ACS Medicinal Chemistry Letters

Letter

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ACS Med. Chem. Lett., **Just Accepted Manuscript** • DOI: 10.1021/acsmedchemlett.9b00114 • Publication Date (Web): 03 Jun 2019

Just Accepted

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Discovery of Imidazoisoindole Derivatives as Highly Potent and Orally Active Indoleamine-2,3-dioxygenase Inhibitors

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KEYWORDS: IDO, immuno-oncology, SAR, lead optimization

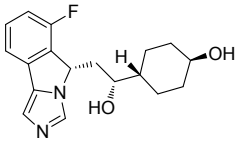
ABSTRACT: A novel series of imidazoisoindoles were identified as potent IDO inhibitors. Lead optimization toward improving potency and eliminating CYP inhibition resulted in the discovery of lead compound **25**, a highly potent IDO inhibitor with favorable pharmacokinetic properties. In the MC38 xenograft model in hPD1 transgenic mouse, **25** in combination with the anti-PD1 monoclonal antibody (**SHR-1210**) achieved a synergistic antitumor effect, superior to each single agent.

The immune system plays a vital role in the regulation of tumor growth, invasion, and metastasis. Immune checkpoint inhibitors that restore the capability of the immune system to recognize and eliminate malignant cells have produced impressive clinical benefit, but many patients across a wide range of malignancies still do not respond.[1] These findings imply there are additional immunoregulators that maintain productive immunosurveillance in cancer. Indeed, tumor cells escape immune elimination through evolving various tactics to evade, subvert, and manipulate innate and adaptive immunity. As a result, combinatory regimens with different targeted therapeutic agents are necessary to produce sustained therapeutic effect.[2,3] One of these agents is a family of tryptophan catabolizing enzymes including indoleamine-2,3-dioxygenase (IDO) and tryptophan 2,3-dioxygenase (TDO) which convert tryptophan first to *N*-formylkynurenine and further to kynurenine and additional metabolites. Both the depletion of tryptophan and signals generated by its metabolites are important contributors to immunosuppresion.[4-6]

Expression of IDO is widespread in human body, being most abundant in antigen-presenting cells such as macrophages and dendritic cells. IDO activity is increased in several tumor types and is correlated with a poor prognosis.[7,8] TDO is exclusively produced in the liver to maintain the systemic tryptophan levels in response to food uptake. Although the major role of IDO in immune regulation has been validated, there are recent evidences that suggest TDO might regulate immunosuppression similar to that of IDO.[9] IDO selective and IDO/TDO dual inhibitors have been the focus of research,[10-13] whereas TDO selective inhibitors remain elusive.[9,14]

Tumor cells hijack the immunosuppressive process by upregulating IDO activity in the tumor microenvironment, which leads to accelerated differentiation of $CD4^+$ T cell into regulatory T cells, as well as suppression of $CD8^+$ effector T cell and impaired dendritic cell functions. On the other hand, tumor cells evade immune-mediated eradication via PD-L1 expression, since the interaction of PD-L1 with PD1 inhibits the secretion of cytotoxic mediators by $CD8^+$ T cells. In addition, IDO was further upregulated upon blockade of PD1/PD-L1 interaction in mice due to compensatory mechanism.[15] Therefore, simultaneous blockade of both pathways may represent an opportunity to accomplish greater antitumor effects by complementary regulations of the cytotoxic T cells.

NLG-919 is one of the IDO/TDO dual inhibitors that have been evaluated in clinical trials alone or in combination with anti-PD-L1 antibody for various solid tumors.[16-18] Herein, we report the synthesis and SAR study of a novel series of imidazoisoindoles as potent IDO inhibitors and the identification of lead compound that synergized with PD1 blockade in a murine tumor model.



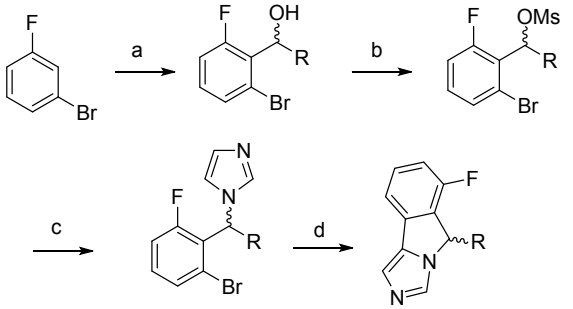
NLG-919

Figure 1. Imidazoleisoindole derivative as IDO inhibitor in clinical trial.

NLG-919 interacts with IDO via imidazoleisoindole core coordinating to the iron center of heme. Hydroxyl group on the side chain engages in an extensive hydrogen bond network and contributes to the biological activity.[19] However, three consecutive chiral centers exert tremendous structural complexities and synthetic challenges. We hypothesized that

modification of the side chain of NLG-919 with imidazoleisoindole core kept intact could offer the best opportunity to fine tune potency and physicochemical properties. Compounds **1-8** were synthesized via the route shown in Scheme 1.[20] Regioselective lithiation of *m*-bromofluorobenzene with LDA followed by nucleophilic addition to a variety of substituted aldehydes gave rise to corresponding alcohols. The resulting alcohols were mesylated and subsequently substituted by imidazole. The final intramolecular Pd-mediated cyclization furnished the tricyclic imidazoleisoindole core decorated with various appendages.

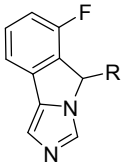
Scheme 1. Synthesis of 5-substituted imidazoleisoindoles [a]

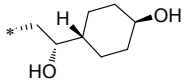
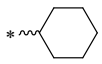
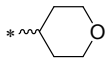
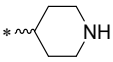
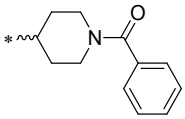
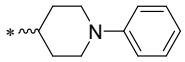
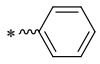
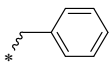


[a]Reagents and conditions: (a) (1) LDA, THF, –78°C, 1 h; (2) RCHO, –78°C, 1 h; (b) NaH, THF, MsCl, reflux, 48 h; (c) imidazole, NaH, DMF, 100°C, 12 h; (d) $Pd(OAc)_2$, PPh_3, Cy_2NMe, DMF, 100°C, 5 h.

The screening assays include enzymatic assays with purified recombinant human IDO/TDO proteins and cellular IDO inhibition assay in the Hela cell line. Cyclohexyl **1** exhibited comparable potency to NLG-919 as shown in Table 1. However, smaller cyclopentyl **2** was less potent in IDO assay. Tetrahydropyranyl **3** was a much weaker inhibitor compared to the all-carbon counterpart **1**. Piperidinyl **4** completely lost potency in all the assays since hydrogen bond donor at this position may not be tolerated. Blocking NH with amide (**5)** didn't improve potency. To our delight, phenylpiperidinyl **6** restored the potency similar to NLG-919. Replacement of cyclohexyl (**1)** with phenyl (**7**) led to a 20-fold drop of potency in the enzymatic assays. And benzyl **8** showed similar potency to phenyl **7**.

Table 1. SAR of 5-substituted imidazoleisoindoles

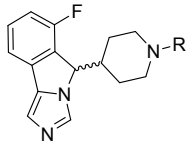


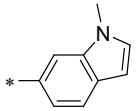
Compd	R	IDO IC_{50} (nM)[a]	TDO IC_{50} (nM)[a]	Hela IC_{50} (nM)[a]
NLG-919		79	247	434
1		108	85	568
2		764	95	1239
3		969	189	5344
4		>10000	>10000	ND[b]
5		3563	1275	ND[b]
6		87	338	544
7		2741	374	ND[b]
8		2734	793	ND[b]

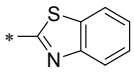
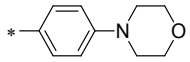
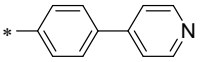
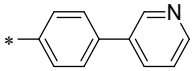
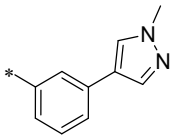
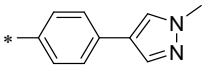
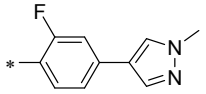
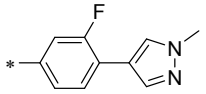
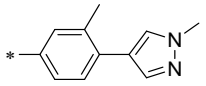
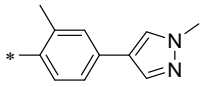
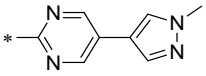
[a]Values are expressed as the mean of three independent determinations. ND[b] = not determined.

Compound **6** was selected as the start for the next round SAR study. A series of substituted piperidinyls were synthesized and assessed (Table 2**)**. In order to lower lipophilicity of compound **6**, replacement of the phenyl group with heteroaryls was investigated. Heteroaryls such as thiadiazole (**9**), indole (**10**) and benzothiazole (**11**) demonstrated enhanced potency toward IDO enzymatic assay but not cellular assay. Simple substituents such as fluorine (**12**) and cyano (**13**) didn't improve potency. The morpholine **14** showed 3-fold lower potency than **6**. Replacement of morpholine with pyridine (**15**) resulted in a 10-fold boost toward IDO activity. Among the aromatic substitutions tested, methylpyrazole **18** was the most potent in all three assays (IDO IC_{50} = 26 nM, TDO IC_{50} = 132 nM, Hela IC_{50} =101 nM, respectively). The *m*-substituted methylpyrazole **17** was 2-fold less potent than **18** due to unfavorable orientation of the aromatic substitution. Fluorine (**19**, **20**) and methyl (**21**, **22**) were tolerated on the central phenyl ring. Fluorine **20** was 2-fold more potent than **18**. And fluorine **19** and methyl **21** showed comparable potency to **18**. In compound **23**, central phenyl ring was replaced by pyrimidine and the potency decreased by 4-fold compared to **18**.

Table 2. SAR of 5-piperidinyl substituted imidazoleisoindoles



Compd	R	IDO IC_{50} (nM)[a]	TDO IC_{50} (nM)[a]	Hela IC_{50} (nM)[a]
9		297	586	571
10		27	376	203

11		19	195	440
12	3-F-Ph	72	371	167
13	4-CN-Ph	81	394	353
14		358	1029	1712
15		33	353	280
16		70	183	240
17		79	639	253
18		26	132	101
19		21	215	57
20		8	65	69
21		14	114	121
22		20	226	691
23		113	579	324

[a]Values are expressed as the mean of three independent determinations.

NLG-919 was reported as a potent inhibitor of CYP3A4 (IC_{50} = 2.8 uM).[21] Consequently, CYP inhibition assay was included at this stage of lead optimization. As shown in Table 3, substitutions on the central phenyl ring inevitably led to inhibition of different CYP isoforms. The most potent compound **20** significantly inhibited CYP3A4 (IC_{50} = 2.6 uM). Compound **18** had a clean CYP profile with only moderate inhibition of CYP3A4.

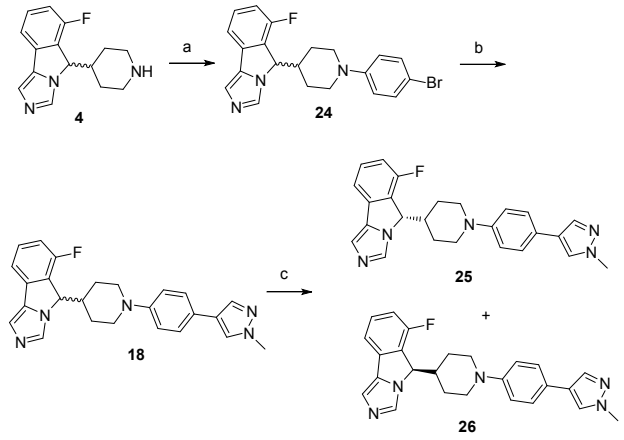
Table 3. CYP inhibition of compounds 18-21

Compd	1A2 IC_{50} (uM)[a]	2C9 IC_{50} (uM)[a]	2C19 IC_{50} (uM)[a]	2D6 IC_{50} (uM)[a]	3A4 IC_{50} (uM)[a] m/t[b]
18	>10	>10	>10	>10	8.4/6.08
19	8.95	0.94	1.15	9.61	0.35/0.95
20	>10	7.4	6.81	>10	4.57/2.6
21	>10	0.3	1.57	4.51	0.21/0.44

[a]Values are expressed as the mean of three independent determinations. [b]Midazolam as substrate/testosterone as substrate.

Lead compound **18** was synthesized by the route shown in Scheme 2. Chiral separation of the racemate **18** gave rise to the desired enantiomer **25**, while another enantiomer **26** was totally inactive in all IDO assays (IC_{50} > 10 μM).

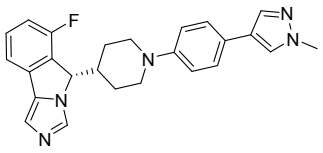
Scheme 2. Synthesis of lead compound 25[a]



[a]Reagents and conditions: (a) 1,4-dibromobenzene, $Pd_2(dba)_3$, BINAP, *t*BuONa, toluene, 80°C, 12 h; (b) 1-methyl-4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1H-pyrazole, $Pd(dppf)Cl_2$, Na_2CO_3, DME/H_2O, microwave ,120°C, 40 min; (c) chiral separation.

Compound **25** was fully profiled *in vitro* and *in vivo* as shown in Table 4. It was a highly potent dual IDO/TDO inhibitor in both enzymatic assays and cellular assay. It was clean in *in vitro* toxicity studies including CYP and hERG. It showed high plasma protein bonding in mouse, dog and human and high liver microsome stability in rat and human. Pharmacokinetic profiling of **25** in mouse, rat and dog demonstrated good oral exposure and bioavailability (F = 59.6%, 60.3%, 27.3%, respectively) in all species.

Table 4. Profiling of compound 25



Assay	25
Enzymatic IDO IC_{50} (nM)[a]	9.7
Enzymatic TDO IC_{50} (nM)[a]	47
Cellular Hela IC_{50} (nM)[a]	76
CYP inhibition (1A2, 2C9, 2C19, 2D6, 3A4)	> 30 uM
hERG	> 30 uM
PPB (mouse/dog/human)	99.6% / 99.2% / 99.7%
Liver microsome stability (rat/human) $T_{1/2}$ (min)	142 / 147

Mouse PK@10 mg/kg	
C_{max}(ng/mL)	3605
AUC (ng/mL*h)	29116
$t_{1/2}$ (h)	4.55
Bioavailability F%	59.6%
Rat PK@10mg/kg	
C_{max}(ng/mL)	1848
AUC (ng/mL*h)	6133
$t_{1/2}$ (h)	1.22
Bioavailability F%	60.3%
Dog PK@2 mg/kg	
C_{max}(ng/mL)	166
AUC (ng/mL*h)	1340
$t_{1/2}$ (h)	6.49
Bioavailability F%	27.3%

[a]Values are expressed as the mean of three independent determinations.

In order to gain insight into the superior potency of **25**, computational study was performed. As shown in Figure 2, **25** coordinated to the iron center of heme in a similar manner to NLG-919, while the side chain of **25** stuck deeper into pocket B. The methylpyrazol ring engaged in a cation-π interaction with Arg231 with a distance of 3.38Å. Cation-π interactions are ubiquitously found in proteins, protein-ligand and protein-DNA complexes, and impose influence on biological structures, molecular recognition and catalysis.[22] Due to the additional cation-π interaction, **25** served as a more potent inhibitor, despite the lack of the hydroxyl group which formed a hydrogen bonding with Ser235 as in NLG-919.

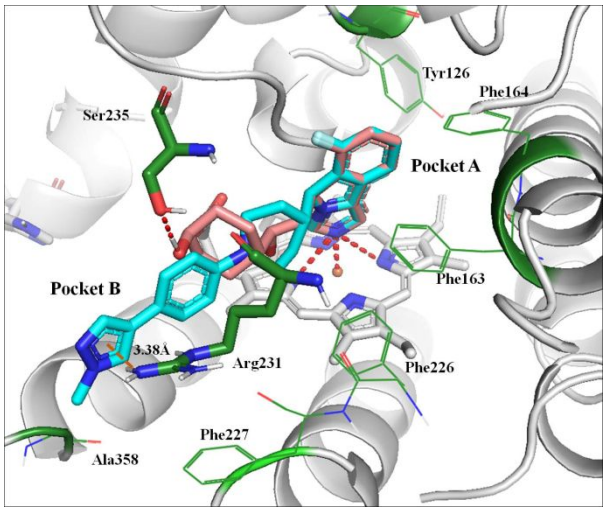


Figure 2. Molecular docking of **25** (cyan) binding to the IDO active site (PDB code: 2D0T). NLG-919 (salmon) and **25** are superimposed for comparison. The key cation-π interaction is indicated by brown dashed line.

Based on the promising *in vitro* activity and pharmacokinetic data, **25** was further evaluated in the *in vivo* pharmacodynamic study. A single dose of 100 mg/kg of **25** was administrated orally to C57 mice. The maximum kynurenine reduction by 57% was observed at 2 h after dosing (see Supporting Information Figure S1).

The hPD1 transgenic mice implanted with MC38 tumors were treated with **25** to evaluate the antitumor efficacy. As shown in Figure 3, oral treatment with **25** (bid × 14) demonstrated dose-dependent tumor growth inhibition (20 mg/kg, TGI = 39%; 50 mg/kg, TGI = 78%). The anti-PD1 monoclonal antibody **SHR-1210** was only modestly efficacious in this tumor model (5 mg/kg, ip, qod × 8, TGI = 65%).[23] Compound **25** was dosed in combination with **SHR-1210** using the following schedule: (**SHR-1210**: 5 mg/kg, ip, qod × 8; **25**: 20 mg/kg or 50 mg/kg, po, bid × 14). The combination regimen at both dose levels achieved significantly enhanced antitumor activity (TGI > 90%) compared to either treatment alone. It was noted that no body weight loss was observed for all the treatment groups.

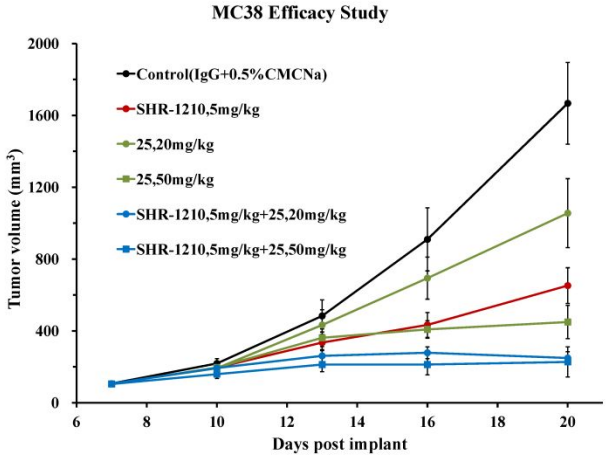


Figure 3. Efficacy study of **25** alone and in combination with **SHR-1210** in the MC38 xenograft model in hPD1 transgenic mouse.

In summary, a highly potent IDO inhibitor **25** with favorable preclinical toxicity and pharmacokinetic properties was discovered through several rounds of SAR studies with the aim of improving potency and eliminating CYP inhibition liabilities. Compound **25** proved to be orally efficacious in the MC38 xenograft model and its combination with anti-PD1 monoclonal antibody showed a synergistic antitumor effect.

ASSOCIATED CONTENT

Supporting Information

Biological assays, pharmacokinetic assays, *in vivo* pharmacodynamic study, *in vivo* efficacy study, computational methods, experimental procedures and analytical data for key compounds are included.

The Supporting Information is available free of charge on the ACS Publications website.

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Author Contributions

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

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

We thank analytical group, DMPK group of the company for their contributions.

ABBREVIATIONS

PD-1, programmed death 1; LDA, lithium diisopropylamide; THF, tetrahydrofuran; DME, 1,2-dimethoxyethane; TEA: triethylamine; MsCl: methanesulfonyl chloride; DMF, dimethyl formamide; BINAP, 2,2'-bis(diphenylphosphino)-1,1'-binaphthyl; dba: dibenzylideneacetone; SAR, structure and activity relationship; CYP, cytochrome p450 enzyme; hERG, human ether-a-go-go-related gene; PPB, plasma protein bonding; PK, pharmacokinetic; po, orally; ip, intraperitoneally; bid, twice daily; qod, every other day; TGI, tumor growth inhibition.

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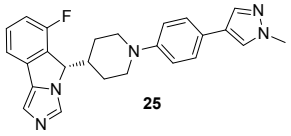

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IDO IC_{50}: 9.7 nM
TDO IC_{50}: 47 nM
Hela IC_{50}: 76 nM
Good bioavailability in preclinical species
Efficacious in MC38 xenograft model
Synergistic antitumor effect in combination with PD1 monoclonal antibody