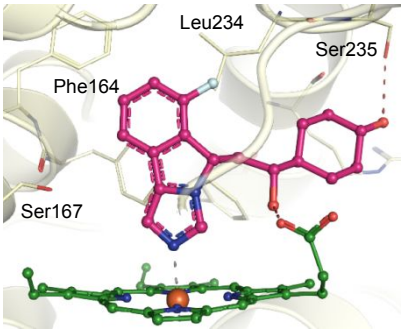
IC_{50} = 28 μM
EC_{50} = >25 μM

37

IC_{50} = 5.7 μM
EC_{50} = >25 μM

46

IC_{50} = 0.135 μM
EC_{50} = 1.1 μM



NLG-919 (magenta) bound in hIDO
IC_{50} = 28 nM; EC_{50} =75 nM; K_i = 5.7 nM