

Conformational-Analysis-Guided Discovery of 2,3-Disubstituted Pyridine IDO1 Inhibitors

Emily C. Cherney,* Liping Zhang, Weiwei Guo, Audris Huang, David Williams, Steven Seitz, Weifang Shan, Xiao Zhu, Johnni Gullo-Brown, Derrick Maley, Tai-an Lin, John T. Hunt, Christine Huang, Zheng Yang, Celia J. D'Arienzo, Lorell N. Discenza, Asoka Ranasinghe, Mary F. Grubb, Sarah C. Traeger, Xin Li, Kathy A. Johnston, Lisa Kopcho, Mark Fereshteh, Kimberly A. Foster, Kevin Stefanski, Diane Delpy, Gopal Dhar, Aravind Anandam, Sandeep Mahankali, Shweta Padmanabhan, Prabhakar Rajanna, Venkata Murali, T. Thanga Mariappan, Shabeerali Pattasser, Roshan Y. Nimje, Zhenqiu Hong, James Kempson, Richard Rampulla, Arvind Mathur, Anuradha Gupta, Robert Borzilleri, Gregory Vite, and Aaron Balog



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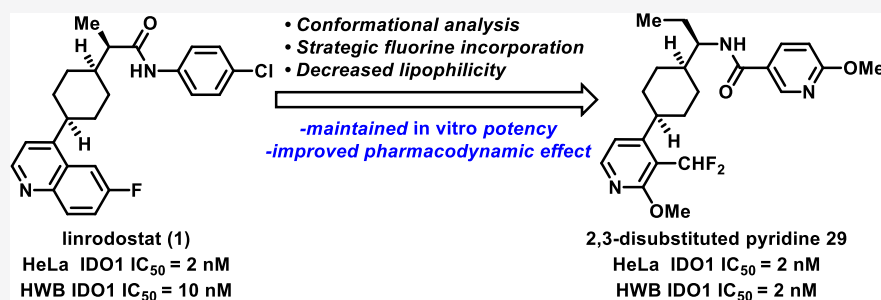
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ABSTRACT: IDO1 inhibitors have shown promise as immunotherapies for the treatment of a variety of cancers, including metastatic melanoma and renal cell carcinoma. We recently reported the identification of several novel heme-displacing IDO1 inhibitors, including the clinical molecules linrodostat (BMS-986205) and BMS-986242. Both molecules contain quinolines that, while being present in successful medicines, are known to be potentially susceptible to oxidative metabolism. Efforts to swap this quinoline with an alternative aromatic system led to the discovery of 2,3-disubstituted pyridines as suitable replacements. Further optimization, which included lowering ClogP in combination with strategic fluorine incorporation, led to the discovery of compound 29, a potent, selective IDO1 inhibitor with robust pharmacodynamic activity in a mouse xenograft model.

KEYWORDS: Indoleamine 2,3-dioxygenase, IDO1, immuno-oncology, cancer immunotherapy, quinoline, metabolism

Over the past several decades, the immune system's role in cancer recognition and killing has been a source of debate. In recent years, as mechanisms of tumoral immune escape have been elucidated and proven in the clinic with checkpoint inhibitors such as Opdivo, Keytruda, and Yervoy, the field has gained mainstream acceptance.¹ Further understanding of the impact of amino acid metabolism on the tumor microenvironment (TME) has led to the identification of immuno-oncology (IO) targets capable of small-molecule perturbation.² One such target, indoleamine 2,3-dioxygenase-1 (IDO1), is a cytosolic enzyme expressed in tumors and tumor-associated cells such as dendritic cells (DCs) and endothelial cells. High IDO1 expression in the tumor or tumor-draining lymph nodes has been associated with poor prognosis.³ Checkpoint inhibitors activate T cells within the tumor and cause antitumor inflammation, which can increase IDO1 expression in the

TME. Preclinically, IDO1 inhibition has been shown to work synergistically with both conventional cancer therapies and checkpoint immunotherapy.^{4–6} This combination of attributes makes IDO1 a promising target for cancer immunotherapy.

IDO1 catalyzes the oxidative cleavage of the 2,3-double bond in D- or L-tryptophan. This oxidative cleavage event is the initial and rate-limiting step of tryptophan catabolism. IDO1 is a monomeric heme-containing dioxygenase, meaning that both atoms of molecular oxygen are incorporated into the

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tryptophan-derived oxidative cleavage product, *N*-formylkynurenine, which can then undergo deformylation with kynurenine formamidase to form kynurenine.⁷ The closely related enzymes IDO2 and tryptophan 2,3-dioxygenase (TDO) are also capable of effecting this transformation. The physiological role of IDO2 has not been fully elucidated, and although it is primarily expressed in hepatocytes, it is not critical for systemic tryptophan homeostasis, as is the case with TDO.⁸ IDO2 possesses only 43% sequence homology with IDO1 and weaker affinity for *L*-tryptophan.⁹ TDO (sometimes called TDO2) is tetrameric as opposed to monomeric, enantiospecific for the catabolism of *L*-tryptophan, and expressed mainly in the liver.^{10,11} TDO is primarily responsible for maintaining homeostasis of dietary *L*-tryptophan, suggesting a role distinct from that of IDO1.¹²

IDO1 expression can be induced in a variety of ways, including type I and type II interferons (including IFN γ), reverse signaling from inhibitory T cell receptors such as binding of CTLA4 to CD80, and COX-2 *via* PGE2 production.¹³ High levels of IDO1 expression in the TME lead to localized *L*-Trp depletion and high kynurenine levels. Low *L*-Trp levels can activate stress response kinase GCN2, leading to biased differentiation of naive T cells into regulatory T (T_{reg}) cells, T cell anergy, and inhibition of T cell proliferation.^{14,15} Additionally, activation of GCN2 can alter DCs to a more immunosuppressive phenotype that produces TGF β and IL-10.¹⁶ Kynurenine can activate the aryl hydrocarbon receptor (AhR) upon binding, which can also cause differentiation of naive T cells into T_{reg} cells and lead to the formation of immunosuppressive DCs.¹⁷ These myriad effector pathways can ultimately lead to immune tolerance toward tumoral antigens.¹⁸

Encouraged by the promising preclinical and clinical data available for IDO1, many companies targeted small-molecule inhibitors for cancer immunotherapy. Several candidates have entered the clinic in combination with chemotherapy, vaccines, and checkpoint immunotherapy.¹⁹ More specifically, Bristol Myers Squibb advanced linrodostat (**1**)²⁰ and closely related BMS-986242 (**2**)²¹ into clinical studies (Figure 1a). The unique binding mode of these inhibitors to *apo*-IDO1 (**3** in Figure 1) and mechanistic studies were previously disclosed.^{22,23}

While our (and others') initial efforts toward identifying an IDO1 inhibitor that was differentiated from **1** focused primarily on structure–activity relationship (SAR) exploration in the amide portion of the molecule,^{21,24a,c} we subsequently sought to modify the quinoline present in both **1** and **2**. Quinolines, while found in many marketed drugs, including antimalarials²⁵ and anticancer drugs,²⁶ are known sites of metabolic activation.²⁷ Indeed, *in vitro* biotransformation studies of **2** in hepatocytes of preclinical species revealed that the quinoline was susceptible to metabolic oxidation. For this reason, efforts were initiated to improve the metabolic stability of this chemotype by either adding electron-withdrawing groups to the quinoline to reduce the *N*-oxidation potential^{24a} or replacing it entirely while simultaneously lowering the lipophilicity, which is the focus of this work.

Quinoline replacements were designed to maintain the π -stacking interaction with Phe270 and probe whether hydrogen bonding with Arg343 was necessary (Figure 1b). Additionally, computer-aided drug design (CADD) conformational studies revealed that the dihedral angle between the quinoline and the cyclohexane in the X-ray cocrystal of **3** resided very close to the dihedral angle of the calculated solution-state intrinsic torsional energy minimum. During analogue design, intrinsic torsional

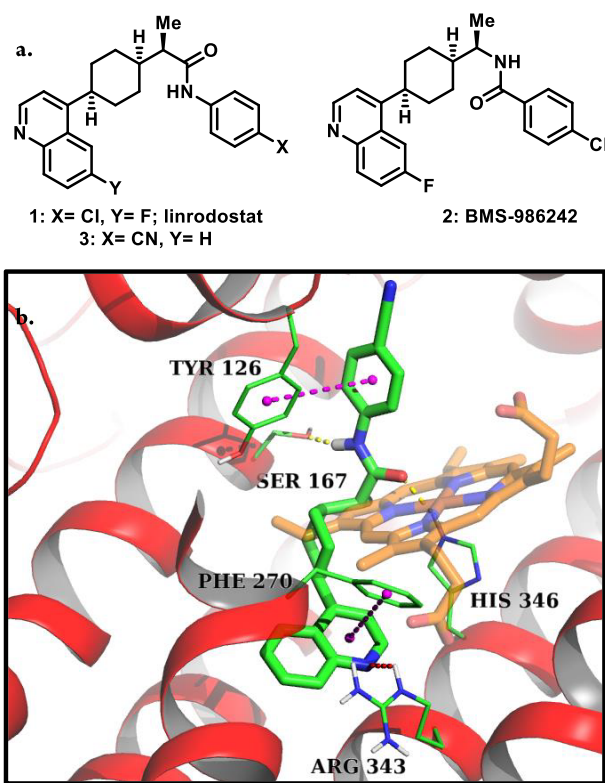


Figure 1. (a) IDO1 inhibitors either clinically investigated by Bristol Myers Squibb (**1**, **2**) or crystallized with hIDO1 protein (**3**). (b) X-ray cocrystal of human IDO1 and compound **3** (in green), a closely related analogue of linrodostat (**1**). Heme (in orange) has been added to show that **3** occupies the heme binding pocket.

energy analysis was used to prioritize compounds biased to reside in an active conformation.²⁸

Docking studies showed that both *cis*-cyclohexanes in conformer I (the cocrystal conformer of **3**) and *trans*-cyclohexanes in conformer III were predicted to bind well (see Figure 2). Modeling also predicted that *cis*-cyclohexanes in conformer II and *trans*-cyclohexanes in conformer IV would not bind. We worried that replacing the *cis*-cyclohexylquinoline in **3**

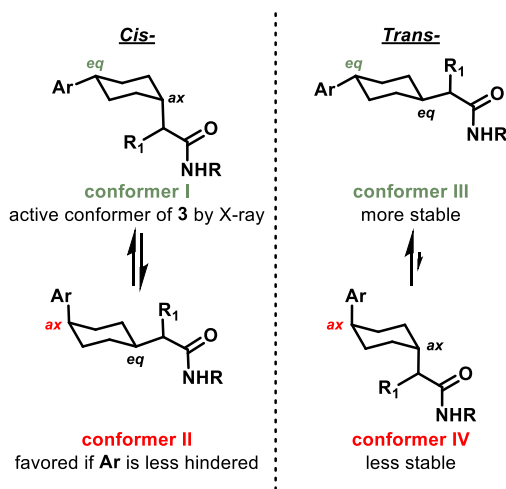
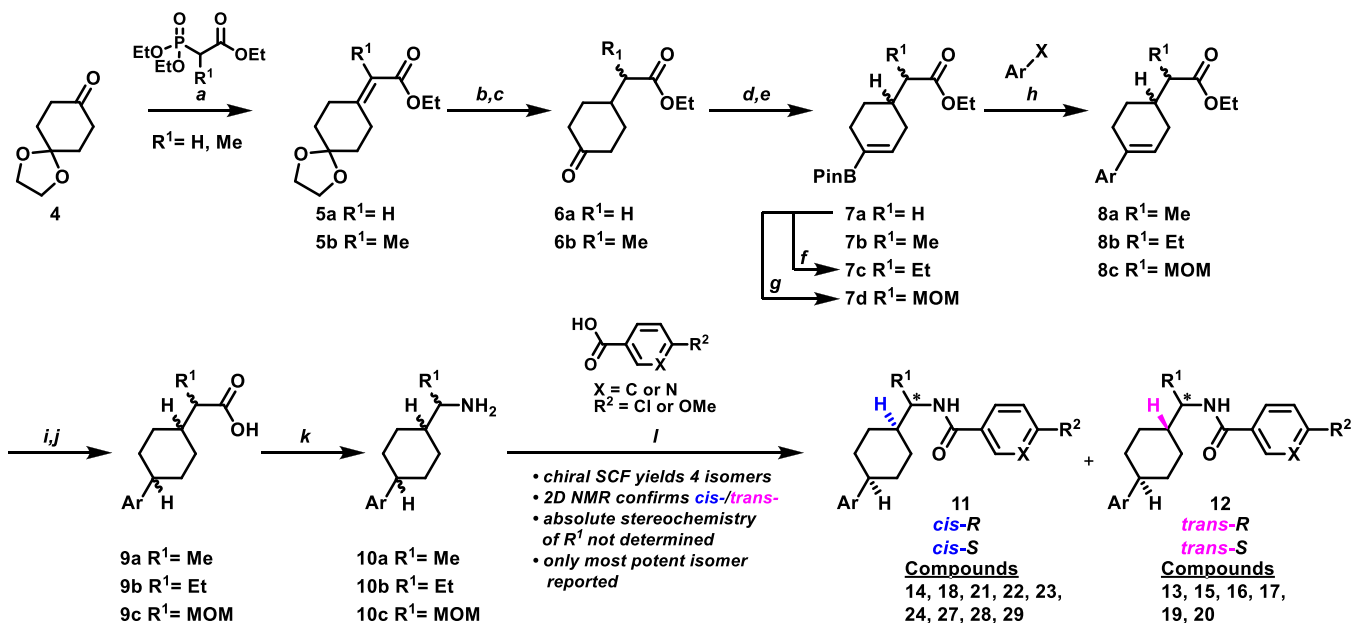


Figure 2. Potential chair conformational isomers of *cis*- and *trans*-cyclohexane analogues: conformers I and III bind to hIDO1 in a docking model, whereas conformers II and IV do not.

Scheme 1. General Synthetic Scheme for Compounds 14–25 and 27–29^a

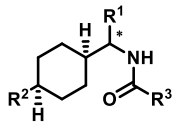
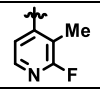
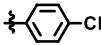
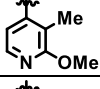
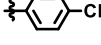
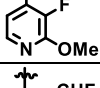
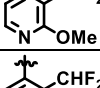
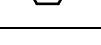
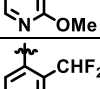
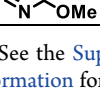
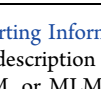
^aReagents and conditions: (a) NaH, THF, 0 °C (H = 96% yield, Me = 78% yield); (b) Pd–C, MeOH, H₂ (balloon to 50 PSI) (H = 98% yield, Me = 89% yield); (c) 4 N HCl, acetone, 45 °C (H = 73% yield, Me = 79% yield); (d) NaHMDS (2 M in THF), *N*-phenyltrifluoromethanesulfonimide, MTBE, –78 °C (H = 76% yield, Me = 96% yield); (e) B₂Pin₂, KOAc, NaBr, Pd(dppf)Cl₂, dioxane, 110 °C (H = 50% yield, Me = 60% yield); (f) LDA, DMPU; EtI, –78 °C (95% yield); (g) LDA, MOMCl, –78 °C (61% yield); (h) Pd(PPh₃)₄, K₂CO₃, dioxane, H₂O, 100 °C (27–99% yield); (i) Pd–C, MeOH, HCOONH₄, 80 °C or Pd–C, MeOH, H₂ (30–99% yield); (j) LiOH, H₂O, THF, 70 °C, 72 h (58% to *ca.* quant. yield); (k) DPPA, TEA, toluene, 70 °C; LiOH, THF (51–78% yield); (l) EDCl, HOBT, DIPEA, DMF, rt or HATU, DIPEA, DMF (3–39% yield).

Table 1. Quinoline Replacements: Broad Structure–Activity Relationships and Metabolic Stability

Cmpd.	R ¹	Cis- or Trans-	R ²	HeLa/ M109 (IC ₅₀ , nM) ^a	Met. Stab. % Rem (H/M) ^b	Cmpd.	R ¹	Cis- or Trans-	R ²	HeLa/ M109 (IC ₅₀ , nM) ^a	Met. Stab. % Rem (H/M) ^b
2	Me ^c	Cis-		2/2	54/21	18	Et	Cis-		475/ >1000	3/1
13	Et	Trans-		26/97	88/65	19	Et	Trans-		48/121	56/8
14	Et	Cis-		43/196	0/0	20	Et	Trans-		40/40	89/56
15	Et	Trans-		14/75	94/90	21	Me	Cis-		12/16	1/1
16	Et	Trans-		80/193	20/42	22	Me	Cis-		7/3	16/15
17	Et	Trans-		19/31	80/28	23	Me	Cis-		4/6	30/3

^aData are reported as averages of ≥2 test results. See the Supporting Information for a description of the assay conditions. ^bPercentage of the parent compound (0.5 μM) remaining after a 10 min incubation with 1 mg/mL HLM or MLM. ^cThe absolute stereochemistry of R¹ is R.

Table 2. 2,3-Disubstituted Pyridines as Quinoline Replacements

	Cmpd	R ¹	R ²	R ³	HeLa/ M109 (IC ₅₀ , nM) ^a	HWB (IC ₅₀ , nM) ^b	Met. Stab. % rem (H/R/M) ^c	ClogP
	24	Me			5/3	47 ± 11 N = 4	0/1/1	5.48
	25	Et			2/7	2.8 ± 1.5 N = 4	71/34/15	5.94
	26	Et			3/22	11 ± 1.1 N = 2	68/21/15	5.54
	27	Et			2/6	4.4 ± 5.8 N = 4	85/57/30	5.85
	28	MOM			2/11	4.1 ± 3.2 N = 2	68/35/15	4.94
	29	Et			2/14	2.3 ± 2.0 N = 22	87/90/60	4.46

^aData are reported as averages of ≥3 test results. See the [Supporting Information](#) for a description of the assay conditions. ^bData are reported as averages of ≥2 test results. See the [Supporting Information](#) for a description of the assay conditions. ^cPercentage of the parent compound (0.5 μM) remaining after a 10 min incubation with 1 mg/mL HLM, RLM, or MLM.

with smaller aryl groups could bias new analogues toward disfavored conformer II. We were also wary of only targeting *cis*-cyclohexanes and potentially overlooking active *trans*-cyclohexanes, which have a strong energetic bias to reside in conformer III. Therefore, both *cis*- and *trans*-cyclohexanes were pursued simultaneously *via* a stereodivergent route during initial SAR exploration ([Scheme 1](#)).

Compounds 13–24 and 27–29 can be synthesized as mixtures of diastereomers from intermediate 7a or 7b starting from cyclohexanedione monoketal 4 following a previously reported five-step sequence.²⁹ Horner–Wadsworth–Emmons olefination followed by reduction and ketal hydrolysis gave ketones 6a or 6b. Formation of the vinyl triflate followed by Miyaura borylation provided 7a or 7b. For 7c or 7d, 7a was alkylated with either ethyl iodide or methoxymethyl chloride, respectively. Aryl halides could be installed *via* Suzuki coupling to give 8a–c. Reduction of the styrenyl olefin *via* transfer hydrogenation led to a mixture of *cis* and *trans* diastereomers. In some cases, diastereomers were separated at this point. Hydrolysis of the ester yielded acids 9a–c. Curtius rearrangement provided amines 10a–c, which were then coupled to the necessary acids to give compounds 13–24 and 27–29 as mixtures of four diastereomers. For the final compounds 25 and 26, an additional displacement with sodium methoxide was performed on advanced intermediates to provide 2-methoxypyridines (see the [Supporting Information](#)). For each of the final compounds reported, chiral resolution was performed, and the most potent enantiomer is reported in [Tables 1](#) and [2](#). While absolute stereochemistry was not routinely determined, 2D NMR techniques were employed to assign relative *cis/trans* stereochemistry for all of the final compounds (see the [Supporting Information](#)).

As stated above, while absolute stereochemistry was not routinely determined, all of the mixtures were chirally resolved, and the relative stereochemistry was assigned by 2D NMR analysis. The most potent enantiomer is reported below in [Table](#)

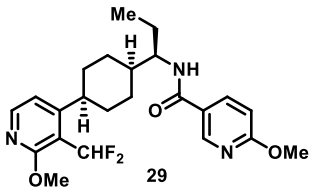
1. On the basis of intrinsic torsional energy analysis, aromatic or heteroaromatic rings containing substitution at positions either *ortho* or *meta* to the cyclohexyl ring were prioritized. Pyridone 13 and benzodioxole 14 were prioritized on the basis of this strategy. Interestingly, compounds 13 and 14 maintained double-digit nanomolar activity in human cellular assays without containing a hydrogen-bond acceptor to interact with Arg343. Unfortunately, both were significantly less potent than BMS-986242 (2), and 14 suffered from very poor metabolic stability. Contrary to our original hypothesis, *para*-substituted arene 15 demonstrated that neither *ortho* nor *meta* substitution is required to maintain double-digit nanomolar human IDO1 potency. Also, the *trans* isomer of 15 was superior to the *cis* isomer (data not shown). This *trans* preference was observed in several compounds where *ortho* substitution was absent (15, 16, 17, 19, and 20), which could be due to a preference for *cis* isomers with these aryl groups to adopt putatively disfavored conformer II ([Figure 2](#)). Compound 21 was an exception to this trend, perhaps because the 4-pyridyl nitrogen can interact with Arg343 to bias 21 to adopt conformer I in the binding pocket. Conversely, when *ortho* substitution was present, the *cis* isomer was usually preferred (compounds 14, 18, 22, and 23), which could be due to a preference for these aryl groups to adopt known active conformer I ([Figure 2](#)). 4-Pyridyl compounds such as 21, 22, and 23 not only had torsional energy minima corresponding to the active conformation of the quinoline but also retained the nitrogen to potentially interact with Arg343. As expected, 21, 22, and 23 were more potent in cellular assays, with 22 and 23 achieving single-digit nanomolar activity. However, they suffered from very poor metabolic stability.

Our focus then turned to improving the metabolic stability of the 4-pyridyl analogues ([Table 2](#)). Analogue 24, with 2-fluoro in place of the 2-methyl group in 23, performed in the same fashion as 23, displaying poor metabolic stability. However, swapping for a 2-methoxy group (25) led to a significant improvement in not only human whole-blood activity but also metabolic

stability. The 3-methyl group in **25** was a potential metabolic soft spot, so it was replaced with a fluorine (**26**) or a difluoromethyl group (**27**) in an attempt to bolster the metabolic stability.³⁰ Gratifyingly, **27** with the difluoromethyl group performed comparably to **25** in cellular and whole-blood assays with further improved metabolic stability. Attempts to introduce more polarity into the molecule and lower ClogP to improve the metabolic stability included adding a MOM group at R¹ (**28**) and a methoxypyridine at R³ (**29**). SAR around the amide portion of the molecule has been discussed in greater detail previously.^{21,24} Modification of the cyclohexyl core to more polar, saturated heterocycles (not shown) did not yield superior leads. While the MOM group in **28** was unsuccessful in improving the metabolic stability, methoxypyridine-containing **29** showed an overall superior profile in terms of human cellular and whole-blood potency as well as metabolic stability across species. Compound **29** has a lower ClogP than prior candidates **1** and **2** (6.39 for **1** vs 5.94 for **2** vs 4.46 for **29**) and other closely related analogues shown in Table 2.

The relative stereochemistry around the cyclohexane in **29** was confirmed to be *cis* via 2D NMR experiments. The absolute stereochemistry at R¹ was confirmed to be *R*, which is consistent with the stereochemistry in **1** and **2** (see the structure in Table 3).³¹ Further *in vitro* profiling revealed that lower ClogP

Table 3. *In Vitro* Profile of Compound 29

	
met. stability CYP (t _{1/2} min)	32 (H), 11 (M), 20 (R), 7 (D), 3 (C)
met. stability UGT (t _{1/2} min)	>120 (H, R, M, D, C)
PAMPA Papp (pH 7.4)	1070 nm/s
Caco Papp (a–b:b–a)	122:56 nm/s
PXR-TA EC ₅₀ (μM)	1.9 (22% Ymax)
human CYP IC ₅₀ (μM)	2C9, 1.89; 2C8, 4.01; 2C19, 9.50; others, >10
hERG IC ₅₀ (μM)	17.4 (61% inh. at 30 μM)
Na ⁺ patch clamp	<18% inh. at 10 μM (1 and 4 Hz)
Ca ²⁺ patch clamp IC ₅₀ (μM)	0.76
protein binding (% free)	H 0.8, M 1

correlated with improved *in vitro* oxidative metabolism in most species (CYP t_{1/2} for **2** = 14 (H), 4 (M), 10 (R), 10 (D), 2 (C)) and lower protein binding (0.8/1% free for H/M for **29** vs <0.1% free for both H/M for **1** vs <0.3/<0.2% free for H/M for **2**). Compound **29** had good intrinsic permeability and only modest PXR activation. Like compound **2**, **29** did show CYP inhibition in some isoforms, but it was less potent (human CYP IC₅₀(μM) for **2**: 0.74 for 2C9; 1.39 for 2C8; 3.37 for 2C19). Compound **29** showed some hERG inhibition at higher concentrations as well as sodium and calcium channel inhibition, but the hERG inhibition was still attenuated compared with **2** (87% inhibition at 10 μM).

In a PK–PD study in a nu/nu mouse xenograft model implanted with human SKOV3 cells (ovarian cancer cell line), compound **29** had lower tumor exposures than **1** at doses of 60, 120, and 180 mg/kg, and the exposures were not dose-proportional for doses from 20 to 180 mg/kg (Table 4). Despite the lower exposure, the 20 mg/kg dose of **29** still caused a significant reduction in tumor kynurenine (58%), possibly due

Table 4. PK–PD study of Compound 29 vs Linrodostat in a Human SKOV3 Xenograft Tumor Mouse Model

cmpd	dose ^a (mg/kg)	PK ^b	PD ^c (%)
29	20 ^c	7.9	58
29	60 ^c	9.2	57
29	120 ^c	13	63
29	180 ^c	13	80
1	60 ^c	18	65

^aQD po dosing. ^bPK is AUC (0–24 h) in μM·h measured in the tumor. ^cPD is percent kynurenine AUEC (0–24 h) reduction measured in the tumor. %Kyn reduction was measured at steady state after the fifth dose (calculated as the area under the Kyn concentration–time curve from 0 to 24 h) and compared with that of the vehicle control.

to lower protein binding (*vide supra*). While the 180 mg/kg dose displays a deeper PD effect (80% reduction), it is unclear why the 120 mg/kg dose, despite reaching similar exposure in the tumor, reached only 63% kynurenine reduction. Pharmacokinetic studies in preclinical species (Table 5) showed moderate

Table 5. PK Parameters for Compound 29

parameter	mouse ^a	rat ^a	dog ^a	cyno ^a
dose (mg/kg) iv/po	1 ^b /10 ^c	0.5 ^b /10 ^c	1 ^d /5 ^e	1 ^d /5 ^e
CL (mL/min/kg) iv	33	15	18	29
V _{ss} (L/kg) iv	6.2	4.0	9.0	4.5
AUC _{total} (μM·h) iv/po	1.2/3.9	1.3/8.7	2.3/3.2	1.3/0.029
t _{1/2} (h) iv	5.0	7.1	14	11
F _{po} (%)	33	34	28	4.5

^aData are reported as averages over ≥3 animals. ^b80% PEG 400, 20% water. ^c5% ethanol; 55% PEG 400; 20% propylene glycol, 20% TPGS. ^d70% PEG 400; 30% water. ^e90% PEG 400; 10% solutol.

to low clearance in mouse and rat, respectively, but higher clearance in dog and particularly cyno, which also showed very low oral bioavailability. High volumes of distribution were observed across species. Additionally, liver accumulation was seen in rats (24 h liver/plasma ratio = 8.3).

In vitro biotransformation studies in hepatocytes (H/R/D/C) and *in vivo* metabolite identification studies in nu/nu mice from the PK–PD study confirmed demethylation of the difluoromethylpyridine as the primary route of metabolism. The demethylated metabolite of **29** was synthesized to confirm its structure. While the metabolite was much less active against IDO1 (IDO1 HWB IC₅₀ > 1000 nM), it was still liable to oxidative metabolism (CYP t_{1/2} = 24 (H), 36 (M), 26 (R), 38 (D), 7 (C)). The –OCD₃ analogue of **29** was synthesized in an attempt to attenuate the demethylation, but the *in vitro* oxidative metabolic stability was not substantially improved (CYP t_{1/2} = 15 (H), 11 (M), 18 (R), 29 (D), 8 (C)).

In summary, structure-based drug design, torsional angle energy minima, and the potential for conformational isomerization of cyclohexane were considered in designing replacements for the quinolines found in linrodostat (**1**) and BMS-986242 (**2**). A stereodivergent synthesis was pursued that identified a variety of successful quinoline replacements with both *cis* and *trans* stereochemistry around the cyclohexane. Strategic incorporation of fluorine and polarity into 2,3-disubstituted 4-pyridyl analogues led to the identification of compound **29**. Despite lower exposures, compound **29** achieved similar or even superior (180 mg/kg dose) kynurenine

reduction in tumors in a human SKOV3 xenograft tumor mouse model compared with 1.

■ ASSOCIATED CONTENT

SI Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsmmedchemlett.1c00236>.

Biological assay protocols, *in vivo* pharmacokinetic–pharmacodynamic study protocols, experimental procedures, and analytical data for all final compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Emily C. Cherney – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States; orcid.org/0000-0003-1977-2900; Phone: 609-252-6064; Email: emily.cherney@bms.com

Authors

Liping Zhang – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States; orcid.org/0000-0002-1797-7227

Weiwei Guo – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Audris Huang – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

David Williams – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Steven Seitz – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Weifang Shan – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Xiao Zhu – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States; orcid.org/0000-0001-7647-4390

Johnni Gullo-Brown – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Derrick Maley – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Tai-an Lin – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

John T. Hunt – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Christine Huang – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Zheng Yang – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Celia J. D'Arienzo – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Lorell N. Discenza – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Asoka Ranasinghe – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Mary F. Grubb – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Sarah C. Traeger – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Xin Li – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Kathy A. Johnston – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Lisa Kopcho – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Mark Fereshteh – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Kimberly A. Foster – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Kevin Stefanski – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Diane Delpy – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Gopal Dhar – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Aravind Anandam – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Sandeep Mahankali – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Shweta Padmanabhan – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Prabhakar Rajanna – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Venkata Murali – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

T. Thanga Mariappan – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Shabeerali Pattasseril – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Roshan Y. Nimje – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India

Zhenqiu Hong – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

James Kempson – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States; orcid.org/0000-0002-9540-3886

Richard Rampulla – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Arvind Mathur – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Anuradha Gupta – Department of Discovery Synthesis, Biocon Bristol Myers Squibb R&D Centre, Syngene International Ltd., Bangalore 560099, India; orcid.org/0000-0002-4211-1441

Robert Borzilleri – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Gregory Vite – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Aaron Balog – Bristol Myers Squibb, Research and Development, Lawrence Township, New Jersey 08648, United States

Complete contact information is available at:

<https://pubs.acs.org/10.1021/acsmmedchemlett.1c00236>

Notes

The authors declare no competing financial interest.

ABBREVIATIONS

TEA, triethylamine; THF, tetrahydrofuran; DPPA, diphenylphosphoryl azide; DMPU, 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidinone; LDA, lithium diisopropylamide; MTBE, methyl *tert*-butyl ether; B₂Pin₂, bis(pinacolato)diboron; EDCl, 1-ethyl-3-(3-(dimethylamino)propyl)carbodiimide; HOBT, hydroxybenzotriazole; DIPEA, *N,N*-diisopropylethylamine; HATU, 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo-[4,5-*b*]pyridinium 3-oxide hexafluorophosphate; DMF, *N,N*-dimethylformamide; EtI, ethyl iodide; MOMCl, methoxymethyl chloride; NaOMe, sodium methoxide; H, human; M, mouse; R, rat; D, dog; C, cyno

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