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Navigating CYP1A Induction and Arylhydrocarbon Receptor Agonism in Drug Discovery. A Case History with $S1P_1$ Agonists

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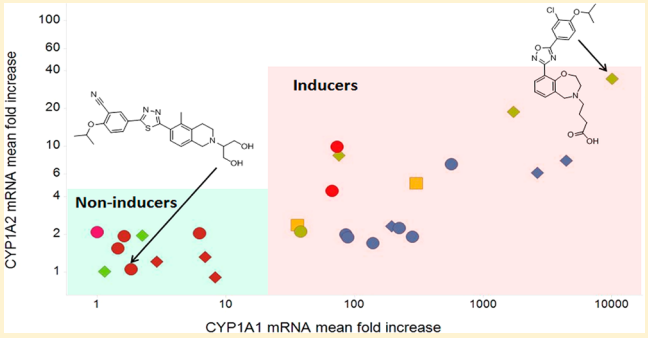
Supporting Information

ABSTRACT: This article describes the finding of substantial upregulation of mRNA and enzymes of the cytochrome P450 1A family during a lead optimization campaign for small molecule $S1P_1$ agonists. Fold changes in mRNA up to 10 000-fold for CYP1A1 in vivo in rat and cynomolgus monkey and up to 45-fold for CYP1A1 and CYP1A2 in vitro in rat and human hepatocytes were observed. Challenges observed with correlating induction in vitro and induction in vivo resulted in the implementation of a short, 4 day in vivo screening study in the rat which successfully identified noninducers. Subtle structure−activity relationships in this series of $S1P_1$ agonists are described extending beyond planarity and lipophilicity, and the impact and considerations of AhR and CYP1A induction in the context of drug development are discussed.



INTRODUCTION

The aryl hydrocarbon receptor (AhR) is a ligand-dependent transcription factor regulating the expression of numerous genes and mediating a diverse range of biological and toxicological responses following exposure to endogenous and exogenous chemicals.[1−3] These genes include those encoding xenobiotic metabolizing enzymes such as the cytochrome P450 (CYP) 1A family, and induction of this enzyme family is one of the most sensitive AhR activation end points.[4] The CYP1A family consists of CYP1A1 and 1A2 in rats, mice, dogs, monkey, and human with strong amino acid sequence conservation across species.[5]

In rats and human, CYP1A1 is expressed only at very low levels in liver and is present mostly as an extrahepatic enzyme in tissues notably the small intestine and lung. CYP1A1 levels are highly variable across human liver and small intestine preparations where CYP1A1 is predominantly expressed after induction.[5] CYP1A1 can oxidize and metabolically activate promutagenic or procarcinogenic substances.[6−9] These include polyaromatic hydrocarbons (PAHs) and aromatic amines and amides, present as environmental contaminants and components of cigarette smoke, charbroiled foods, and agricultural chemicals, respectively. These chemicals are metabolized by CYP1A and result in electrophillic reactive intermediates capable of binding to cellular macromolecules, DNA, and RNA, causing cytotoxic processes.[10−14]

CYP1A2 is expressed mainly in the liver across species and is only weakly expressed in extrahepatic tissues. In human liver CYP1A2 accounts for about 13% of total CYP content and metabolizes about 4% of therapeutic drugs including caffeine, theophylline, and phenacetin.[12]

Details on the mechanisms of AhR activation causing CYP1A enzyme induction can be found in numerous articles.[1,15−17] Differences exist in the relative sensitivities to induction of CYP1A1 and 1A2 across species with humans reported as being less responsive and sensitive to CYP1A1 activity and induction than rats but having a more robust response to CYP1A2.[4]

In addition to enzyme regulation numerous other physiological functions mediated via the AhR have emerged including development and regulation of cell differentiation and cycling, hormonal and nutritional homeostasis, coordination of cell stress responses (including inflammation and apoptosis), immune responses and aging and cancer promotion including regulation of cell proliferation and apoptosis.[18,19]

The development of drugs that possess off-target agonism of AhR therefore represents a challenge to the drug discovery scientist. For example the induction of CYP1A may result in raised levels of reactive intermediates and increased risk of carcinogenicity following exposure to environmental contam-

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inants. Additionally, upregulation of CYP1A is considered a clinically relevant drug−drug interaction liability as has been demonstrated via the impact of smoking on the increased metabolism, and therefore reduced exposure to CYP1A2 substrates such as theophylline, caffeine, and clozapine.[20,21] Other clinically relevant effects of CYP1A2 regulation include the effect of rifampicin and carbamazepine to increase caffeine and clozapine metabolism, respectively. The consumption of cruciferous vegetables and charbroiled meat is also known to increase CYP1A2 activity in human. Details on these interactions are covered in a review by Zhou et al.[11] These drug−drug interactions can often be managed in the clinic through dosage adjustment, therapeutic monitoring, and control of co-medications as is routinely applied when CYP1A2 levels are altered through smoking and sudden smoking cessation. However, avoiding other consequences of AhR activation, particularly regulation of cell proliferation, apoptosis, and potential carcinogenesis could reduce risk in drug development. Furthermore, the emergence of tumorogenicity may not occur until late in a drug development campaign, in carcinogenicity studies, or even in clinical development, where attrition comes after significant investment.

The details of our lead campaign to discover $S1P_1$ agonists have been extensively published[22−26] with successful optimization of parameters such as potency, selectivity, PK, and predicted human dose illustrated. This article details the finding of CYP1A induction observed during the optimization and preclinical development of selective $S1P_1$ agonists. Figure 1 illustrates exemplar compounds from these series for which the optimization and profile have been previously published.[22−25] Consistent with the high in vitro potency and good pharmacokinetic properties, these molecules showed efficacy at low oral doses in an acute dosing rat lymphopenia assay and a chronic dosing rat collagen induced arthritis model. Due to their promising developability profiles and predicted human PK and dose, these compounds were further progressed to 7-day repeat dose safety studies in rats.

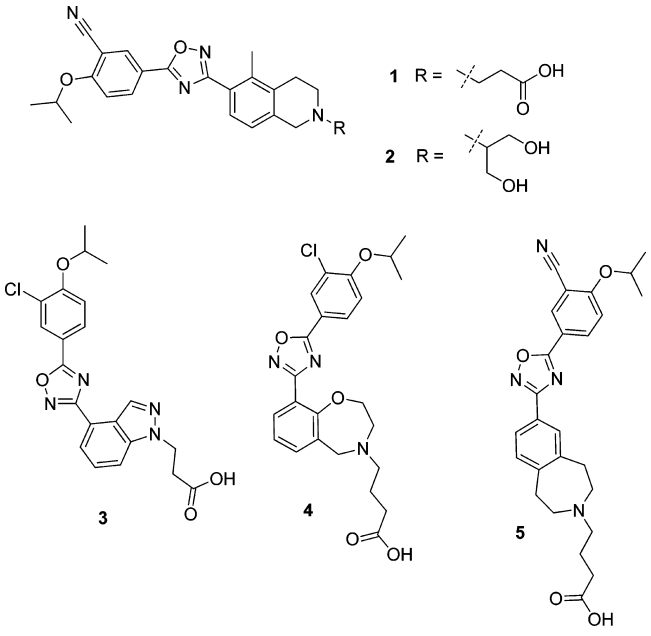

Figure 1. $S1P_1$ agonists progressed to 7-day rat safety studies.

RESULTS AND DISCUSSION

AhR Panel Activation and CYP1A mRNA Upregulation in Vivo. As part of early safety assessment studies in rats, toxicogenomic data are frequently collected in addition to histopathology to highlight cellular pathways and processes affected by the new chemical entity (NCE). Transcript changes in the liver can be associated with hepatotoxicity, and through evaluations of panels of genes, it is possible to elucidate the mechanism of action of the hepatotoxicity. One example is the induction of drug metabolizing enzymes, such as the CYP1A family. The regulation of this enzyme family is classically via the arylhydrocarbon receptor, and upregulation of this receptor is associated with increased expression of genes including CYP1A1, CYP1A2, NAD(P)H quinone oxidoreductase (NQO1) and epoxide hydrolase (Ephx). This collection of genes is referred to as the AhR panel. A positive response for this panel is alerted when upregulation of each gene reaches a threshold value.

Following 7-day repeat oral administration of $S1P_1$ agonists **3**−**5** to rats, marked increases in the expression of genes associated with the AhR panel were observed (Figure 2).

The induction of the mRNA was dose related, and the magnitude of the changes associated with CYP1A1 was up to 10 000-fold the control value. Interestingly, a retrospective analysis of the entire GSK data set revealed that these compounds stood out as the most prolific inducers of this gene panel.

Increases in CYP1A1 mRNA showed the most change and were considered representative of the entire gene panel. Additionally, for these compounds, the extent of CYP1A1 mRNA upregulation was found to correlate well with unbound systemic exposure (Figure 3). Although upregulation of mRNA was observed, there was no change to the exposure of the test compound on repeat dosing, suggesting the CYP1A family was not the primary clearance mechanism of these compounds in the rat.

Compounds **4** and **5** were terminated for developability reasons unrelated to AhR or enzyme induction, though compound **3** was further progressed to non-rodent safety studies. Following repeat oral 30 mg/kg administration to cynomolgus monkeys and in contrast to findings in the rat, a significant reduction in systemic exposure was observed between day 1 and day 7 (Figure 4). Consistent with the rat, Taqman data revealed a substantial increase in hepatic CYP1A1 and CYP1A2 mRNA. To further investigate the cause of the reduced exposure, microsomes were prepared from the livers obtained from cynomolgus monkeys in the control and treated groups and the in vitro clearance of compound **3** was determined. The in vitro clearance in microsomes prepared from the control animals showed compound **3** to be metabolically stable, whereas a high in vitro clearance of 11 mL/min/g tissue was observed in microsomes prepared from treated animals. Taken together these data indicate that compound **3** induced CYP1A and was also a substrate for this enzyme in the cynomolgus monkey, an autoinducer. There was no reduction in exposure observed in the rat on repeat administration, despite high levels of mRNA induction, suggesting a species difference in the routes of clearance for compound **3**.

CYP1A Upregulation in Vitro. Although upregulation of genes encoding the AhR panel was substantial in the rat, the human profile is of ultimate importance. The potential for **3**

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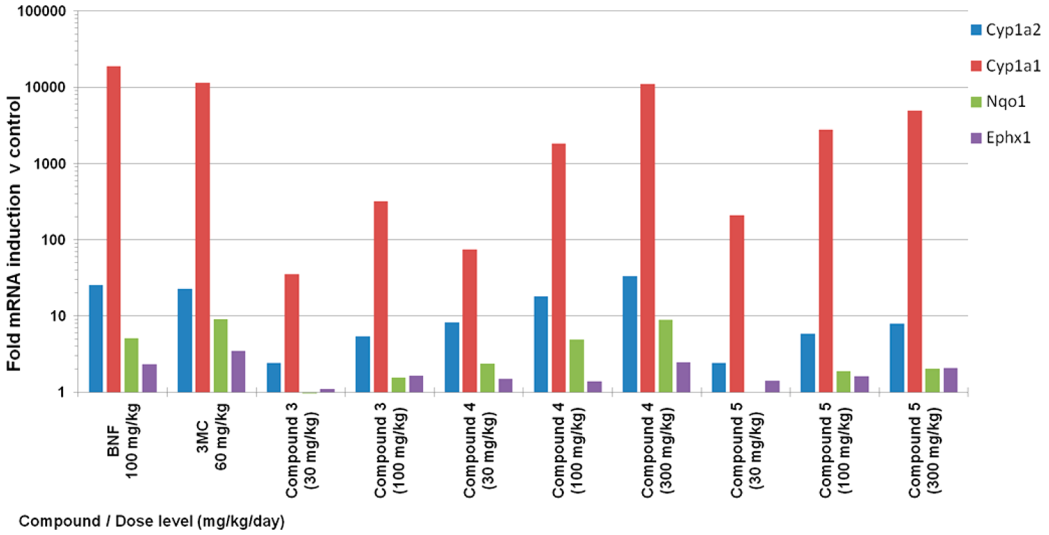

Figure 2. Induction of the AhR gene panel (hepatic mRNA fold induction versus control animals) for compounds **3**, **4**, and **5** following 7 days of oral dosing to rats. The response to prototypical inducers β-napthoflavone (BNF) and 3-methylcholanthrene (3MC) after 4 days of administration is included for reference. Cyp: cytochrome P450. NQO1: NAD(P)H quinone oxidoreductase. Ephx1: epoxide hydrolase.

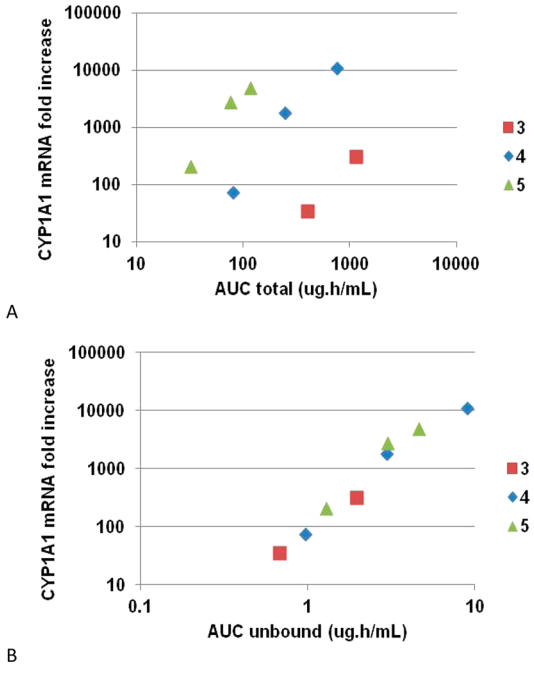

Figure 3. Correlation between CYP1A1 mRNA fold induction and exposure (AUC) for compounds **3**−**5** following 7-day administration to the rat. Doses administered per day were 30 and 100 mg/kg for compound **3**; 30, 100, and 300 mg/kg for compounds **4** and **5**. Part A shows the correlation with total exposure and part B shows the correlation with unbound exposure, corrected using the unbound fraction in blood.

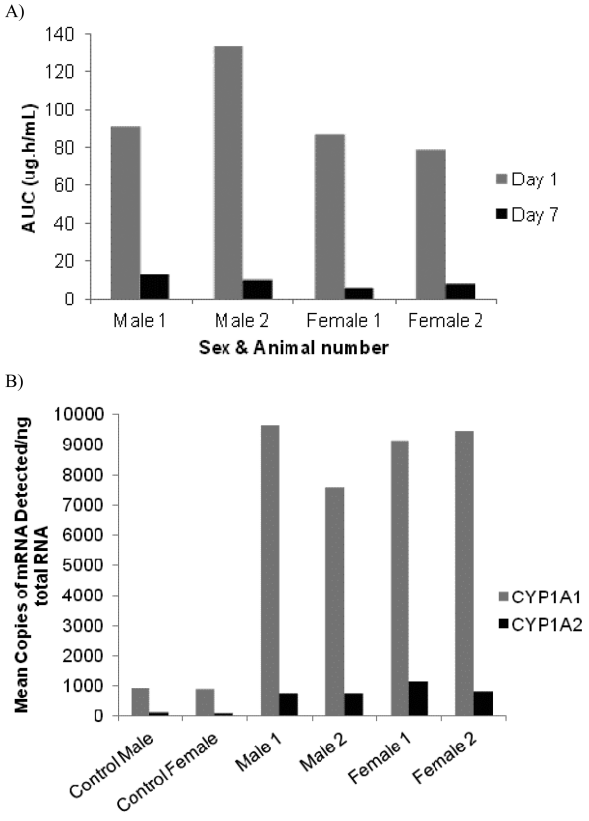

Figure 4. (A) Exposure data for compound **3** on days 1 and 7 in monkeys following daily dosing at 30 mg/kg. (B) Taqman analysis for CYP1A1 and CYP1A2 from the livers of treated animals versus animals receiving vehicle control.

and **4** to interact with the human AhR and cause induction was studied in human hepatocytes using end points of CYP1A2 mRNA and catalytic enzyme activity (determined using the rate of CYP1A mediated deethylation of the CYP1A probe substrate 7-ethoxyresorufin) as markers of enzyme induction. As mentioned previously, because of the low level of hepatic CYP1A1 expression and the predominant location of CYP1A1 extrahepatically the study of human CYP1A1 induction in vitro is less straightforward due to the challenges of obtaining metabolically competent cells from tissues where CYP1A1 is inducible, such as the lung. We therefore used an available human hepatocyte assay with CYP1A2 mRNA and activity as the end point, with the assumption that changes in 1A2 also represent 1A1.

Compound **3** was shown to cause a 45-fold increase in CYP1A2 human mRNA levels (Table 1) with an associated

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Table 1. Comparison of the CYP1A1, CYP1A2 (Rat), and CYP1A2 (Human) Induction Observed across Various in Vivo and in Vitro Assay Formats[a]

	rat in vitro[b]		rat in vivo[c]		human in vitro[d]	
compd	CYP1A1 mRNA	CYP1A2 mRNA	CYP1A1 mRNA	CYP1A2 mRNA	CYP1A2 mRNA	catalytic activity (EROD)
1	<1	<1	3	1	0.2	1.1
3	<1	<1	35	2	45	7.7
4	5	3	75	8	0.6	1.1
5	3	2	210	2		
6	43	19	2	2		
7	32	8	1	1		

[a]All data expressed as fold-change versus control to enable comparison. [b]Rat in vitro assays conducted at 10 μM. [c]Compounds **1**–**5**: 7-day dosing 30 mg/kg. Compounds **6** and **7**: 4-day dosing 20 mg/kg. [d]mRNA assay at 10 μM, EROD assay at 5 μM. EROD: 7-ethoxyresorufin *O*-deethylase activity.

increase in CYP1A2 catalytic enzyme activity. This finding was consistent with the CYP1A1 and CYP1A2 mRNA upregulation observed in rat and cynomolgus monkey substantiating the evidence for an interaction with the AhR across the species. However, in contrast to findings in the rat, compound **4** did not cause upregulation of CYP1A2 mRNA in human hepatocytes, highlighting a species difference. Although mRNA levels for CYP1A2 were not increased by compound **4** in human hepatocytes, the low levels of expression of CYP1A1 in the human liver complicate the evaluation of CYP1A1 upregulation. The possibility still existed that compound **4** was interacting with the AhR, but no observation could be made using human hepatocytes.

Due to the upregulation of CYP1A2 in human hepatocytes and substantial reduction in systemic exposure on repeat administration to the monkey, compound **3** was unable to be progressed further. Compound **1** was subsequently identified and entered safety testing in rats. Offering other developability advantages but having structural similarity with compounds **3**–**5**, compound **1** was also expected to upregulate the genes of the AhR panel. However, inspection of the Taqman data following 7-day administration of compound **1** showed levels of gene expression within the normal range, suggesting a reduced or absent interaction with the AhR in rat. Compound **1** was therefore positively differentiated from the previous compounds **3**–**5** (Figure 5).

As we had now discovered a compound that did not induce mRNA changes associated with the AhR gene panel in the rat and also did not upregulate CYP1A2 in human hepatocytes (Table 1), we progressed compound **1** to further rodent and non-rodent safety studies. The program strategy required the identification of backup compounds that would also need to be devoid of any potential interaction with the AhR gene panel. We therefore embarked on a series of experiments with the objectives of understanding the structure–activity-relationships and to establish a screening cascade to filter out the AhR gene panel induction.

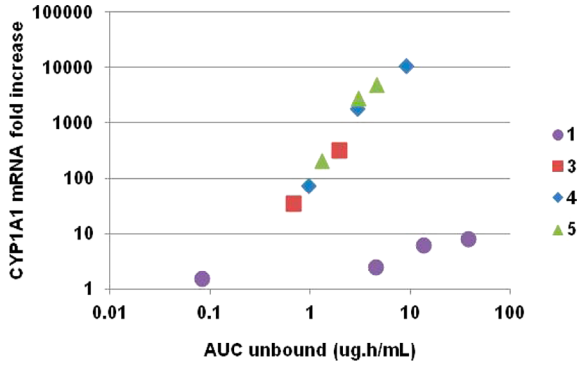

Figure 5. Correlation between CYP1A1 mRNA fold induction and unbound exposure (AUC) for compound **1** (noninducer) compared to compounds **3**–**5** (inducers) following 7-day administration to the rat. Doses administered per day were 1, 30, 100, and 300 mg/kg for compound **1**; 30 and 100 mg/kg for compound **3**; 30, 100, and 300 mg/kg for compounds **4** and **5**.

Due to the complexities of species differences in expression and upregulation of enzymes encoded by the AhR, coupled with the developability risks associated with AhR agonism, a decision was taken to design out mRNA upregulation and CYP1A enzyme induction in all species as a way of mitigating the risk of AhR agonism. The most sensitive marker of this response for our compounds was CYP1A1 mRNA upregulation in the rat, and this end point was selected to optimize compounds.

"Screening" for CYP1A Induction. Utilizing an in vitro assay system to predict effects in vivo is common approach to screening in drug discovery. We initially investigated the utility of rat hepatocytes as a system to predict the in vivo findings. Compounds **1**, **3**, **4**, and **5** were incubated with rat hepatocytes, and the increase in mRNA levels for CYP1A1 and CYP1A2 was quantified using Taqman analysis. The induction of CYP1A1 and CYP1A2 mRNA is presented in Table 1 where it can be seen that the extent of induction observed in vivo was not reflected in vitro. Refinements to the in vitro assay system were conducted and multiple methods of data analysis attempted (including % change compared to positive control, EC_{50}, and maximum induction), but an in vitro–in vivo correlation of CYP1A enzyme induction was not established.

As the in vitro CYP1A induction assay in rat hepatocytes was not able to predict the extent of induction in vivo, we introduced a short-term in vivo screen in the rat. Data from Baldwin et al.[27] demonstrated that hepatic CYP1A mRNA induction by β-napthoflavone was observed after three daily administrations. We therefore established an in vivo screening protocol where test compounds were administered orally to rats at a single nominal dose level of 30 mg/kg for 4 days. The dose level was selected to represent the most likely lowest dose in a 7-day safety assessment study and was manageable with respect to the availability of drug substance where no greater than 150 mg of compound was required to dose $n = 3$ rats. The systemic exposure was measured on days 1 and 4, the animals were then culled, the livers were harvested, and the extent of mRNA induction of genes in the AhR gene panel was quantified.

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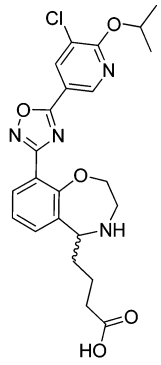
The first two molecules to enter such studies were the enantiomeric pair **6** and **7** (Figure 6) for which the in vitro and



6 enantiomer 1
7 enantiomer 2

Figure 6. Benzoxazepine $S1P_1$ agonists devoid of CYP1A mRNA upregulation in vivo.

in vivo profile have already been reported.[25] Good systemic exposure was observed for both compounds, and TaqMan analysis revealed no significant change in hepatic CYP1A mRNA levels. Interestingly, these two compounds showed some mRNA upregulation in the in vitro rat hepatocytes assay (Table 1), further highlighting the discrepancy between in vitro and in vivo data.

The identification of compounds from the same triaryl series with a marked difference in their ability to activate the AhR in the rat prompted us to investigate the impact of subtle structural changes. A survey of the literature demonstrated that a significant proportion of compounds described as AhR agonists are planar hydrophobic polyaromatics such as dioxin **8** (TCDD, Figure 7) or β-benzoflavone **9**,[28] even if disruption of

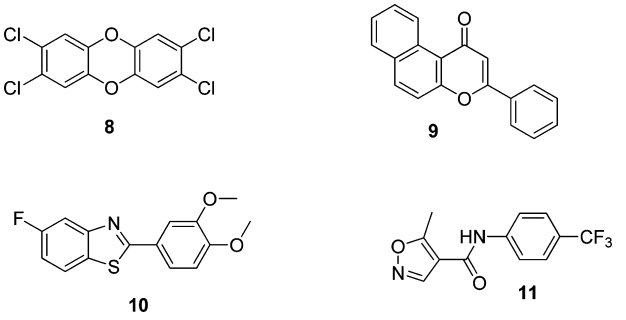


Figure 7. Examples of reported AhR agonists.

planarity has been demonstrated to be tolerated in some occasions.[29] "Nonclassical"[16] AhR agonists such as benzothiazole 5F 203, **10**,[30,31] or leflunomide **11**[28] have also been reported.

Crystallographic data for compounds **1** and **4** highlighted that one of the aromatic rings in compound **1** (considered AhR negative) was out of plane when compared with compound **4** (AhR positive). The dihedral angles between the oxadiazole ring and the phenyl closest to the basic nitrogen were 18° versus 5°, respectively (Figure 8). We postulated that the ortho-methylated THIQ (**1**) was sufficiently out of plane to avoid binding the AhR. This assumption was in part reinforced by the fact that the main difference between compounds **4**, **6**, and **7** was the presence of a carboxylic chain out of the triaryl plain in

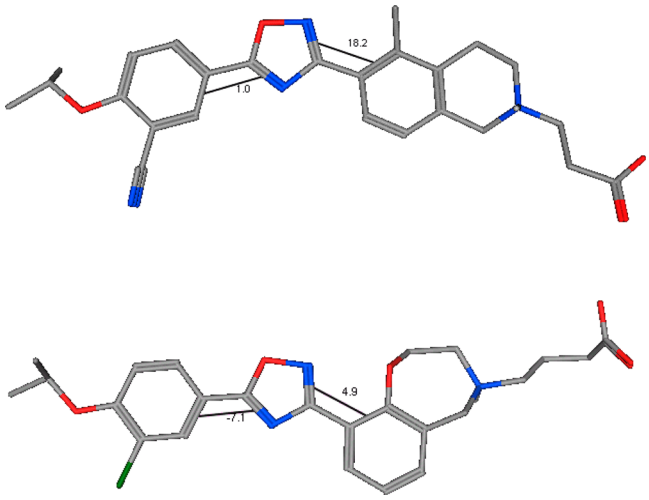


Figure 8. Crystallographic data for compounds **1** and **4**. The values represent measured dihedral angles.

the case of the noninducers because of the presence of an asymmetric carbon. However, it is also possible that the change from phenyl to pyridine in the distal ring may also impact AhR binding.

Second Generation S1P1 Agonists. We have presented the identification of zwitterionic $S1P_1$ agonists (**1**, **6**, and **7**) devoid of CYP1A induction with an overall profile suitable for progression to preclinical development. In addition to effects of $S1P_1$ agonists to cause a reduction in peripheral circulating lymphocytes, emerging data with FTY720[32,33] suggested that efficacy in human multiple sclerosis and its animal model may also involve direct effects on S1P receptor-mediated signaling in CNS. This prompted us to explore a subsequent generation of compounds with CNS penetration properties. We explored this using the analogous amines to the zwitterions previously described, aware of the known ability of lipophillic amines to cross the blood–brain barrier.[34]

Compound **12** (Table 2) was the first druglike triarylamine identified with good in vitro potency and selectivity. Pharmacokinetic properties suitable for oral delivery were confirmed, and maximum efficacy was demonstrated in the rat lymphopenia assay at an oral dose of 1 mg/kg. This molecule, analogous to the AhR negative compound **1**, was therefore progressed to the in vivo rat 4-day induction screen using an oral dose level of 30 mg/kg. Unfortunately, TaqMan analysis revealed an average 62-fold increase in CYP1A1 mRNA. This demonstrated that the lack of planarity per se does not systemically lead to non AhR agonists.

Without a predictive in vitro assay system and being unable to demonstrate consistent SAR with AhR gene panel induction, our path forward was restricted to systematic progression of compounds with the requisite in vitro pharmacology, PK, efficacy profile, and human dose prediction into the 4-day repeat dose rat study. Compounds devoid of AhR gene panel activation potential were considered as candidates for preclinical development. The compounds progressed into the 4-day study are highlighted in Figure 9.

Chemistry. The general route to access these compounds (with the exception of compound **16**) is highlighted in Scheme 1 and uses the formation of the oxadiazole (via condensation and dehydration) as a key step. Once the amines **26** are deprotected in acidic condition, the final compounds can be

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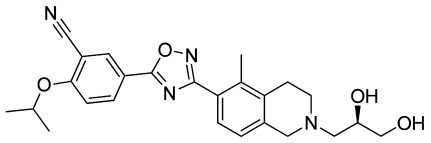
Table 2. In Vitro and in Vivo Properties of Compound 12



12

MW, CHI LogD @ pH 7.4, PSA		448, 2.16, 116
$S1P_1$ pEC_{50} (β-arrestin)		8.1
$S1P_3$ pEC_{50} (GTPγS)		< 4.5
CLi hepatocytes; rat, dog, human (mL/min/g liver)		2.1, 7.1, <0.85
Rat PK n=3 ± SD	CL_b (mL/min/kg)[a]	53 ± 6
	V_{ss} (L/kg)[a]	6.6 ± 0.2
	$t_{½}$ (h)[a]	1.7 ± 0.2
	Oral bioavailability[b] (%)	56 ± 7
Rat 4 day induction study: CYP1A1 mRNA Mean Fold increase		62

[a]1 mg/kg iv DMSO/10% (w/v) Kleptose HPB 0.9% saline (aqueous) (2%:98% v/v). [b]3 mg/kg oral. 1% (w/v) methylcellulose 400 (aqueous).

obtained via reductive amination using optionally protected dihydroxy aldehyde (see Experimental Section).

The syntheses of the key intermediates to access compounds **2**, **12**−**15**, **17**, **22**, and **23** have already been described. The syntheses of **27** and **28**, key building blocks to access compounds **18**, **21**, and **24**, are described in Scheme 2 and 3, respectively.

The synthesis of **27** uses benzoic acid **29** as starting material. Straightforward modifications of the acid lead to acetal **30** which cyclizes to **31** in acidic conditions. Reduction of the double bond and the amide functionality of **31** followed by demethylation of the phenol leads, after protection of the secondary amine, to **32**. The nitrile of **27** is inserted using palladium chemistry from the phenol triflate of **32**. All transformations are high yielding and can be performed on multigram quantities.

Compound **28** is obtained from resorcinol **33** which can be monoformylated. Reductive amination using ethanolamine leads, after protection of the secondary amine, to **34**. The benzoxapine **35** is then obtained via a Mitsunobu reaction. As for **27**, the nitrile of **28** is introduced using palladium chemistry.

Compound **16** is easily obtained from triaryl **40** (Scheme 4). The latter is obtained via Suzuki coupling between **37** and **39**, obtained respectively from **36** and **38** whose syntheses have already been published.[37]

Profile of Basic S1P1 Agonists. The physicochemical properties of these compounds are shown in Table 3. As can been seen from these data, the compounds progressed had similar properties with respect to molecular weight and polar surface area, with measured CHILogD at pH 7.4 ranging from 2.15 to 2.84. With the exception of compound **15** (cLogP = 2.35), the cLogP is within a 1.1 unit window. The main difference could be considered the pK_a of these compounds, mainly driven by the nature of the bicyclic amine (aza-THIQ, THIQ, benzazepine, and benzoxazepine), as neither the nature of the amine substituent nor the nature of the other aromatic rings and their substituents has a significant impact on the measured pK_a (compare **2**, **12**−**14**, and **16**−**17** or **18**−**23**).

When measured, in vivo rat pharmacokinetics demonstrated oral exposure with distribution into tissues and a moderate blood clearance (Table 4).

Basic S1P1 Agonists and CYP1A mRNA Upregulation in Vivo. Compounds **2**, **12**−**24** were progressed into the rat 4-day in vivo CYP1A induction screening study. All data associated with these studies are shown in Table 5, and the CYP1A1 mRNA upregulation is additionally depicted in Figure 10. Doses used were in the range 15−30 mg/kg, selected to be representative of the lower end of the dose required in safety testing but also determined by compound availability. The AUC and C_{max} values indicated systemic exposure remained constant over the 4-day study period, providing no evidence for autoinduction in the rat. Total exposure was generally within 2-fold (see compounds **14**−**24**), though it is noted that the AUC/D values for compounds **2**, **12**, and **13** are lower. It is clear from this data set that systemic exposure is not the sole determinant of the extent of induction, as exemplified by noninducer compounds **15**, **16**, and **17** having a higher exposure than the inducers **12** and **13** within the THIQ template. Furthermore, the data set confirms the subtle nature of the SAR with compounds having relatively equivalent properties, showing marked differences in their ability to cause CYP1A induction.

The extent of induction of CYP1A1 versus CYP1A2 for all compounds described is shown in Figure 11. A compound was considered an inducer when CYP1A1 mRNA increases >20-fold, and CYP1A2 mRNA increases >5-fold were observed. Looking more broadly across the templates all the amine benzazepines and benzoxazepines tested showed substantial CYP1A induction of >40-fold (compounds **18**−**24**). Lower levels of induction were only observed with some (aza-) THIQ derivatives. Of the seven (aza-) THIQ compounds tested, only compounds **12** and **13** showed some upregulation. The greater induction observed with **12** and **13** is intriguing, given the structural similarity with compound **2**, the only difference residing in the arrangement of the hydrophilic side chain. The observation that such a small modification can result in a large difference in vivo is difficult to interpret when other small modifications from **2** (aza-THIQ **15** or thiazole **16**) or when more drastic shape modifications (compound **17**) have no effect on CYP1A1 induction.

Similar comments can also be made when comparing the structure of the benzazepine (compounds **18**−**23**) or the benzoxazepine (compound **24**) with the noninducers THIQs. Compounds **20** and **24** differ from **2** by one carbon and one oxygen, respectively, and showed induction. When compared with the THIQ series, modification of the hydrophilic side chain in the benzazepines has no notable impact with all compounds (compare **18** and **19** with **20**) considered substantial inducers. The effect of increasing planarity (compound **22**) appears to lead to increased induction (600-fold versus 275 for compound **20**), but the increased exposure may contribute to this observation. Lowering lipophilicity on the distal ring (compound **21**) may appear to reduce induction (80-fold versus 275 for compound **20**), but this effect is not dramatic and other contributing factors may be considered.

One may be tempted to look at these data as a "template effect", with the benzazepines and benzoxazepine showing generally more induction than the THIQs. Moreover, due to the similarity of intrinsic properties between **2** and **12**−**24**

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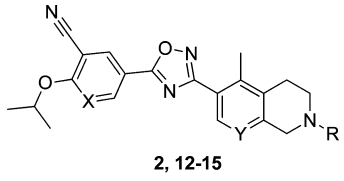
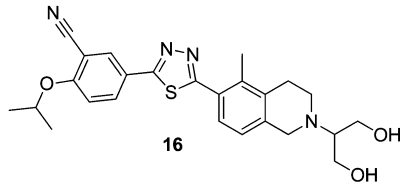
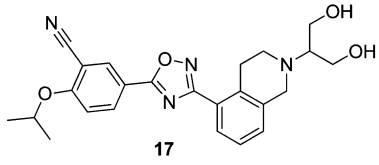
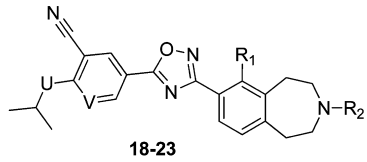
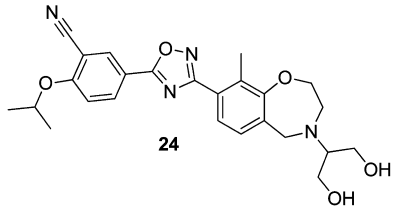










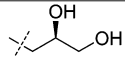
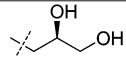
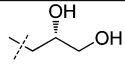
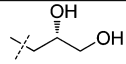
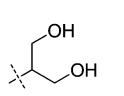
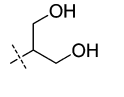
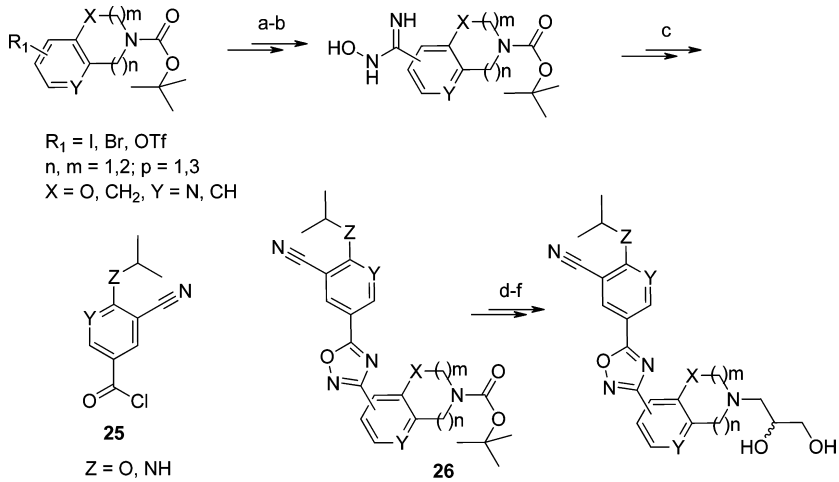
Cpd	X	Y	R	Cpd	U	V	R_1	R_2
12	CH	CH		18	O	CH	CH_3	
13	CH	CH		19	O	CH	CH_3	
2	CH	CH		20	O	CH	CH_3	
14	N	CH		21	NH	N	CH_3	
15	CH	N		22	O	CH	H	
				23	NH	N	H	

Figure 9. Structures of compounds **2**, **12**–**24**.

Scheme 1. General Route To Access Oxadiazole Containing S1P1 Agonists[a]



[a]Reagents and conditions: (a) palladium-mediated nitrile formation; (b) addition of hydroxylamine; (c) **25**, base, heat; (d) HCl; (e) reductive amination; (f) alcohol deprotection.

(cLogP, PSA, CHILogD), we may consider that the induction is related to pK_a. Indeed the benzazepines (pK_a range 7.8–8.4) are more basic than the THIQs (pK_a range 7.2–7.7, aza-THIQ **15** pK_a of 6.2). However, this argument is not supported by the

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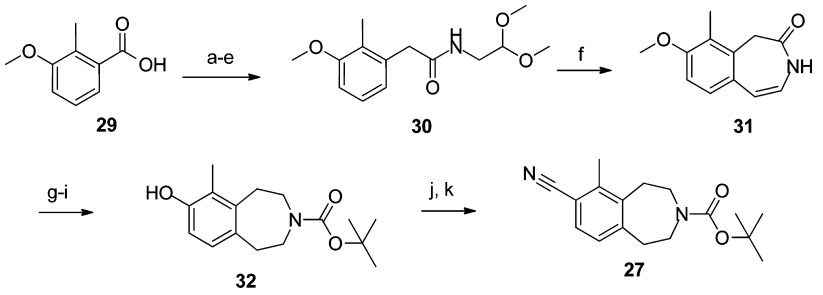
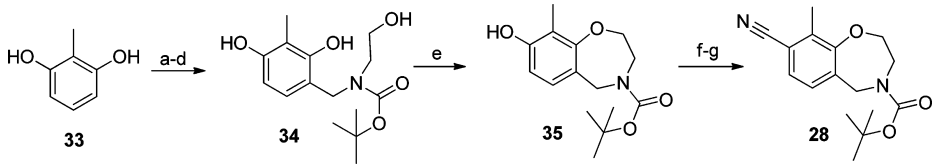
Scheme 2. Synthesis of Benzazepine Intermediate 27[a]



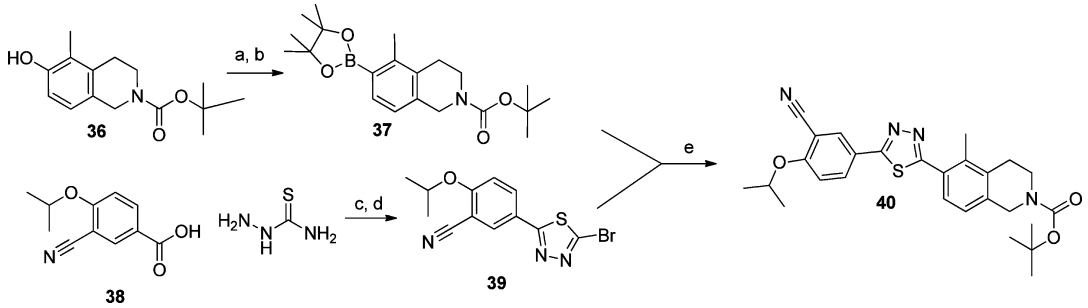
[a]Reagents and conditions: (a) BH_3·THF, THF, 0 °C to room temperature, 95%; (b) MsCl, NEt_3, CH_2Cl_2, 0 °C to room temperature, 77%; (c) NaCN, DMF, 90 °C, 86%; (d) NaOH, EtOH, reflux, 97%; (e) $(COCl)_2$, cat. DMF, CH_2Cl_2, room temperature, then NEt_3, $NH_2CH_2CH(OCH_3)_2$, 98%; (f) conc HCl, AcOH, room temperature, 76%; H_2, Pd/C, MeOH, room temperature, 98%; (h) BH_3·THF, THF, room temperature, 96%; (i) aqueous HBr, 100 °C, then Boc_2O, NEt_3, CH_2Cl_2/THF, room temperature, 52%; (j) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, −50 °C to room temperature, 85%; (k) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 120 °C, 95%.

Scheme 3. Synthesis of Benzoxazepine Intermediate 28[a]



[a]Reagents and conditions: (a) $POCl_3$, DMF, 0 °C to room temperature, 40%; (b) $NH_2(CH_2)_2OH$, EtOH, room temperature; (c) $NaHB(OAc)_3$, THF, room temperature; (d) Boc_2O, NEt_3, MeOH, room temperature, 35% (3 steps); (e) PPh_3, DIAD, THF, 0 °C, 84%; (f) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, 0 °C, 100%; (g) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 100 °C, 91%.

Scheme 4. Synthesis of Thiadiazole Containing S1P1 Agonists[a]



[a]Reagents and conditions: (a) $(CF_3SO_2)_2O$, pyridine, CH_2Cl_2, room temperature, 100%; (b) bis(pinacolato)borane, KOAc, $PdCl_2$(dppf), 1,4-dioxane, reflux, 88%; (c) $POCl_3$, 90 °C, 99%; (d) $CuBr_2$, isoamyl nitrite, CH_3CN, room temperature, 63%; (e) Na_2CO_3, $PdCl_2$(dppf)·CH_2Cl_2, DME/H_2O, reflux, 71%.

level of induction observed with benzoxazepine **24**, with a measured pK_a of 6.3.

CONCLUSION

Overall, the data presented lead to the conclusion that the SAR between structure and AhR gene panel upregulation was complex and undoubtedly multifactorial. The structural relationships were subtle and extended beyond general properties such as planarity, basicity, or lipophilicity.

This program of work demonstrated a finding of marked upregulation of genes encoding the AhR during profiling of numerous $S1P_1$ agonist compounds. CYP1A1 mRNA upregulation was largely used as a marker for AhR activation, and the magnitude of upregulation of CYP1A1 mRNA in the rat by certain compounds was one of the largest observed within the GSK data set to date. We explored the structure−activity relationships of this finding in the rat in vivo and demonstrated subtle structural changes could impact the extent of induction observed. A robust relationship between in vitro and in vivo induction in the rat was not established. Due to species differences in the extent of AhR activation, a decision was taken to design out evidence of induction in all species, and due to the lack of in vitro−in vivo correlation, a short-term rat in vivo model was successfully established as a decision making tool. This strategy allowed us to identify compounds devoid of CYP1A induction that were progressed to further development.

It is acknowledged that adopting the strategy to only progress compounds devoid of induction in both rat and human could be considered conservative. However, there was no precedence within GSK for development of a compound with this type of profile. Additionally, we felt the magnitude of the upregulation observed justified our conservative approach. Evaluation of AhR activity in early safety studies is essential to understand the developability risks associated with a candidate

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Table 3. Physicochemical Profiles of Amines 2, 12−24[a]

compd	MW	cLogP	CHILogD, pH 7.4	PSA	template	measured pK_a
2	449	3.75	2.76	116	THIQ	7.3
12	449	3.66	2.70	116	THIQ	7.7
13	449	3.66	2.70	116	THIQ	7.7
14	449	2.97	2.84	129	THIQ	7.3
15	486	2.35	2.15	129	aza-THIQ	6.2
16	501	3.66	2.33	102	THIQ	7.2
17	471	3.55	2.58	116	THIQ	7.3
18	463	3.83	2.36	116	BZ	8.4
19	463	3.83	2.36	116	BZ	8.4
20	463	3.95	2.64	116	BZ	8.2
21	499	3.02	2.44	131	BZ	8.3
22	449	3.75	2.78	116	BZ	7.8
23	449	2.82	2.63	131	BZ	8.1
24	464	3.57	2.86	125	BZO	6.3

[a]THIQ: tetrahydroisoquinoline. BZ: benzazepine. BZO: benzoxazepine.

Table 4. Pharmacokinetics of Compounds 12, 22, 23, and 24 in the Rat[a]

	compd			
	12	**22**	**23**	**24**
CL_b (mL/min/kg)[b]	53 ± 6 (n = 3)	21 ± 3 (n = 3)	22 (n = 1)	54 (n = 1)
V_{ss} (L/kg)[b]	6.6 ± 0.2	7.6 ± 0.4	10.9	4.0
$T_{1/2}$ (h)[b]	1.7 ± 0.2	5.3 ± 0.3	8.1	1.0
oral bioavailability[c] (%)	56 ± 7 (n = 3)	68 ± 18	67 ± 14	69 ± 11

[a]Data are for n = 1 or are the mean ± SD at n = 3. [b]1 mg/kg iv DMSO/10% (w/v) Kleptose HPB 0.9% saline (aqueous) (2%:98% v/v). [c]3 mg/kg oral in 1% (w/v) methylcellulose 400 (aqueous).

compound. While the drug−drug interaction risks associated with CYP1A enzyme induction may be considered manageable in the clinic, there are further potential and emerging risks associated with AhR agonism and CYP1A enzyme induction, for example, on cellular proliferation and tumorogenesis.[18]

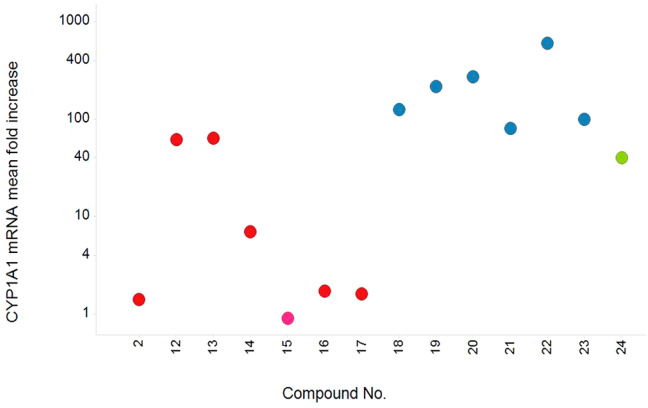

Figure 10. The upregulation of CYP1A1 mRNA following repeat administration of basic $S1P_1$ agonists to the male CD rat. The compounds are categorized by template: THIQ (red), aza-THIQ (pink), benzazepines (blue), benzoxazepines (green).

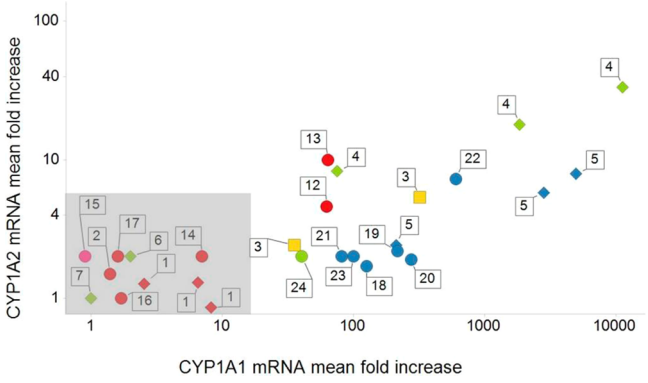

Figure 11. Upregulation of CYP1A1 and CYP1A2 mRNA following repeat administration to the male CD rat. The compounds are categorized by template, THIQ (red), aza-THIQ (pink), indazoles (yellow), benzazepines (blue), benzoxazepines (green), and shaped by class, acid (square), amine (circle), zwitterions (diamond). The shaded area indicates the boundaries considered a noninducer (20-fold CYP1A1 and 5-fold CYP1A2).

Table 5. CYP1A1 mRNA Upregulation and Exposure Data following 4-Day Repeat Administration of Selected Amines to the Male SD Rat[a]

					C_{max} (μg/mL)		AUC (μg·h/mL)		AUC/D (min·kg/L)	
compd	template	dose (mg/kg)	Cyp1A1 mean fold increase	Cyp1A2 mean fold increase	day 1	day 4	day 1	day 4	day 1	day 4
2	THIQ	30	1.4	1.5	0.44 ± 0.06	0.79 ± 0.24	4.42 ± 0.13	7.64 ± 0.71	9 ± 0.4	16 ± 1.2
12	THIQ	30	62	4.6	0.72 ± 0.27	0.71 ± 0.13	7.31 ± 2.1	7.0 ± 1.6	15 ± 4	15 ± 3
13	THIQ	30	64	10	0.77 ± 0.26	0.50 ± 0.05	4.44 ± 1.1	3.6 ± 0.9	9 ± 2	9 ± 2
14	THIQ	22	7	2	2.1 ± 0.5	2.73 ± 1.0	24.3 ± 1.2	28.8 ± 3.7	66 ± 4	79 ± 10
15	aza-THIQ	17	0.9	2	1.61 ± 0.17	2.17 ± 1.0	12.9 ± 1.5	16.8 (n = 2)	46 ± 5	59 (n = 2)
16	THIQ	19	1.7	1	1.75 ± 0.06	2.32 ± 0.54	19.1 ± 2.7	27.8 ± 4.2	61 ± 9	89 ± 10
17	THIQ	15	1.6	2	0.90 ± 0.12	0.94 ± 0.01	12.1 ± 1.5	13.9 ± 1.8	48 ± 6	56 ± 6
18	BZ	19	126	1.7	1.29 ± 0.08	1.53 ± 0.45	20.8 ± 2.6	26 ± 6.3	65 ± 8	83 ± 21
19	BZ	25	215	2.2	0.73 ± 0.32	0.87 ± 0.11	13.2 ± 5.6	15.6 ± 2.2	31 ± 13	38 ± 5
20	BZ	15	275	1.9	0.55 ± 0.03	0.67 ± 0.05	9.1 ± 0.57	11.5 ± 1.3	36 ± 2	46 ± 6
21	BZ	24	81	2	1.14 ± 0.2	1.35 ± 0.21	17 ± 3.9	24.9 ± 4.1	43 ± 9	63 ± 11
22	BZ	30	604	7.3	1.60 ± 0.11	1.38 ± 0.12	23 ± 0.4	23.1 ± 1.7	48 ± 2	59 ± 4
23	BZ	24	100	2	1.01 ± 0.05	1.22 ± 0.12	15.3 ± 0.9	21.3 ± 1.8	38 ± 1	55 ± 5
24	BZO	25	40	2	0.87 ± 0.06	1.14 ± 0.08	8.84 ± 1.7	12.6 ± 0.4	22 ± 4	30 ± 2

[a]PK data are the mean ± SD, n = 3, unless otherwise stated.

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Understanding the consequences of AhR activation is a rapidly expanding area of scientific research including its utility as a therapeutic target.[35] While we are not advocating to avoid AhR agonism completely, we suggest caution and the operation of an appropriate strategy to understand and mitigate potential risks while the implications of substantial AhR agonism are more thoroughly understood.

EXPERIMENTAL SECTION

In Vitro Pharmacology. Materials used were the following: DMEM F12 catalog no. Sigma D6421 or Invitrogen 11320; charcoal stripped FBS catalog no. Hyclone SH30068.03. Base buffer was prepared from Sigma kit H1387, supplemented with 20 mM HEPES (Sigma H0887) and 4.16 mM $NaHCO_3$ (Sigma S8761), and pH was adjusted to 7.4. Loading buffer was base buffer supplemented with 0.1% pluronic acid F-68 solution (Gibco/Invitrogen 24040-032) and 0.1% BSA (Calbiochem 126609). Assay buffer/diluent buffer was base buffer supplemented with 0.1% pluronic acid F-68 solution (Gibco/Invitrogen 24040-032). Coelenterazine was catalog no. Invitrogen C6780 or Biotium BT10110-1.

$S1P_1$ β-Arrestin Recruitment Assay. β-Arrestin recruitment assays were carried out using the PathHunter CHO-K1 EDG1 β-arrestin cell line (DiscoveRx Corporation) in a chemiluminescence detection assay. This cell line stably expresses β-arrestin 2 and $S1P_1$ fused to complementing portions of β-galactosidase ("EA" and "prolink", respectively) which associate upon arrestin recruitment to form functional β-galactosidase enzyme. Cells were grown to 80% confluency in growth medium (F12 nutrient HAMS supplemented with 10% heat-inactivated USA FBS, 1% L-Glutamax, 800 μg/mL Geneticin, and 300 μg/mL hygromycin). Cells were harvested from the flask using enzyme free cell dissociation buffer (Gibco) and washed from flasks with Optimem solution (Gibco). Cells were then centrifuged at 1000 rpm for 2−3 min and resuspended in assay buffer (prepared from Sigma kit H1387 supplemented with 20 mL/L HEPES, 4.7 mL/L $NaHCO_3$, 0.1% pluronic acid F-68 solution, 0.1% BSA and adjusted to pH 7.4 using sodium hydroxide at 1×10^6 cells/mL). Cells were dispensed into assay plates containing compounds (100 nL/well of a solution of test compound in 100% DMSO) at 1×10^4 cells/well and incubated at 37 °C/5% CO_2 for 90 min followed by 15 min at room temperature. An amount of 5 μL of detection mix (1 part Galacton Star, 5 parts Emerald II, 19 parts assay buffer; DiscoveRx) was added per well, and the plates were incubated at room temperature for 60 min. Luminescence was quantified using a Viewlux plate reader.

Membrane Preparation for $S1P_3$ GTPγS Assay. All steps were performed at 4 °C. Cells were homogenized within a glass Waring blender for two bursts of 15 s in 200 mL of buffer (50 mM HEPES, 1 mM leupeptin, 25 μg/mL bacitracin, 1 mM EDTA, 1 mM PMSF, 2 μM pepstatin A). The blender was plunged into ice for 5 min after the first burst and 10−40 min after the final burst to allow foam to dissipate. The material was then spun at 500*g* for 20 min and the supernatant spun for 3 min at 48 000*g*. The resultant pellet was resuspended in the same buffer without PMSF and pepstatin A but containing 10% w/v sucrose. The membrane suspension was then layered on top of buffer without PMSF and pepstatin A containing 40% w/v sucrose and spun at 100 000*g* for 60 min. The cloudy interface between the two sucrose layers was removed and resuspended in buffer without PMSF and pepstatin A. The material was spun at 48 000*g* for 45 min. The resultant cell pellet was resuspended in the required volume in buffer without PMSF and pepstatin A (usually ×4 the volume of the original cell pellet), aliquoted, and stored frozen at −80 °C.

$S1P_3$ GTPγS Assay. $S1P_3$ expressing RBL membranes (0.44 μg/well) purified through a sucrose gradient were homogenized by passing through a 23G needle. These were then adhered to WGA-coated SPA beads (GE Healthcare 0.5 mg/well) in assay buffer (HEPES 20 mM, $MgCl_2$ 10 mM, NaCl 100 mM and pH adjusted to 7.4 using KOH 5 M). An amount of 2 μg/well of saponin was added. After 30 min of precoupling on ice, 5 μM GDP final assay concentration was added to the bead and membrane suspension. The bead, membrane, saponin, and GDP suspension was mixed with [^{35}S]GTPγS (PerkinElmer, 0.3 nM final radioligand concentration) made in assay buffer (HEPES 20 mM, $MgCl_2$ 10 mM, NaCl 100 mM and pH adjusted to 7.4 using KOH 5 M). The bead, membrane, and radioligand suspension was dispensed into white Greiner polypropylene 384-well plates (45 μL/well), containing 0.5 μL of a solution of test compound in 100% DMSO. The final assay cocktail (45.5 μL) was then sealed, spun on a centrifuge, then read on a Viewlux instrument following a 3 h incubation of plates at room temperature.

In Vitro DMPK. Rat and Human Hepatocyte Induction Studies. Cryopreserved rat and human hepatocytes were purchased from Celsius (Baltimore, MD, U.S.) and Invitrogen (Paisley, U.K.). The hepatocytes were removed from liquid nitrogen storage, immediately thawed at 37 °C for 90 s, and transferred in prewarmed cryopreserved hepatocyte recovery medium. The cells were centrifuged at 100*g* for 10 min and resuspended in cryopreserved hepatocyte plating medium (at 4 °C) before being counted under a microscope using a hemocytometer. The viability was assessed using the trypan blue exclusion method, and only cell suspensions with viability of >80% were used. Cells were seeded according to the supplier's protocol and cultured in Williams medium E (WME) supplemented with fetal bovine serum, dexamethasone, gentamycin, insulin, L-glutamine, and HEPES (plating supplement pack, Cellzdirect) and allowed to acclimatize in a humidified CO_2 incubator (5% CO_2) at 37 °C for 4 h. The medium was then replaced with WME containing ITS + (insulin, human transferrin, and selenous acid), dexamethasone, gentamycin, L-glutamine, and HEPES (cell maintenance supplement pack, Cellzdirect) and 0.25 mg/mL Matrigel. The hepatocytes were allowed to acclimatize in a humidified CO_2 incubator (5% CO_2) at 37 °C for 1 day. Following acclimatization, the medium was removed and replaced with prewarmed culture medium solutions containing test compound or positive control (in duplicate). Hepatocytes were also exposed to solvent control in quadruplicate. All cells were then incubated for a further 48 h with one change of drug/controls after 24 h. At the end of the incubation period, the medium was removed and the cells were lysed. Isolation and analysis of mRNA levels of specific P450 genes were conducted using the methods described by Baldwin et al.[27] The mRNA level for each specific CYP was expressed as a mean ratio of treated over solvent control. The EROD assays were performed to assess CYP1A2 activity in human hepatocytes. Cells were washed with Williams medium E prior to incubation with 0.5 μM ethoxyresorufin in a serum-free medium. Following incubation for 10 min at 37 °C, the fluorescence was measured, with excitation at 530 nm and emission at 590 nm, using a CytoFluor microplate reader. The CYP activity was collated as fluorescence units per min in each sample and expressed as fold increase over control (solvent only).

Microsomal Intrinsic Clearance and mRNA Analysis from Cynomolgus Monkey Livers. Materials. HPLC-grade DMSO, phenacetin, furafylline, glucose 6 phosphate, nicotinamide adenine dinucleotide phosphate, glucose 6-phosphate dehydrogenase, sodium bicarbonate, sodium chloride, sucrose, Tris-HCl, pH 7.4, were purchased from Sigma (St. Louis, MO). Potassium phosphate dibasic and monobasic were obtained from Mallinckrodt (St. Louis, MO). **3** was prepared by GSK. HPLC-grade acetonitrile was purchased from EMD Chemicals, Inc. (Gibbstown, NJ). The 96-well sample tubes were purchased from Matrix Technologies (Hudson, NH). Pierce BCA protein assay reagent kit was purchased from Thermo Scientific (Waltham, MA).

Methods: Microsome Preparation. Microsomes from control and treated cynomolgous monkey livers were prepared using the method described by Jewell et al.[36] Control and induced monkey livers were washed in ice-cold saline (0.9% w/v sodium chloride) and then homogenized in the presence of sucrose/Tris buffer. Homogenate was centrifuged at 10 000*g* for 20 min at approximately 4 °C. The supernatant was removed and centrifuged at 105 000*g* for 60 min at 4 °C. The remaining pellet was resuspended in fresh sucrose/Tris buffer and then centrifuged at 105 000*g* for 60 min at 4 °C. The supernatant was discarded and the pellet resuspended in 50 mM potassium phosphate buffer and stored at −80 °C prior to use. The protein

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concentration was determined using the method described by Lowry et al.[37]

Metabolic Stability. The compound was incubated with isolated liver microsomes of both control and induced cynomolgous monkey livers at 37 °C for 30 min in 50 mM potassium phosphate buffer (pH 7.4) and 50 μL of NADPH generating system cofactor. At 0, 3, 6, 9, 12, 18, and 30 min, 50 μL of the incubation mix was removed and quenched with 200 μL of solvent containing an internal standard. Appropriate control incubations were also conducted simultaneously. The samples were analyzed by LC−MS/MS, and the intrinsic clearance values were determined according to Clarke et al.[38]

In Vitro Metabolic Stability in Hepatocytes. *Materials.*The metabolic stability of test compounds (0.5 μM) was determined in pooled human, Sprague−Dawley rat, beagle dog, and CD-1 mouse cryopreserved primary hepatocytes (CellzDirect Invitrogen Corporation, Durham, NC, USA). Hepatocytes were a pool from at least 50 donors.

Methods. A 5 mM stock solution of test compound was prepared in DMSO and diluted to 100 μM with DMSO. An aliquot was taken and further diluted to 1 μM with Williams medium E (WME) and prewarmed at 37 °C. The cryopreserved hepatocytes for each species were thawed in the manner recommended by the supplier. Cell concentration and viability were ascertained using the Trypan blue exclusion method. The cell suspension was then diluted to 1.4 million cells per mL with prewarmed (37 °C) WME. An amount of 300 μL of cell suspension was added to each well of a 12-well plate to which 300 μL of 1 μM test compound solution was also added to give a final incubation concentration of 0.5 μM test compound and 0.7 million cells/mL. The incubations were maintained at 37 °C for the duration of the experiment. At set intervals up to 120 min 25 μL of cell suspension was transferred to a 96-well block containing 100 μL of stopping solution containing internal standard. The samples were centrifuged, and the resulting supernatant was analyzed by LC−MS/MS. Intrinsic clearance was calculated by nonlinear regression analysis of peak area ratio vs time using Grafit, version 5.0.8 (Erithacus software, U.K.) to determine the first order elimination rate constant (k). To scale the hepatocyte clearance, a hepatocyte yield of 1.2×10^8 cells per gram of liver in human, rat, monkey, and mouse and 2.4×10^8 cells per gram of liver in the dog were used.[39]

In Vivo Studies. All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Laboratory Animals. For all studies, the temperature and humidity were nominally maintained at 21 °C ± 2 °C and 55% ± 10%, respectively. The diet for rodents was 5LF2 Eurodent diet 14% (PMI Labdiet, Richmond, IN). There were no known contaminants in the diet or water at concentrations that could interfere with the outcome of the studies.

Rat PK Studies. Male CD rats were supplied by Charles River UK Ltd. and were surgically prepared under anesthesia at GSK with implanted cannulae in the femoral vein (for drug administration) and jugular vein (for blood sampling). Each rat was allowed to recover from surgery for at least 2 days prior to dosing. Rats had free access to food and water throughout. Where definitive PK was required studies were conducted using a crossover design with $n = 3$ animals over two dosing occasions, with 3 days between dose administrations. Other data presented are from single animals only. On dosing day 1, $n = 3$ male rats each received a 1 h intravenous infusion of the test compound formulated in DMSO and 10% (w/v) Kleptose in saline (≤5%:≥95%) at a concentration of 0.2 mg/mL. If necessary the pH of the dose solution was adjusted to 8.0 using 0.1 mM NaOH, and the dose was filtered using a ∼0.2 μm syringe filter unit. Test compounds were administered as a 1 h iv infusion at 5 mL kg^{-1} h^{-1} to achieve a target dose of 1 mg/kg. On dosing day 2, the same three rats each received an oral administration of test compound suspended in 1% (w/v) methylcellulose aq at a concentration of 0.6 mg/mL administered by gavage at 5 mL/kg to achieve a target dose of 3 mg/kg. At the end of the study the rats were euthanised by administration of sodium pentobarbital (Euthatal) through the jugular vein cannula.

Rat 4 Day Oral CYP1A mRNA Induction Study. Male CD rats (226−325 g, supplied by Charles River UK Ltd.) were selected for study based on health. Rats had free access to food and water throughout. Rat PK studies were conducted as a repeat dose design, with once daily oral administration for 4 days. On each study day, $n = 3$ male rats received an oral gavage of the test compound formulated in a 1% (w/v) methylcellulose aq suspension at a concentration of 3−6 mg/mL administered by gavage at 5 mL/kg to achieve a target dose of 15−30 mg/kg. Tail vein blood sampling was conducted on days 1 and 4 up to 24 h postdose and frozen prior to sample analysis. At the end of the study the rats were exsanguinated via abdominal aorta under isofluorane anesthesia. At necropsy, a liver section was removed from the left lateral lobe and cut into six approximately equal pieces and mixed with RNALater and placed on crushed wet ice for RNA/Hepatotaq analysis.

Blood Sample Analysis. Diluted blood samples (1:1 with water) were extracted using protein precipitation with acetonitrile containing an analytical internal standard. An aliquot of the supernatant was analyzed by reverse phase LC−MS/MS using a turbo ionspray interface in positive ion mode. Samples were assayed against calibration standards prepared in diluted (1:1 with water) control blood.

Hepatotaq Gene Expression Analysis. Gene expression analysis of liver samples was conducted according to the methods described in Swain et al.[40] Liver samples on ice were homogenized with TRIzol before RNA purification, DNasing of extract, and conversion to cDNA took place. The prepared samples were analyzed on low density arrays to provide Cyp1a1 data.

PK Data Analysis. PK parameters were obtained from the blood concentration−time profiles using noncompartmental analysis with WinNonlin Professional 4.1a (Pharsight, Mountain View, CA).

Chemistry. General Methods. All solvents were purchased from Sigma-Aldrich (Hy-Dry anhydrous solvents), and commercially available reagents were used as received. All reactions were followed by TLC analysis (TLC plates GF254, Merck) or LCMS (liquid chromatography−mass spectrometry) using a Waters ZQ instrument. NMR spectra were recorded on a Bruker nanobay 400 MHz or a Bruker AVII+ 600 MHz spectrometers and are referenced as follows: ^{1}H NMR (400 or 600 MHz), internal standard TMS at $\delta = 0.00$; ^{13}C NMR (100.6 or 150.9 MHz), internal standard CDCl3 at $\delta = 77.23$ or DMSO-d_6 at $\delta = 39.70$. Column chromatography was performed on prepacked silica gel columns (30−90 mesh, IST) using a Biotage SP4. Mass spectra were recorded on Waters ZQ (ESI-MS) and Q-Tof 2 (HRMS) spectrometers. Mass directed autoprep was performed on a Waters 2767 with a MicroMass ZQ mass spectrometer using Supelco LCABZ++ column.

Synthetic Methods and Characterization of Compounds. Abbreviations for multiplicities observed in NMR spectra are the following: s; singlet; br s, broad singlet; d, doublet; t, triplet; q, quadruplet; p, pentuplet; spt, septuplet; m, multiplet. The purity of all compounds was determined by LCMS and ^{1}H NMR and was always >95%.

LCMS. UPLC analysis was conducted on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm, i.d. 1.7 μm packing diameter) at 40 °C. Flow rate was 1 mL/min. For the formate method, solvents employed were A = 0.1% v/v solution of formic acid in water and B = 0.1% v/v solution of formic acid in acetonitrile. For the high pH method, the solvents employed were A = 10 mM ammonium hydrogen carbonate in water adjusted to pH 10 with ammonia solution and B = acetonitrile. For both methods the gradient employed is indicated in Table 6.

The UV detection was a summed signal from wavelength of 210 to 350 nm. Mass spectra were obtained on a Waters ZQ instrument: ionization mode, alternate-scan positive and negative electrospray; scan range 100−1000 amu; scan time 0.27 s; interscan delay 0.10 s.

MDAP (Mass-Directed Automatic Preparative Purification). For the formate method, HPLC analysis was conducted on either a Sunfire C18 column (100 mm × 19 mm, i.d. 5 μm packing diameter) or a Sunfire C18 column (150 mm × 30 mm, i.d. 5 μm packing diameter) at ambient temperature. The solvents employed were A =

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Table 6. Gradient Used in UPLC Analysis

time (min)	flow rate (mL/min)	% A	% B
0	1	99	1
1.5	1	3	97
1.9	1	3	97
2.0	1	0	100

0.1% v/v solution of formic acid in water and B = 0.1% v/v solution of formic acid in acetonitrile. Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm × 19 mm, i.d. 5 μm packing diameter) or 40 mL/min (150 mm × 30 mm, i.d. 5 μm packing diameter).

For the high pH method, HPLC analysis was conducted on either an Xbridge C18 column (100 mm × 19 mm, i.d. 5 μm packing diameter) or a Xbridge C18 column (100 mm × 30 mm, i.d. 5 μm packing diameter) at ambient temperature. The solvents employed were A = 10 mM ammonium bicarbonate in water, adjusted to pH 10 with ammonia solution and B = acetonitrile. Run as a gradient over either 15 or 25 min (extended run) with a flow rate of 20 mL/min (100 mm × 19 mm, i.d. 5 μm packing diameter) or 40 mL/min (100 mm × 30 mm, i.d. 5 μm packing diameter).

For both methods the UV detection was a summed signal from wavelength of 210 to 350 nm. Mass spectra were obtained on a Waters ZQ instrument: ionization mode, alternate-scan positive and negative electrospray; scan range 100−1000 amu; scan time 0.50 s; interscan delay 0.20 s.

(*R*)-5-(3-(2-(2,3-Dihydroxypropyl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (12). To a suspension of L-(−)-glyceraldehyde (127 mg, 1.41 mmol) and 2-[(1-methylethyl)oxy]-5-[3-(5-methyl-1,2,3,4-tetrahydro-6-isoquinolinyl)-1,2,4-oxadiazol-5-yl]-benzonitrile hydrochloride (as obtained in ref 36, 187 mg, 0.46 mmol) in CH_2Cl_2 (10 mL) under nitrogen, was added AcOH (0.039 mL, 0.68 mmol) followed by sodium triacetoxyborohydride (436 mg, 2.06 mmol). The resulting mixture was stirred at room temperature for 20 h. Further sodium triacetoxyborohydride (193 mg, 0.91 mmol) was added to the mixture, and stirring was continued for a further 72 h. The solution was then treated with a saturated $NaHCO_3$ aqueous solution (5 mL), and the resulting biphasic mixture was vigorously stirred for 30 min and then was concentrated in vacuo. The residue was partitioned between a saturated $NaHCO_3$ aqueous solution (5 mL) and AcOEt (5 mL), and the layers were separated. The aqueous phase was extracted with EtOAc (3 × 4 mL). The combined organic phases were dried using a hydrophobic frit and concentrated in vacuo. Purification of the residue by mass-directed autopreparative reverse-phase HPLC gave (*R*)-5-(3-(2-(2,3-dihydroxypropyl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (147 mg, 72%) as a pale yellow gum. LCMS (method formate): retention time 0.86 min, $[M + H]^+$ = 449.3. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.41 (d, J = 2.0 Hz, 1H), 8.34 (dd, J = 9.0, 2.3 Hz, 1H), 7.86 (d, J = 8.0 Hz, 1H), 7.12−7.20 (m, 2H), 4.84 (spt, J = 6.0 Hz, 1H), 4.62−4.77 (m, 3H), 4.48−4.61 (m, 2H), 4.43 (br s, 1H), 3.59−3.87 (m, 3H), 3.40 (d, J = 10.3 Hz, 1H), 3.17−3.32 (m, 3H), 2.51−2.61 (m, 3H), 1.51 (d, J = 6.0 Hz, 6H).

(*S*)-5-(3-(2-(2,3-Dihydroxypropyl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (13). (*S*)-5-(3-(2-(2,3-Dihydroxypropyl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile was obtained as a cream colored gum in an analogous manner to compound **12**, using D-glyceraldehyde: 96 mg, 44% yield. LCMS (method formate): retention time 0.87 min, $[M + H]^+$ = 449.3. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.40 (d, J = 2.0 Hz, 1H), 8.33 (dd, J = 8.9, 2.1 Hz, 1H), 7.85 (d, J = 8.0 Hz, 1H), 7.13−7.22 (m, 2H), 4.83 (spt, J = 6.1 Hz, 1H), 4.51−4.65 (m, 1H), 4.45 (br s, 1H), 3.59−4.04 (m, 7H), 3.42 (br s, 1H), 3.17−3.35 (m, 3H), 2.55 (s, 3H), 1.48−1.55 (d, J = 6.1 Hz, 6H).

5-(3-(2-(1,3-Dihydroxypropan-2-yl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxynicotinonitrile Hydrochloride (14). *Step 1.* To make 1,1-dimethylethyl 5-methyl-6-oxo-3,4,6,7,8,8a-hexahydro-2(1*H*)-isoquinolinecarboxylate, 1,1-dimethylethyl 4-oxo-1-piperidinecarboxylate (70 g, 350 mmol) and pyrrolidine (43.6 mL, 530 mmol) were dissolved in toluene (310 mL), and the resulting mixture was refluxed under Dean−Stark conditions for 24 h and then concentrated in vacuo. The residue was dissolved in anhydrous toluene (270 mL) and treated with hydroquinone (0.40 g) and 1-penten-3-one (29.6 g, 350 mmol). The resulting solution was refluxed for 24 h and then diluted with EtOAc (300 mL). The mixture was washed with HCl (0.5 N in water, 500 mL) and the aqueous phase extracted with EtOAc (300 mL). The combined organic phases were dried ($MgSO_4$) and concentrated. Purification of the residue by flash chromatography on a silica cartridge (1.5 kg) gave 1,1-dimethylethyl 5-methyl-6-oxo-3,4,6,7,8,8a-hexahydro-2(1*H*)-isoquinolinecarboxylate (55.2 g, 59.2%) as pale yellow oil which crystallized on standing. LCMS (method formate): retention time 1.04 min, $[M + H]^+$ = 266.24. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 4.16−4.02 (m, 2H), 3.08−3.01 (m, 1H), 2.77−2.71 (m, 1H), 2.58−2.49 (m, 3H), 2.39−2.26 (m, 2H), 2.06−2.00 (m, 1H), 1.79 (s, 3H), 1.59−1.52 (m, 1H), 1.49 (s, 9H).

Step 2. Lithium bis(trimethylsilyl)amide (1 M in THF, 246 mL, 246 mmol) was added dropwise to a solution of 1,1-dimethylethyl 5-methyl-6-oxo-3,4,6,7,8,8a-hexahydro-2(1*H*)-isoquinolinecarboxylate (54.4 g, 210 mmol) in THF (200 mL) at −63 °C, and the mixture was stirred for an additional 30 min. Chloro(trimethyl)silane (31.4 mL, 250 mmol) was added dropwise, and the resulting mixture was stirred for 2 h at −70 °C. The reaction was warmed to room temperature over 20 min and diluted with diethyl ether (800 mL). The reaction was added to a saturated sodium carbonate solution, and the phases were separated. The aqueous phase was extracted with diethyl ether (300 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated in vacuo. The residue was dissolved in acetonitrile (200 mL), and palladium(II) acetate (46.0 g, 210 mmol) was added. The resulting mixture was cooled (water bath) to maintain a reaction temperature below 35 °C and stirred overnight. The reaction was filtered through Celite and the residue rinsed with EtOAc (3 × 300 mL). The filtrate was further filtered through a 1 in. pad of silica gel and concentrated. The residue was dissolved in EtOAc (500 mL), treated with tetrabutylammonium fluoride (1 M in THF, 200 mL). The resulting mixture was allowed to stand for 30 min, washed with HCl (0.5 N in water, 300 mL) and a 10% sodium thiosulfate solution, dried ($MgSO_4$), and concentrated. Purification of the residue by flash chromatography on silica gel (300 g), eluting with an EtOAc/cyclohexane gradient (0−60%), gave 1,1-dimethylethyl 6-hydroxy-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (29.9 g, 55%) as a white solid. LCMS (method formate): retention time 1.07 min, $[M + H]^+$ = 264.12. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.03 (s, 1H), 6.75 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 8.1 Hz, 1H), 4.36 (s, 2H), 3.52 (t, J = 6.1 Hz, 2H), 2.62 (t, J = 6.1 Hz, 2H), 2.01 (s, 3H), 1.41 (s, 9H). HRMS calculated for $C_{15}H_{22}NO_3$: 264.1600. Found: 264.1605.

Step 3. To a solution of 1,1-dimethylethyl 6-hydroxy-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (3.16 g, 12 mmol) in CH_2Cl_2 (50 mL) at room temperature under nitrogen was added pyridine (1.94 mL, 24 mmol), and the resulting solution was cooled to −30 °C before trifluoromethanesulfonic anhydride (2.23 mL, 13.20 mmol) was added dropwise. The resulting mixture was stirred for 40 min at this temperature, then warmed to room temperature and concentrated in vacuo. The residue was dissolved in EtOAc, and the organic phase was washed sequentially with HCl (1 N in water), a saturated $NaHCO_3$ aqueous solution, and brine. The solution was then dried ($MgSO_4$) and concentrated in vacuo to give 1,1-dimethylethyl 5-methyl-6-{[(trifluoromethyl)sulfonyl]oxy}-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (4.85 g, 102%) as a red oil which was used in the next step without further purification. LCMS (method high pH): retention time 1.46 min, $[M - H]^-$ = 394.2. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.10 (d, J = 8.1 Hz, 1H), 7.02 (d, J = 8.1 Hz, 1H), 4.58 (s, 2H), 3.68 (t, J = 5.8 Hz, 2H), 2.76 (t, J = 5.8 Hz, 2H), 2.25 (s, 3H), 1.50 (s, 9H).

Step 4. A solution of 1,1-dimethylethyl 5-methyl-6-{[(trifluoromethyl)sulfonyl]oxy}-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (26.1 g, 66 mmol) in DMF (200 mL) was degassed for 10 min under vacuum and then flushed with nitrogen. The solution was

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treated with tetrakis(triphenylphosphine)palladium (7.6 g, 6.6 mmol) and zinc cyanide (10.1 g, 86 mmol), and the resulting mixture was stirred at 100 °C under nitrogen for 6 h and cooled to room temperature. The mixture was filtered, the residue washed with EtOAc, and most of the solvent evaporated in vacuo. The residue was dissolved in EtOAc and washed with a saturated sodium hydrogen carbonate aqueous solution (×2). The combined aqueous phases were extracted with EtOAc (×2), and the combined organic phases were washed with brine, dried ($MgSO_4$), and concentrated in vacuo. Purification of the residue by flash chromatography, eluting with an EtOAc/cyclohexane gradient, gave 1,1-dimethylethyl 6-cyano-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (16.6 g, 92%) as a white solid. LCMS (method high pH): retention time 1.24 min, $[M + H]^+$ = 273.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.57 (d, J = 8.1 Hz, 1H), 7.22 (d, J = 8.1 Hz, 1H), 4.56 (s, 2H), 3.59 (t, J = 6.1 Hz, 2H), 2.72 (t, J = 6.1 Hz, 2H), 2.39 (s, 3H), 1.44 (s, 9H). HRMS calculated for $C_{16}H_{21}N_2O_2$: 273.1603. Found: 273.1608.

Step 5. A mixture of 1,1-dimethylethyl 6-cyano-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (16.6 g, 61 mmol), sodium hydrogen carbonate (30.7 g, 370 mmol), and hydroxylamine hydrochloride (25.4 g, 370 mmol) in ethanol (250 mL) was refluxed for 28.5 h and allowed to cool to room temperature. The reaction was filtered and the residue washed with ethanol. The combined filtrate and washings were concentrated in vacuo. The residue was poured into water (100 mL) and stirred at room temperature for 20 min. The precipitated solid was isolated by filtration and dried under vacuum at 40 °C for 16 h to give 1,1-dimethylethyl 6-[(hydroxyamino)(imino)-methyl]-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (16 g, 86%) as a white solid. LCMS (method high pH): retention time 0.93 min, $[M + H]^+$ = 306.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.21 (s, 1H), 7.07 (d, J = 8.9 Hz, 1H), 6.99 (d, J = 8.9 Hz, 1H), 5.65 (s, 2H), 4.48 (s, 2H), 3.58 (t, J = 5.9 Hz, 2H), 2.67 (t, J = 5.9 Hz, 2H), 2.20 (s, 3H), 1.43 (m, 9H). HRMS calculated for $C_{16}H_{24}N_3O_3$: 306.1818. Found: 306.1817.

Step 6. A mixture of methyl 5-cyano-6-oxo-1,6-dihydro-3-pyridine-carboxylate (2.98 g, 16.7 mmol), isopropyl iodide (6.68 mL, 66.9 mmol), and silver carbonate (9.23 g, 33.5 mmol) in $CHCl_3$ (100 mL) was stirred at 60 °C for 4 h under nitrogen and then was cooled to room temperature and filtered using a pad of Celite. Most of the solvent was removed in vacuo. Purification of the residue by flash chromatography on silica gel (0−30% EtOAc in cyclohexane) gave methyl 5-cyano-6-isopropoxynicotinate (3.2 g, 87%) as a colorless solid. LCMS (method formate): retention time 1.08 min, $[M + H]^+$ = 220.0. ^{1}H NMR (600 MHz, $CDCl_3$) δ ppm 8.97 (d, J = 2.2 Hz, 1H), 8.47 (d, J = 2.2 Hz, 1H), 5.54 (spt, J = 6.2 Hz, 1H), 3.96 (s, 3H), 1.45 (d, J = 6.2 Hz, 6H).

Step 7. A solution of LiOH (1.74 g, 72.7 mmol) in water (10 mL) was added to a solution of methyl 5-cyano-6-[(1-methylethyl)oxy]-3-pyridinecarboxylate (3.2 g, 14.5 mmol) in MeOH (30 mL), and the resulting mixture was stirred at room temperature for 4 h. Most of MeOH was removed in vacuo, and the residual aqueous phase was acidified to pH 1 with a 2 N HCl aqueous solution and then was extracted with EtOAc (3 × 50 mL). The combined organic phases were washed with brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give 5-cyano-6-isopropoxynicotinic acid (3.05 g, 95%) as a colorless solid. LCMS (method formate): retention time 0.90 min, $[M + H]^+$ = 206. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 13.48 (br s, 1H), 8.92 (d, J = 2.3 Hz, 1H), 8.61 (d, J = 2.5 Hz, 1H), 5.45 (spt, J = 6.2 Hz, 1H), 1.37 (d, J = 6.2 Hz, 6H).

Step 8. Oxalyl chloride (1.94 mL, 22.1 mmol) was added at room temperature to a suspension of 5-cyano-6-[(1-methylethyl)oxy]-3-pyridinecarboxylic acid (3.04 g, 14.7 mmol) in CH_2Cl_2 (50 mL), followed by DMF (0.011 mL, 0.15 mmol), and the resulting mixture was stirred at this temperature for 3 h. The resulting homogeneous, pale yellow solution was concentrated in vacuo and the residue was azeotroped with toluene (3 × 10 mL) to give 5-cyano-6-isopropoxy-nicotinoyl chloride (3.5 g, 95%) as a pale yellow oil which solidified on standing under vacuum. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.06 (d, J = 2.5 Hz, 1H), 8.53 (d, J = 2.5 Hz, 1H), 5.59 (spt, J = 6.1 Hz, 1H), 1.47 (d, J = 6.3 Hz, 6H).

Step 9. 5-Cyano-6-[(1-methylethyl)oxy]-3-pyridinecarbonyl chloride (660 mg, 2.94 mmol) was added portionwise to a suspension of 1,1-dimethylethyl 6-[(hydroxyamino)(imino)methyl]-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (855 mg, 2.80 mmol) in toluene (10 mL) and pyridine (10 mL) at room temperature under nitrogen, and the resulting mixture was stirred at this temperature for 20 min and then was stirred at 120 °C for 2 h before being cooled to room temperature and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (0−50% EtOAc in cyclohexane) gave *tert*-butyl 6-(5-(5-cyano-6-isopropoxypyridin-3-yl)-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (1.24 g, 86%) as colorless foam. LCMS (method formate): retention time 1.54 min, $[2M + H]^+$ = 951; $[M - C_4H_8]^+$ = 420. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.16 (d, J = 2.3 Hz, 1H), 8.65 (d, J = 2.5 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.13 (d, J = 8.1 Hz, 1H), 5.52−5.64 (m, 1H), 4.66 (s, 2H), 3.74 (t, J = 5.8 Hz, 2H), 2.87 (t, J = 5.7 Hz, 2H), 2.54 (s, 3H), 1.53 (s, 9H), 1.49 (d, J = 6.3 Hz, 6H).

Step 10. A solution of 1,1-dimethylethyl 6-(5-{5-cyano-6-[(1-methylethyl)oxy]-3-pyridinyl}-1,2,4-oxadiazol-3-yl)-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (1.12 g, 2.35 mmol) in CH_2Cl_2 (5 mL) at 0 °C was treated with trifuoroacetic acid (0.91 mL, 11.8 mmol) dropwise. The resulting mixture was allowed to warm to room temperature and was stirred at this temperature for 16 h. Most of the solvent was removed in vacuo and the residue was triturated with Et_2O (2 × 5 mL) and then filtered off to give 2-[(1-methylethyl)oxy]-5-[3-(5-methyl-1,2,3,4-tetrahydro-6-isoquinolinyl)-1,2,4-oxadiazol-5-yl]-3-pyridinecarbonitrile trifluoroacetate (1.1 g, 96%) as a colorless solid. LCMS (method formate): retention time 0.96 min, $[M + H]^+$ = 376. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.21 (d, J = 2.5 Hz, 1H), 9.13 (br s, 2H), 8.99 (d, J = 2.5 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.30 (d, J = 8.1 Hz, 1H), 5.51 (spt, J = 6.2 Hz, 1H), 4.38 (br s, 2H), 3.49 (br s, 2H), 2.99 (t, J = 6.2 Hz, 2H), 2.48 (s, 3H), 1.42 (d, J = 6.1 Hz, 6H).

Step 11. 2,2-Dimethyl-1,3-dioxan-5-one (199 mg, 1.53 mmol) was added to a stirred solution of 2-[(1-methylethyl)oxy]-5-[3-(5-methyl-1,2,3,4-tetrahydro-6-isoquinolinyl)-1,2,4-oxadiazol-5-yl]-3-pyridine-carbonitrile trifluoroacetate (150 mg, 0.31 mmol) in 1,2-dichloro-ethane (2 mL) and THF (2 mL). The reaction mixture was stirred at room temperature for 30 min, and then sodium triacetoxyborohydride (325 mg, 1.53 mmol) was added. Stirring at room temperature was continued for 4 h. Further portions of 2,2-dimethyl-1,3-dioxan-5-one (199 mg, 1.53 mmol) and sodium triacetoxyborohydride (325 mg, 1.53 mmol) were added, and stirring continued for 16 h at room temperature. Further portions of 2,2-dimethyl-1,3-dioxan-5-one (199 mg, 1.53 mmol) and sodium triacetoxyborohydride (325 mg, 1.53 mmol) were added, and stirring was continued for 6 h. A saturated $NaHCO_3$ aqueous solution (10 mL) was added and the mixture extracted with EtOAc (2 × 10 mL). The combined extracts were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (10−40% EtOAc in isohexane] gave 5-(3-(2-(2,2-dimethyl-1,3-dioxan-5-yl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxynicotinonitrile (30 mg, 20%) as a colorless solid after trituration with Et_2O. LCMS (method formate): retention time 1.04 min, $[M + H]^+$ = 490. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.20 (s, 1H), 8.99 (s, 1H), 7.66 (d, J = 3.0 Hz, 1H), 7.11 (d, J = 3.0 Hz, 1H), 5.50−5.53 (m, 1H), 3.97−4.00 (m, 2H), 3.81−3.85 (m, 3H), 2.89−2.91 (m, 2H), 2.73−2.76 (m, 2H), 2.66−2.68 (m, 2H), 2.43 (s, 3H), 1.41 (d, J = 7.8 Hz, 6H), 1.28−1.32 (m, 6H).

Step 12. A 2 N HCl aqueous solution (2 mL) was added to a solution of 5-{3-[2-(2,2-dimethyl-1,3-dioxan-5-yl)-5-methyl-1,2,3,4-tetrahydro-6-isoquinolinyl]-1,2,4-oxadiazol-5-yl}-2-[(1-methylethyl)-oxy]-3-pyridinecarbonitrile (30 mg, 0.06 mmol) in THF (2 mL). The resulting mixture was stirred at room temperature for 2 h and then was diluted with EtOAc (10 mL) and basified by the addition of a saturated $NaHCO_3$ aqueous solution. The aqueous phase was separated and extracted with EtOAc (10 mL). The combined organics were dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in THF and treated with a 4 N HCl solution in 1,4-dioxan (1 mL, excess). The solvent was concentrated in vacuo. Trituration of the residue with Et_2O gave **14** (20 mg, 73%) as a colorless solid. LCMS

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(method formate): retention time 0.91 min, $[M + H]^+$ = 450. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.32−10.45 (m, 1H), 9.21 (d, J = 2.0 Hz, 1H), 8.99 (d, J = 2.0 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.29 (d, J = 8.1 Hz, 1H), 5.41−5.57 (m, 3H), 4.68−4.81 (m, 1H), 4.52−4.65 (m, 1H), 3.90 (br s, 5H), 3.49−3.63 (m, 1H), 3.14 (br s, 2H), 2.47 (s, 3H), 1.42 (d, J = 6.1 Hz, 6H).

5-(3-(7-(2,2-Dimethyl-1,3-dioxan-5-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (15). *Step 1.* 5-(3-(7-(2,2-Dimethyl-1,3-dioxan-5-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile was obtained from 2-isopropoxy-5-(3-(4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)benzonitrile (synthesis published in ref 38) using a similar procedure as described in step 11 for compound **14**. LCMS (method formate): retention time 0.91 min, $[M + H]^+$ = 490.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.80 (s, 1H), 8.52 (s, 1H), 8.41 (dd, J = 9.0, 2.3 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 4.96−5.01 (m,1H), 4.01−4.04 (m, 2H), 3.83−3.87 (m, 4H), 3.35 (s, 1H), 2.92−2.95 (m, 2H), 2.74−2.80 (m, 2H), 2.49 (s, 3H), 1.34−1.41 (m, 12H).

Step 2. **15** was obtained as a white foam from 5-(3-(7-(2,2-dimethyl-1,3-dioxan-5-yl)-4-methyl-5,6,7,8-tetrahydro-1,7-naphthyridin-3-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile using a similar procedure to the one described in step 12 for compound **14**: 62 mg, 18% yield (2 steps). LCMS (method formate): retention time 0.80 min, $[M + H]^+$ = 450.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.80 (br s, 1H), 8.93 (s, 1H), 8.56 (d, J = 2.3 Hz, 1H), 8.44 (dd, J = 9.0, 2.3 Hz, 1H), 7.59 (d, J = 9.0 Hz, 1H), 5.92−6.91 (br s, 2H), 5.00 (spt, J = 6.0 Hz, 1H), 4.85 (dd, J = 16.1, 8.0 Hz, 1H), 4.56 (d, J = 15.8 Hz, 1H), 3.86−4.07 (m, 4H), 3.47−3.70 (m, 2H), 3.08−3.33 (m, 2H), 2.56 (s, 3H), 1.40 (d, J = 6.0 Hz, 6H).

5-(5-(2-(1,3-Dihydroxypropan-2-yl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,3,4-thiadiazol-2-yl)-2-isopropoxybenzonitrile Hydrochloride (16). *Step 1.* To make *tert*-butyl 5-methyl-6-(4,4,5-trimethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate, a solution of 1,1-dimethylethyl 5-methyl-6-{[(trifluoromethyl)sulfonyl]oxy}-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (step 3, compound **14**, 0.791 g, 2 mmol) in 1,4-dioxane (10 mL) was degassed under vacuum for 10 min and then was treated with $PdCl_2$(dppf) (0.146 g, 0.200 mmol), potassium acetate (0.785 g, 8.00 mmol), and bis(pinacolato)diboron (0.609 g, 2.400 mmol). The resulting mixture was refluxed for 5 h and then was cooled to room temperature, and most of the solvent was removed in vacuo. The residue was partitioned between EtOAc and water. The biphasic mixture was filtered through a pad of Celite, and then the layers were separated. The aqueous phase was extracted with EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (0−10% MeOH in CH_2Cl_2) gave *tert*-butyl 5-methyl-6-(4,4,5-trimethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (660 mg, 88%) as a pale yellow solid. LCMS (method formate): retention time 1.56 min, $[M + H]^+$ = 374 (weak), $[2M + H]^+$ = 747. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.62 (d, J = 7.8 Hz, 1H), 6.97 (d, J = 7.8 Hz, 1H), 4.54−4.58 (br s, 2H), 3.61−3.70 (m, 2H), 2.70−2.80 (m, 2H), 2.48 (s, 3H), 1.50 (s, 9H), 1.38 (s, 12H).

Step 2. Phosphoric trichloride (90 g, 587 mmol) was added to a mixture of 3-cyano-4-[(1-methylethyl)oxy]benzoic acid (20.9 g, 102 mmol) and hydrazinecarbothioamide (13.9 g, 153 mmol), and the resulting mixture was stirred at 90 °C for 3 h, then was cooled to room temperature and slowly poured into a ice-cooled 5 M NaOH aqueous solution (600 mL), making sure that the temperature was maintained below 35 °C. The solution was cooled to 0 °C, and concentrated aqueous HCl was slowly added to adjust the pH to 10. The beige solid formed was filtered off and then dissolved in CH_2Cl_2 (1 L) and MeOH (50 mL). The organic phase was washed with water (500 mL), dried over $MgSO_4$, and concentrated in vacuo to give 5-(5-amino-1,3,4-thiadiazol-2-yl)-2-isopropoxybenzonitrile (26.5 g, 99% yield) as a pale yellow solid which was used in the next step without further purification. LCMS (method formate): retention time 0.82 min, $[M + H]^+$ = 261. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 7.99−8.04 (m, 2H), 7.37 (d, J = 7.8 Hz, 1H), 7.35 (br s, 2H), 4.83−4.89 (m, 1H), 1.35 (d, J = 8.0 Hz, 6H).

Step 3. A solution of 5-(5-amino-1,3,4-thiadiazol-2-yl)-2-[(1-methylethyl)oxy]benzonitrile (6.36 g, 24.4 mmol) in CH_3CN (60 mL) was treated with copper(II) bromide (10.9 g, 48.9 mmol) and isoamyl nitrite (6.53 mL, 48.9 mmol), and the resulting mixture was stirred at room temperature for 1 h and then was concentrated in vacuo. The residue was dissolved in EtOAc, and the organic phase was filtered through a pad of Celite. The insoluble material was washed with EtOAc and the combined organics were washed with a 2 N HCl aqueous solution (100 mL), then with brine (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give 5-(5-bromo-1,3,4-thiadiazol-2-yl)-2-isopropoxybenzonitrile (4.98 g, 63%) as a yellow solid. LCMS (method formate): retention time 1.14 min, $[M + H]^+$ = 324, 326 (1 Br). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.33 (s, 1H), 8.22 (dd, J = 8.0, 3.0 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 4.91−4.96 (m, 1H), 1.35 (d, J = 7.8 Hz, 6H).

Step 4. A solution of 1,1-dimethylethyl 5-methyl-6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (300 mg, 0.80 mmol) and 5-(5-bromo-1,3,4-thiadiazol-2-yl)-2-[(1-methylethyl)oxy]benzonitrile (313 mg, 0.96 mmol) in 1,2-dimethoxyethane (6 mL) and water (2 mL) was treated with Na_2CO_3 (426 mg, 4.02 mmol) and Pd(dppf)Cl_2·CH_2Cl_2 (1.13 g, 1.39 mmol). The resulting mixture was refluxed under nitrogen for 3 h and then was cooled to room temperature, and most of the solvent was removed in vacuo. The residue was partitioned between EtOAc (100 mL) and water (100 mL), and the biphasic mixture was filtered through a pad of Celite and the insoluble washed with EtOAc. The combined layers were separated, and the organic phase was washed with water (100 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (5−30% EtOAc in hexanes) gave *tert*-butyl 6-(5-(3-cyano-4-isopropoxyphenyl)-1,3,4-thiadiazol-2-yl)-5-methyl-3,4-dihydroisoquinoline-2(1*H*)-carboxylate (394 mg, 71%) as a pale yellow foam. LCMS (method formate): retention time 1.46 min, $[M + H]^+$ = 491. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.25 (d, J = 8.3 Hz, 1H), 8.18 (s, 1H), 7.46 (d, J = 8.1 Hz, 1H), 7.30−7.35 (m, 1H), 7.11 (d, J = 8.6 Hz, 1H), 4.74−4.84 (m, 1H), 4.66 (s, 2H), 3.74 (t, J = 5.6 Hz, 2H), 2.86 (br s, 2H), 2.48 (s, 3H), 1.52 (s, 9H), 1.48 (d, J = 6.1 Hz, 6H).

Step 5. A solution of 1,1-dimethylethyl 6-(5-{3-cyano-4-[(1-methylethyl)oxy]phenyl}-1,3,4-thiadiazol-2-yl)-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (912 mg, 1.86 mmol) in CH_2Cl_2 (10 mL) was treated with trifluoroacetic acid (5.00 mL, 64.9 mmol) at room temperature. The resulting mixture was stirred for 30 min and then was concentrated in vacuo. The residue obtained was subsequently taken up twice in toluene and concentrated in vacuo, then was triturated with Et_2O and filtered off to give 2-isopropoxy-5-(5-(5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,3,4-thiadiazol-2-yl)-benzonitrile trifluoroacetate (808 mg, 86%) as a brown solid which was used in the next step without further purification. LCMS (method formate): retention time 0.82 min, $[M + H]^+$ = 391. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.20 (br s, 2H), 8.38 (s, 1H), 8.30 (br s, 1H), 7.56 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 9.1 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 4.87−5.03 (m, 1H), 4.38 (br s, 2H), 3.48 (br s, 2H), 2.99 (br s, 2H), 2.39 (s, 3H), 1.38 (d, J = 5.8 Hz, 6H).

Step 6. A solution of 2-[(1-methylethyl)oxy]-5-[5-(5-methyl-1,2,3,4-tetrahydro-6-isoquinolinyl)-1,3,4-thiadiazol-2-yl]benzonitrile trifluoroacetate (795 mg, 1.58 mmol) and 2,2-dimethyl-1,3-dioxan-5-one (0.56 mL, 4.73 mmol) in CH_2Cl_2 (20 mL) was treated with sodium triacetoxyborohydride (1.67 g, 7.88 mmol) portionwise. The resulting mixture was stirred for 18 h at room temperature and then was treated with a saturated $NaHCO_3$ aqueous solution. The resulting mixture was stirred for 15 min, and then the layers were separated. The aqueous layer was extracted three times with CH_2Cl_2. The combined organic layers were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (2−5% MeOH in CH_2Cl_2) gave 5-(5-(2-(2,2-dimethyl-1,3-dioxan-5-yl)-5-methyl-1,2,3,4-tetrahydroisoquinolin-6-yl)-1,3,4-thiadiazol-2-yl)-2-isopropoxybenzonitrile (824 mg, 99%) as a yellow solid. LCMS (method formate): retention time 0.89 min, $[M + H]^+$ = 505. ^{1}H

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NMR (400 MHz, $CDCl_3$) δ ppm 8.08−8.14 (m, 2H), 7.33 (d, J = 8.1 Hz, 1H), 7.05 (d, J = 9.1 Hz, 1H), 6.94 (d, J = 8.1 Hz, 1H), 4.71 (spt, J = 6.1 Hz, 1H), 4.03 (dd, J = 11.9, 5.1 Hz, 1H), 3.98 (dd, J = 12.1, 2.5 Hz, 1H), 3.88 (dd, J = 11.9, 7.8 Hz, 1H), 3.79 (s, 2H), 3.67 (dd, J = 12.0, 3.9 Hz, 1H), 3.49−3.55 (m, 1H), 2.87−2.93 (m, 2H), 2.77 (t, J = 6.1 Hz, 2H), 2.36 (s, 3H), 1.35−1.42 (m, 12H).

Step 7. A solution of 5-{5-[2-(2,2-dimethyl-1,3-dioxan-5-yl)-5-methyl-1,2,3,4-tetrahydro-6-isoquinolinyl]-1,3,4-thiadiazol-2-yl}-2-[(1-methylethyl)oxy]benzonitrile (824 mg, 1.63 mmol) in THF (15 mL) was treated with a 2 N HCl aqueous solution (15 mL), and the resulting mixture was stirred at room temperature for 2 h and then was concentrated in vacuo and the residue coevaporated twice with toluene. The residue obtained was triturated with Et_2O and the precipitate formed was filtered off and dried under vacuum to give **16** (502 mg, 60%) as a light yellow solid. LCMS (method formate): retention time 0.80 min, $[M + H]^+$ = 465. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 10.29 (br s, 1H), 8.39 (d, J = 2.3 Hz, 1H), 8.31 (dd, J = 8.8, 2.3 Hz, 1H), 7.57 (d, J = 8.1 Hz, 1H), 7.50 (d, J = 9.3 Hz, 1H), 7.28 (d, J = 8.1 Hz, 1H), 5.43−5.56 (m, 2H), 4.94 (s, 1H), 4.67−4.82 (m, 1H), 4.53−4.65 (m, 1H), 3.90 (br s, 4H), 3.52−3.64 (m, 1H), 3.30 (d, J = 5.6 Hz, 2H), 3.13 (br s, 2H), 2.39 (s, 3H), 1.38 (d, J = 6.1 Hz, 6H).

5-(3-(2-(1,3-Dihydroxypropan-2-yl)-1,2,3,4-tetrahydroisoquinolin-5-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile Hydrochloride (17). *Step 1.* 5-(3-(2-(2,2-Dimethyl-1,3-dioxan-5-yl)-1,2,3,4-tetrahydroisoquinolin-5-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile was obtained as a yellow solid from 2-isopropoxy-5-(3-(1,2,3,4-tetrahydroisoquinolin-5-yl)-1,2,4-oxadiazol-5-yl)benzonitrile (synthesis described in ref 39) using a similar procedure as described in step 11 for compound **14**. LCMS (method formate): retention time 0.94 min, $[M + H]^+$ = 475.2. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.41 (br s, 1H), 8.32 (d, J = 8.1 Hz, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.25−7.33 (m, 1H), 7.20 (d, J = 6.6 Hz, 1H), 7.12 (d, J = 9.1 Hz, 1H), 4.72−4.85 (m, 1H), 4.01−4.15 (m, 1H), 3.91−3.99 (m, 1H), 3.88 (br s, 2H), 3.75 (d, J = 9.3 Hz, 1H), 3.26 (br s, 2H), 2.94 (br s, 2H), 2.83 (br s, 2H), 1.36−1.54 (m, 12H).

Step 2. **17** was obtained as a white solid from 5-(3-(2-(2,2-dimethyl-1,3-dioxan-5-yl)-1,2,3,4-tetrahydroisoquinolin-5-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile using a similar procedure as the one described in step 12 for compound **14**: 233 mg, 51% yield (2 steps). LCMS (method formate): retention time 0.84 min, $[M + H]^+$ = 435.2. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 10.37 (br s, 1H), 8.51−8.55 (m, 1H), 8.39−8.44 (m, 1H), 8.03−8.09 (m, 1H), 7.55−7.59 (m, 1H), 7.47−7.55 (m, 2H), 5.45−5.58 (m, 2H), 4.93−5.04 (m, 1H), 4.72−4.83 (m, 1H), 4.58−4.69 (m, 1H), 3.83−3.99 (m, 5H), 3.45−3.60 (m, 4H), 1.39 (d, J = 6.1 Hz, 6H).

(*R*)-5-(3-(3-(2,3-Dihydroxypropyl)-6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile Hydrochloride (18). *Step 1.* To make (3-methoxy-2-methylphenyl)methanol, to a solution of 2-methyl-3-(methyloxy)benzoic acid (24.9 g, 150 mmol) in THF (300 mL) at 0 °C under nitrogen was slowly added borane−tetrahydrofuran complex (1 M in THF, 308 mL, 308 mmol) via canula over 15 min. After 10 min, the mixture was allowed to warm to room temperature and stirred for 3 h. The solution was then cooled to 0 °C and slowly treated with a 2 N HCl aqueous solution (200 mL) so that the temperature was kept below 20 °C (CAUTION: formation of hydrogen!). Most of the THF was removed in vacuo, and the residue was partitioned between EtOAc and brine. The layers were separated, and the aqueous phase was extracted twice with EtOAc. The combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give (3-methoxy-2-methylphenyl)methanol (22.8 g, 95%) as a brown solid used as it was in the next step without further purification. LCMS (method formate): retention time 0.79 min, $[M + H]^+$ = 153. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.15−7.24 (m, 1H), 7.01 (d, J = 7.6 Hz, 1H), 6.85 (d, J = 8.3 Hz, 1H), 4.72 (s, 2H), 3.85 (s, 3H), 2.24 (s, 3H). OH was not observed.

Step 2. Methanesulfonyl chloride (23.0 mL, 295 mmol) was added dropwise at 0 °C to a solution of [2-methyl-3-(methyloxy)phenyl]-methanol (51 g, 268 mmol) and NEt_3 (44.8 mL, 322 mmol) in CH_2Cl_2 (1 L), and the resulting mixture was allowed to warm to room temperature over 2 h and then was washed with water (500 mL) and brine (200 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (750 g column, 0−30% EtOAc in cyclohexane) gave 1-(chloromethyl)-3-methoxy-2-methylbenzene (35.2 g, 77%) as pale yellow oil. LCMS (method formate): retention time 1.17 min, $[M + H]^+$ = 171. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.19 (t, J = 8.0 Hz, 1H), 6.97 (d, J = 7.6 Hz, 1H), 6.88 (d, J = 8.1 Hz, 1H), 4.64 (s, 2H), 3.79−3.93 (m, 3H), 2.32 (s, 3H).

Step 3. A solution of 1-(chloromethyl)-2-methyl-3-(methyloxy)-benzene (35 g, 205 mmol) in DMF (200 mL) at room temperature was treated with sodium cyanide (17 g, 347 mmol), and the resulting mixture was stirred at 90 °C for 4 days, then was cooled to room temperature and diluted with water (1 L). The aqueous phase was extracted with Et_2O (1 L) and the organic phase was washed with water (500 mL), dried over $MgSO_4$, and concentrated in vacuo to give 2-(3-methoxy-2-methylphenyl)acetonitrile (28.5 g, 86%) as a pale yellow oil which was used in the next step without further purification. LCMS (method formate): retention time 0.96 min, $[M + H]^+$ = 162. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.21 (t, J = 8.0 Hz, 1H), 6.99 (d, J = 7.6 Hz, 1H), 6.87 (d, J = 8.3 Hz, 1H), 3.86 (s, 3H) 3.69 (s, 2H) 2.13−2.31 (m, 3H).

Step 4. A solution of [2-methyl-3-(methyloxy)phenyl]acetonitrile (28 g, 174 mmol) in EtOH (200 mL) was treated with a 5 N NaOH aqueous solution (174 mL, 868 mmol), and the resulting mixture was heated at reflux for 2 days and then was cooled to room temperature and concentrated in vacuo to half its volume. The residue was diluted with water (100 mL), and the aqueous phase was washed with Et_2O (200 mL) and then was acidified with a 5 N HCl aqueous solution to pH 2. The resulting suspension was extracted with CH_2Cl_2 (2 × 200 mL). The combined organics were washed with water (100 mL), dried over $MgSO_4$, and concentrated in vacuo to give 2-(3-methoxy-2-methylphenyl)acetic acid (30.3 g, 97% yield) as a white solid. LCMS (method formate): retention time 0.83 min, $[M + H]^+$ = 181. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.16 (t, J = 8.0 Hz, 1H), 6.84 (t, J = 7.6 Hz, 2H), 3.79−3.92 (m, 3H), 3.70 (s, 2H), 2.20 (s, 3H). COOH was not observed.

Step 5. A solution of [2-methyl-3-(methyloxy)phenyl]acetic acid (30.2 g, 168 mmol) in CH_2Cl_2 (300 mL) was treated with oxalyl chloride (29.3 mL, 335 mmol) and then with DMF (0.2 mL, catalytic), and the resulting mixture was stirred at room temperature for 2 h until the effervescence ceased. Most of the solvent was evaporated in vacuo to give a dark purple liquid which was dissolved in CH_2Cl_2 (100 mL) and added dropwise at room temperature to a solution of 2,2-dimethoxyethanamine (19.38 g, 184 mmol) and NEt_3 (30.4 mL, 218 mmol) in CH_2Cl_2 (200 mL). The resulting mixture was stirred at this temperature for 1 h, then was washed successively with water (200 mL), a 10% aqueous AcOH solution (200 mL), and a saturated $NaHCO_3$ aqueous solution (300 mL), then was dried over $MgSO_4$ and concentrated in vacuo to give *N*-(2,2-dimethoxyethyl)-2-(3-methoxy-2-methylphenyl)acetamide (43.9 g, 98% yield) as a beige crystalline solid. LCMS (method formate): retention time 0.81 min, $[M + H]^+$ = 268. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.17 (t, J = 8.0 Hz, 1H), 6.82 (t, J = 8.2 Hz, 2H), 5.49−5.63 (m, 1H), 4.31 (t, J = 5.4 Hz, 1H), 3.85 (s, 3H), 3.61 (s, 2H), 3.28−3.39 (m, 8H), 2.16 (s, 3H).

Step 6. *N*-[2,2-Bis(methyloxy)ethyl]-2-[2-methyl-3-(methyloxy)-phenyl]acetamide (44 g, 165 mmol) was dissolved in a mixture of concentrated aqueous HCl (100 mL, 3.29 mol) and acetic acid (100 mL, 1.75 mol), and the resulting solution was stirred at room temperature for 3 h, then added to ice (500 g). The aqueous phase was extracted with CH_2Cl_2 (1 L). The organic phase was washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give 8-methoxy-9-methyl-1*H*-benzo[*d*]azepin-2(3*H*)-one (25.4 g, 76%) as a dark brown solid which was used in the next step without further purification. LCMS (method formate): retention time 0.84 min, $[M + H]^+$ = 204. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 9.52 (br s, 1H), 7.11 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 8.6 Hz, 1H), 6.25−6.34 (m, 1H), 6.16−6.25 (m, 1H), 3.79 (s, 3H), 3.35 (s, 2H), 2.23 (s, 3H).

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Step 7. A solution of 9-methyl-8-(methyloxy)-1,3-dihydro-2*H*-3-benzazepin-2-one (24 g, 118 mmol) in MeOH (1.2 L) was treated with palladium on carbon (10% w/w, 50% wet; 2 g), and the resulting mixture was stirred under an atmosphere of hydrogen for 16 h. The catalyst was filtered off under nitrogen and washed with MeOH. The combined organics were concentrated in vacuo to give 8-methoxy-9-methyl-4,5-dihydro-1*H*-benzo[*d*]azepin-2(3*H*)-one (23.8 g, 98%) as pale green solid which was used in the next step without further purification. LCMS (method formate): retention time 0.76 min, $[M + H]^+$ = 206. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.99 (d, J = 8.2 Hz, 1H), 6.73 (d, J = 8.2 Hz, 1H), 6.01 (br s, 1H), 3.89 (s, 2H), 3.82 (s, 3H), 3.43−3.53 (m, 2H), 3.05−3.17 (m, 2H), 2.29 (s, 3H).

Step 8. A solution of 9-methyl-8-(methyloxy)-1,3,4,5-tetrahydro-2*H*-3-benzazepin-2-one (21.1 g, 103 mmol) in THF (100 mL) at room temperature under nitrogen was treated dropwise with borane−tetrahydrofuran complex (206 mL, 206 mmol), and the resulting mixture was stirred at 60 °C for 16 h and then was cooled to 0 °C. The mixture was carefully treated with a 5 N HCl aqueous solution (20 mL), and then most of the solvent was removed in vacuo. The aqueous residue was diluted with water (40 mL), and the aqueous phase was washed with Et_2O (2 × 50 mL). The aqueous layer was then basified with a 10 N NaOH aqueous solution to pH 10 and extracted first with CH_2Cl_2 and then with EtOAc. The combined organics were dried over $MgSO_4$ and concentrated in vacuo to give 7-methoxy-6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepine (18.9 g, 96%) as a pale yellow solid. LCMS (method formate): retention time 0.80 min, $[M + H]^+$ = 192. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.93 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 8.1 Hz, 1H), 3.82 (s, 3H), 2.82−3.03 (m, 8H), 2.21 (s, 3H), 1.85 (br s, 1H).

Step 9. A solution of 6-methyl-7-(methyloxy)-2,3,4,5-tetrahydro-1*H*-3-benzazepine (18.9 g, 99 mmol) in aqueous HBr (48% w/w, 100 mL) was stirred at 100 °C for 3 h, then was cooled to room temperature. Most of the water was removed in vacuo to give a beige solid. This residue was suspended in THF (100 mL) and CH_2Cl_2 (100 mL), and the resulting solution was treated with NEt_3 (41.3 mL, 296 mmol), then with di-*tert*-butyl dicarbonate (31.0 mL, 133 mmol). The resulting mixture was stirred at room temperature for 2 h, then was concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (200 mL) and the organic phase was washed with 0.5 N HCl aqueous solution (200 mL), then brine (200 mL), dried over $MgSO_4$, and concentrated in vacuo to give a mixture of the desired phenol and the byproduct *tert*-butyl carbonate. The residue was dissolved in MeOH (200 mL) and treated at room temperature with a 2 N NaOH aqueous solution (200 mL). The resulting mixture was stirred at room temperature for 2 h. Half of the solvent was then removed in vacuo, and the residual aqueous phase was washed with Et_2O (200 mL). The aqueous layer was then acidified with a 2 N HCl aqueous solution and then was extracted with EtOAc (2 × 200 mL). The combined organic phases were dried over $MgSO_4$ and concentrated in vacuo to give a brown gum. Purification of this residue by flash chromatography on silica gel (330 g column, 0−50% EtOAc in cyclohexane) gave *tert*-butyl 7-hydroxy-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (12.0 g, 44%) as a white solid. The Et_2O layer previously kept was washed with a 2 N NaOH aqueous solution (100 mL), and the aqueous phase was acidified with a 5 M HCl aqueous solution before being extracted with CH_2Cl_2 (2 × 50 mL). The combined organics were dried over $MgSO_4$ and concentrated in vacuo to give a second batch of *tert*-butyl 7-hydroxy-6-methyl-4,5-dihydro-1*H*-benzo[*d*]-azepine-3(2*H*)-carboxylate (2.1 g, 7.7%). LCMS (method formate): retention time 1.10 min, $[M + H]^+$ = 278. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 6.84 (d, J = 8.1 Hz, 1H), 6.58 (d, J = 8.1 Hz, 1H), 4.76 (s, 1H), 3.56 (m, 4H), 2.80−2.99 (m, 4H), 2.23 (s, 3H), 1.47 (s, 9H).

Step 10. A solution of 1,1-dimethylethyl 7-hydroxy-6-methyl-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (12 g, 43.3 mmol) and pyridine (7.00 mL, 87 mmol) in CH_2Cl_2 (200 mL) at −50 °C under nitrogen was treated with triflic anhydride (9.50 mL, 56.2 mmol). The resulting mixture was allowed to warm to room temperature over 1 h and then was washed with water and a 1 N HCl aqueous solution, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (330 g silica column, eluting with 0−50% EtOAc in cyclohexane) gave *tert*-butyl 6-methyl-7-(((trifluoromethyl)sulfonyl)oxy)-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (15.0 g, 85%) as a colorless solid. LCMS (method formate): retention time 1.45 min, $[M + H]^+$ = 410. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.05 (s, 2H), 3.59 (br s, 4H), 2.84−3.06 (m, 4H), 2.33 (s, 3H), 1.45 (s, 9H).

Step 11. A solution of 1,1-dimethylethyl 6-methyl-7-{[(trifluoromethyl)sulfonyl]oxy}-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (13 g, 32 mmol) in DMF (150 mL) was degassed using vacuum using nitrogen (3 purges), and then zinc cyanide (4.85 g, 41.3 mmol) and palladium tetrakis (3.67 g, 3.18 mmol) were added and the resulting mixture was stirred under nitrogen at 120 °C for 6 h and then was cooled to room temperature. The mixture was diluted with EtOAc (400 mL) and filtered through a pad of Celite. The insolubles were rinsed with EtOAc, and the combined organics were washed with water (2 × 200 mL), dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (330 g silica column, eluting with 0−50% EtOAc/cyclohexane) gave *tert*-butyl 7-cyano-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (8.6 g, 95% yield) as a white solid. LCMS (method formate): retention time 1.21 min, $[M + H]^+$ = 287. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 7.42 (d, J = 7.8 Hz, 1H), 7.08 (d, J = 7.6 Hz, 1H), 3.58 (d, J = 4.0 Hz, 4H), 2.86−3.08 (m, 4H), 2.56 (s, 3H), 1.44 (s, 9H).

Step 12. Oxalyl chloride (6.4 mL, 73 mmol) was added to a solution of 3-cyano-4-[(1-methylethyl)oxy]benzoic acid (10.7 g, 52 mmol) in CH_2Cl_2 (100 mL) followed by the addition of DMF (0.044 mL, 0.57 mmol), and the resulting mixture was stirred at room temperature for 4 h. The reaction mixture was concentrated in vacuo. The residue was coevaporated with cyclohexane (2 × 50 mL) to give 3-cyano-4-[(1-methylethyl)oxy]benzoyl chloride (11.7 g, 100%) as pale yellow oil which solidified on standing. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.37 (s, 1H), 8.26−8.29 (m, 1H), 7.07 (d, J = 7.7 Hz, 1H), 4.79−4.85 (m, 1H), 1.49 (t, J = 8 Hz, 6H).

Step 13. A mixture of *tert*-butyl 7-cyano-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (3.0 g, 10.5 mmol), hydroxylamine hydrochloride (2.18 g, 31.4 mmol), and $NaHCO_3$ (2.64 g, 31.4 mmol) in EtOH (50 mL) was refluxed for 24 h. Hydroxylamine hydrochloride (1 g) and $NaHCO_3$ (1 g) were further added, and the reflux was continued for 24 h. The reaction mixture was then cooled to room temperature, and the insolubles were filtered off. The solvent was evaporated in vacuo, and the residue was dissolved in water (50 mL). The aqueous phase was extracted with EtOAc (2 × 50 mL) and the combined organics were washed with water and brine, dried over $MgSO_4$, and concentrated in vacuo to give *tert*-butyl 7-(*N*′-hydroxycarbamimidoyl)-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (3.3 g, 99%) as colorless foam. LCMS (method formate): retention time 0.94 min, $[M + H]^+$ = 320. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.21 (s, 1H), 7.61 (br s, 1H), 7.30 (br s, 1H), 6.92−7.10 (m, 2H), 3.46 (br s, 4H), 2.81−2.98 (m, 4H), 2.27 (s, 3H), 1.37 (s, 9H).

Step 14. 3-Cyano-4-[(1-methylethyl)oxy]benzoyl chloride (1.84 g, 8.22 mmol) was added portionwise to a stirred solution of 1,1-dimethylethyl 7-[(hydroxyamino)(imino)methyl]-6-methyl-1,2,4,5-tetrahydro-3*H*-3-benzazepine-3-carboxylate (2.5 g, 7.83 mmol) in toluene (20 mL) and pyridine (10 mL). 10 min after the addition was complete, the resulting mixture was warmed and stirred at reflux for 3 h and then was cooled to room temperature and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (5−25% EtOAc in isohexane) gave *tert*-butyl 7-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (2.7 g, 71%) as a colorless solid. LCMS (method formate): retention time 1.49 min, $[M + H]^+$ = 489. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.49 (d, J = 2.3 Hz, 1H), 8.39 (m, 1H), 7.52−7.70 (m, 2H), 7.27 (d, J = 7.8 Hz, 1H), 4.90−5.08 (m, 1H), 3.43−3.59 (m, 4H), 2.97 (m, 4H), 2.48 (s, 3H), 1.39 (d, J = 5.8 Hz, 6H), 1.30 (s, 9H).

Step 15. A solution of *tert*-butyl 7-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (1.9 g, 3.9 mmol) in CH_2Cl_2 (15 mL) was slowly

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treated with trifluoroacetic acid (5 mL), and the resulting mixture was stirred at room temperature for 2 h and then was concentrated in vacuo. The residue was coevaporated twice with toluene and triturated with Et_2O to give 2-isopropoxy-5-(3-(6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)benzonitrile trifluoroacetate (1.7 g, 87%) as a colorless solid. LCMS (method formate): retention time 0.98 min, $[M + H]^+$ = 389.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.90 (br s, 2H), 8.49 (d, *J* = 2.3 Hz, 1H), 8.39 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.52−7.70 (m, 2H), 7.27 (d, *J* = 7.8 Hz, 1H), 4.98 (dt, *J* = 12.1, 6.1 Hz, 1H), 3.02−3.32 (m, 8H), 2.48 (s, 3H), 1.39 (d, *J* = 6.1 Hz, 6H).

Step 16. **18** was obtained as a colourless solid from 2-isopropoxy-5-(3-(6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)benzonitrile using L-glyceraldehyde in an procedure analogous to the one described for compound **12**: 214 mg, 43% yield. LCMS (method formate): retention time 0.85 min, $[M + H]^+$ = 463.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.11 (br s, 1H), 8.50 (d, *J* = 2.3 Hz, 1H), 8.39 (dd, *J* = 8.8, 2.3 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 9.1 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 5.62 (br s, 1H), 4.92−5.07 (m, 2H), 3.94−4.07 (m, 1H), 3.75 (br s, 2H), 3.40−3.53 (m, 2H), 3.21−3.39 (m, 4H), 2.97−3.20 (m, 4H), 2.50 (s, 3H), 1.39 (d, *J* = 6.1 Hz, 6H).

(*S*)-5-(3-(3-(2,3-Dihydroxypropyl)-6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile Hydrochloride (19). This compound was obtained as a colorless solid from 2-isopropoxy-5-(3-(6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)benzonitrile using D-glyceraldehyde in an procedure analogous to the one described for compound **13**: 112 mg, 47% yield. LCMS (method formate): retention time 0.89 min, $[M + H]^+$ = 463.3. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.11 (br s, 1H), 8.49 (s, 1H), 8.39 (d, *J* = 8.8, 1H), 7.57−7.64 (m, 2H), 7.26 (d, *J* = 8.8 Hz, 1H), 4.95−5.01 (m,1H), 3.99−4.15 (m, 1H), 3.02−3.43 (m, 14H), 2.50 (s, 3H), 1.39 (d, *J* = 6.1 Hz, 6H).

5-(3-(3-(1,3-Dihydroxypropan-2-yl)-6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile Hydrochloride (20). Sodium triacetoxyborohydride (1.05 g, 4.98 mmol) was added portionwise to a stirred solution of 2-isopropoxy-5-(3-(6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)benzonitrile (500 mg, 1 mmol) and 2,2-dimethyl-1,3-dioxan-5-one (388 mg, 3 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 16 h and then was treated with a saturated $NaHCO_3$ aqueous solution (20 mL), and the resulting mixture was vigorously stirred for 15 min. The layers were separated. The aqueous phase was extracted with CH_2Cl_2 (10 mL), and the combined organic phases were dried using a phase separator and concentrated in vacuo. The residue was dissolved in THF (3 mL), and the solution was treated with a 2 N HCl aqueous solution (3 mL). The resulting mixture was stirred at room temperature for 5 h and then was treated with a saturated $NaHCO_3$ aqueous solution (3 mL). The resulting mixture was stirred for 10 min, and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (10 mL), and the combined organics were dried using a phase separator and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (0−6% MeOH in CH_2Cl_2) gave a compound which was treated with 1 N HCl solution in Et_2O (2 mL). The solvent was then evaporated and the residue triturated with Et_2O to give **20** (203 mg, 41%) as a colorless solid. LCMS (method formate): retention time 0.92 min, $[M + H]^+$ = 463. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.95−10.10 (br s, 1H), 8.50 (d, *J* = 2.3 Hz, 1H), 8.39 (dd, *J* = 9.1, 2.3 Hz, 1H), 7.65 (d, *J* = 7.8 Hz, 1H), 7.56 (d, *J* = 9.3 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 5.41 (br s, 2H), 4.93−5.03 (m, 1H), 3.80 (d, *J* = 4.5 Hz, 6H), 3.51−3.62 (m, 1H), 3.40 (br s, 2H), 3.24 (d, *J* = 10.6 Hz, 2H), 3.02−3.12 (m, 1H), 2.51 (s, 3H), 1.39 (d, *J* = 6.1 Hz, 6H).

5-(3-(3-(1,3-Dihydroxypropan-2-yl)-6-methyl-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-(isopropylamino)nicotinonitrile (21). *Step 1.* To make ethyl 6-chloro-5-cyanonicotinate, a flask was charged with ethyl 5-cyano-6-oxo-1,6-dihydro-3-pyridinecarboxylate (10 g, 52.0 mmol) and then filled under nitrogen with phosphorus oxychloride (50 mL, 536 mmol), and the resulting suspension was stirred at 70 °C for 20 h and then was cooled to room temperature and carefully added to a mixture of ice and water (800 g). The precipitate formed was filtered off and dried under vacuum at 40 °C to give a brown solid. This residue was dissolved in CH_2Cl_2 and the organic phase was washed with water, dried using a phase separator, and concentrated in vacuo to give ethyl 6-chloro-5-cyanonicotinate (9.75 g, 89%) as a very pale gray solid. LCMS (method formate): retention time 0.98 min, $[M + H]^+$ = 211. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.13 (d, *J* = 2.3 Hz, 1H), 8.92 (d, *J* = 2.3 Hz, 1H), 4.38 (q, *J* = 7.1 Hz, 2H), 1.35 (t, *J* = 7.1 Hz, 3H).

Step 2. A flask was charged with ethyl 6-chloro-5-cyano-3-pyridinecarboxylate (2.11 g, 10 mmol), isopropylamine (1.8 mL, 21.01 mmol), and triethylamine (2.8 mL, 20.09 mmol) and then filled with EtOH (14 mL), and the resulting solution was stirred under nitrogen at 120 °C for 25 min under microwave irradiation and then was cooled to room temperature and concentrated in vacuo, The residue was partitioned between EtOAc and a saturated $NaHCO_3$ aqueous solution, and the layers were separated. The aqueous phase was extracted with EtOAc and the combined organic phases were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give ethyl 5-cyano-6-(isopropylamino)nicotinate (2.2 g, 95%) as a very pale gray solid which was used in the next step without any further purification. LCMS (method formate): retention time 1.13 min, $[M + H]^+$ = 233. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.15 (d, *J* = 2.0 Hz, 1H), 7.50 (d, *J* = 2.3 Hz, 1H) 6.52 (s, 1H) 4.60 (m, 1H) 3.53−3.78 (m, 3H) 0.48−0.69 (m, 8H).

Step 3. A solution of ethyl 5-cyano-6-[(1-methylethyl)amino]-3-pyridinecarboxylate (9.85 g, 42.2 mmol) in MeOH (130 mL) at room temperature was treated with 1 N LiOH aqueous solution (127 mL, 127 mmol), and the resulting mixture was stirred at this temperature for 2.5 h. Most of MeOH was then removed in vacuo. The aqueous layer was cooled at 0 °C, and the solution was treated with AcOH (12.1 mL, 211 mmol). The precipitate formed was filtered off and dried at 40 °C under vacuum to give 5-cyano-6-(isopropylamino)-nicotinic acid (7.6 g, 88%) as a pale yellow solid. LCMS (method formate): retention time 0.47 min, $[M + H]^+$ = 206. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 12.13−13.65 (br s, 1H), 8.73 (d, *J* = 2.3 Hz, 1H), 8.23 (d, *J* = 2.3 Hz, 1H), 7.44 (d, *J* = 8.1 Hz, 1 H), 4.40 (dspt, J = 6.3, 8.1 Hz, 1H), 1.15−1.25 (d, J = 6.3 Hz, 6H).

Step 4. A mixture of 5-cyano-6-(isopropylamino)nicotinic acid (1 g, 4.87 mmol), *N*-ethylmorpholine (1.12 g, 1.23 mL, 9.75 mmol), HATU (2.22 g, 5.85 mmol), and *tert*-butyl 7-(*N*′-hydroxycarbamimidoyl)-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (1.87 g, 5.85 mmol) in DMF (10 mL) was stirred at room temperature for 4 h and then was diluted with EtOAc (50 mL). The organic phase was washed successively with a saturated $NaHCO_3$ aqueous solution, water, and brine, then was dried over $MgSO_4$ and concentrated in vacuo. The residue was dissolved in toluene (40 mL) and pyridine (10 mL), and the resulting mixture was refluxed for 2 h and then was cooled to room temperature and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (20% EtOAc in hexanes) gave *tert*-butyl 7-(5-(5-cyano-6-(isopropylamino)pyridin-3-yl)-1,2,4-oxadiazol-3-yl)-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (1.31 g, 55%) as a colorless solid. LCMS (method formate): retention time 1.51 min, $[M + H]^+$ = 489. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.99 (d, *J* = 2.3 Hz, 1H), 8.57 (d, *J* = 2.3 Hz, 1H), 7.81 (d, *J* = 8.1 Hz, 1H), 7.48−7.60 (m, 1H), 7.17 (d, *J* = 8.1 Hz, 1H), 4.46 (m, 1H), 3.41−3.59 (m, 4H), 2.90−3.03 (m, 4H), 2.47 (s, 3H), 1.31 (br s, 9H), 1.24 (d, *J* = 6.6 Hz, 6H).

Step 5. A suspension of *tert*-butyl 7-(5-(5-cyano-6-(isopropylamino)pyridin-3-yl)-1,2,4-oxadiazol-3-yl)-6-methyl-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate (1.3 g, 2.66 mmol) in 1,4-dioxane (5 mL) at room temperature was treated with a 4 N HCl solution in 1,4-dioxane (10 mL), and the resulting mixture was stirred at this temperature for 7 h before being diluted with Et_2O (70 mL). The solid formed was filtered off, washed with Et_2O, and dried under vacuum to give 2-[(1-methylethyl)amino]-5-[3-(6-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-1,2,4-oxadiazol-5-yl]-3-pyridinecarbonitrile hydrochloride (1.05 g, 93%) as a colorless solid. LCMS

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(method formate): retention time 0.89 min, $[M + H]^+$ = 389. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.28 (br s, 2H), 9.00 (d, J = 2.3 Hz, 1H), 8.58 (d, J = 2.3 Hz, 1H), 7.84 (d, J = 7.8 Hz, 1H), 7.54−7.68 (m, 1H), 7.18−7.34 (m, 1H), 4.48 (spt, J = 6.6 Hz, 1H), 3.08−3.31 (m, 8H), 2.52 (s, 3H), 1.24 (d, J = 6.6 Hz, 6H).

Step 6. Sodium triacetoxyborohydride (748 mg, 3.53 mmol) was added portionwise to a stirred solution of 2-[(1-methylethyl)amino]-5-[3-(6-methyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)-1,2,4-oxadiazol-5-yl]-3-pyridinecarbonitrile hydrochloride (300 mg, 0.71 mmol) and 2,2-dimethyl-1,3-dioxan-5-one (276 mg, 253 μL, 2.12 mmol) in CH_2Cl_2 (10 mL). The reaction mixture was stirred at room temperature for 16 h and then was treated with a saturated $NaHCO_3$ aqueous solution (20 mL), and the resulting mixture was vigorously stirred for 15 min. The layers were separated. The aqueous phase was extracted with CH_2Cl_2 (2 × 10 mL), and the combined organic phases were dried using a phase separator and concentrated in vacuo. The residue was dissolved in THF (5 mL), and the solution was treated with a 2 N HCl aqueous solution (5 mL). The resulting mixture was allowed to stand at room temperature for 60 h and then was treated with a saturated $NaHCO_3$ aqueous solution (10 mL). The resulting mixture was stirred for 10 min, and the layers were separated. The aqueous phase was extracted with CH_2Cl_2 (3 × 10 mL), and the combined organics were dried using a phase separator and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (0−8% MeOH in CH_2Cl_2) gave a compound which was treated with 1 N HCl solution in Et_2O (5 mL). The solvent was then evaporated and the residue triturated with Et_2O to give **21** (181 mg, 51%) as a colorless solid. LCMS (method formate): retention time 0.89 min, $[M + H]^+$ = 463. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 10.04 (br s, 1H), 9.00 (d, J = 2.3 Hz, 1H), 8.58 (d, J = 2.3 Hz, 1H), 7.83 (d, J = 7.8 Hz, 1H), 7.62 (d, J = 7.8 Hz, 1H), 7.26 (d, J = 8.1 Hz, 1H), 5.40 (br s, 2H), 4.46 (dd, J = 14.3, 6.7 Hz, 1H), 3.69−3.88 (m, 6H), 3.51−3.62 (m, 1H), 3.38 (d, J = 6.8 Hz, 2H), 3.34 (s, 3H), 3.18−3.31 (m, 2H), 3.01−3.10 (m, 1H), 1.24 (d, J = 6.6 Hz, 6H).

5-(3-(3-(1,3-Dihydroxypropan-2-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile (22). *Step 1.* 5-(3-(3-(2,2-Dimethyl-1,3-dioxan-5-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile was obtained as a pale yellow gum from 2-isopropoxy-5-(3-(2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)benzonitrile (synthesis described in ref 39) using a similar procedure that the one described for compound **14** (step 11). LCMS (method formate): retention time 1.09 min, $[M + H]^+$ = 489. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.45 (d, J = 2.0 Hz, 1H), 8.35 (dd, J = 9.0, 2.1 Hz, 1H), 7.87−7.99 (m, 2H), 7.22−7.32 (m, 1H), 7.14 (d, J = 9.1 Hz, 1H), 4.73−4.87 (m, 1H), 4.05−4.14 (m, 4H), 3.73−3.81 (m, 5H), 3.50−3.59 (m, 2H), 2.82 (d, J = 10.1 Hz, 2H), 1.32−1.56 (m, 12H).

Step 2. **22** was obtained as a cream colored crunchy foam from 5-(3-(3-(2,2-dimethyl-1,3-dioxan-5-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]-azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-isopropoxybenzonitrile using a similar procedure as the one described for compound **14** (step 12): 66 mg, 33% yield (2 steps). LCMS (method formate): retention time 0.88 min, $[M + H]^+$ = 448. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 8.46 (d, J = 2.0 Hz, 1H), 8.37 (dd, J = 8.8, 2.3 Hz, 1H), 7.92−7.98 (m, 2H), 7.28−7.32 (m, 1H), 7.16 (d, J = 9.0 Hz, 1H), 4.84 (spt, J = 6.1 Hz, 1H), 3.78 (d, J = 6.3 Hz, 4H), 3.53 (s, 2H), 2.84−3.33 (m, 9H), 1.52 (d, J = 6.0 Hz, 6H).

5-(3-(3-(1,3-Dihydroxypropan-2-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-(isopropylamino)-nicotinonitrile (23). *Step 1.* *tert*-Butyl 7-(5-(5-cyano-6-(isopropylamino)pyridin-3-yl)-1,2,4-oxadiazol-3-yl)-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate was obtained as a pale yellow solid from 5-cyano-6-(isopropylamino)nicotinic acid and *tert*-butyl 7-(*N*′-hydroxycarbamimidoyl)-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate using a similar procedure as described for compound**21** (step 4): 1.24 g, 73% yield. LCMS (method formate): retention time 1.11 min, $[M + H]^+$ = 475. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.97−9.04 (m, 1H), 8.55−8.62 (m, 1H), 7.79−7.92 (m, 3H), 7.34−7.42 (m, 1H), 4.42−4.60 (m, 1H), 3.42−4.53 (m, 4H), 2.80−3.02 (m, 4H), 1.46 (s, 9H), 1.25 (d, J = 6.5 Hz, 6H).

Step 2. 2-(Isopropylamino)-5-(3-(2,3,4,5-tetrahydro-1*H*-benzo[*d*]-azepin-7-yl)-1,2,4-oxadiazol-5-yl)nicotinonitrile was obtained as a white solid from *tert*-butyl 7-(5-(5-cyano-6-(isopropylamino)pyridin-3-yl)-1,2,4-oxadiazol-3-yl)-4,5-dihydro-1*H*-benzo[*d*]azepine-3(2*H*)-carboxylate using a similar procedure as described for compound **21** (step 5): 887 mg, 91% yield. LCMS (method formate): retention time 0.83 min, $[M + H]^+$ = 375. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.32 (br s, 2H), 9.00 (d, J = 2.3 Hz, 1H), 8.58 (d, J = 2.3 Hz, 1H), 7.92 (s, 1H), 7.87−7.91 (m, 1H), 7.85 (d, J = 8.1 Hz, 1H), 4.47 (spt, J = 6.0 Hz, 1H), 3.22 (m, 8H), 1.24 (d, J = 6.6 Hz, 6H).

Step 3. 5-(3-(3-(2,2-Dimethyl-1,3-dioxan-5-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-(isopropylamino)-nicotinonitrile was obtained as a yellow oil from 2-(isopropylamino)-5-(3-(2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-nicotinonitrile using a similar procedure as described for compound **21** (step 6). LCMS (method formate): retention time 0.98 min, $[M + H]^+$ = 488.

Step 4. **23** was obtained as a white foam from 5-(3-(3-(2,2-dimethyl-1,3-dioxan-5-yl)-2,3,4,5-tetrahydro-1*H*-benzo[*d*]azepin-7-yl)-1,2,4-oxadiazol-5-yl)-2-(isopropylamino)nicotinonitrile using a similar procedure as described for compound **21** (step 6): 29 mg, 13% yield (2 steps). LCMS (method formate): retention time 0.67 min, $[M + H]^+$ = 448. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 9.00 (d, J = 2.5 Hz, 1H), 8.58 (d, J = 2.5 Hz, 1H), 7.76−7.85 (m, 3H), 7.31 (d, J = 7.8 Hz, 1H), 4.47 (spt, J = 6.6 Hz, 1H), 4.24 (dd, J = 5.9, 4.4 Hz, 2H), 3.36−3.52 (m, 4H), 2.88−3.00 (m, 4H), 2.80 (br s, 4H), 2.71 (qt, J = 6.5 Hz, 1H), 1.24 (d, J = 6.6 Hz, 6H).

5-(3-{4-[2-Hydroxy-1-(hydroxymethyl)ethyl]-9-methyl-2,3,4,5-tetrahydro-1,4-benzoxazepin-8-yl}-1,2,4-oxadiazol-5-yl)-2-[(1-methylethyl)oxy]benzonitrile (24). *Step 1.* To make 2,4-dihydroxy-3-methylbenzaldehyde, $POCl_3$ (8.02 mL, 86 mmol) was slowly added over 20 min to DMF (26 mL, 336 mmol) in a two-neck flask with internal thermometer, using an ice bath to make sure the temperature remained between 10 and 20 °C. Once the addition was completed, the resulting mixture was added dropwise to a solution of 2-methyl-1,3-benzenediol (4.84 g, 39 mmol) in DMF (26 mL), making sure using an ice bath that the temperature did not exceed 30 °C. The resulting yellow solution was stirred at room temperature for 16 h and then was cautiously added to an ice cold solution of 5 N NaOH aqueous solution (15 mL of 5 M NaOH aqueous solution were used per mL of $POCl_3$), making sure the temperature did not rise above 30 °C. The aqueous phase was extracted with Et_2O and then slowly acidified with concentrated aqueous HCl. An ice bath was used to make sure that the temperature was kept below 50 °C. The aqueous phase was extracted 3 times with EtOAc. The combined organic phases were washed slowly with a saturated $NaHCO_3$ aqueous solution (warning: CO_2 evolution), then brine, dried over $MgSO_4$, and concentrated in vacuo. NMR analysis of the crude material showed mainly a ∼2/1 mixture of product and starting material. Trituration of this residue with CH_2Cl_2 gave 2,4-dihydroxy-3-methylbenzaldehyde (2.37 g, 40%) as a white solid. LCMS (method formate): retention time 0.43 min, $[M + H]^+$ = 153.1. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 11.60 (s, 1H), 10.78 (br s, 1H), 9.71 (s, 1H), 7.43 (d, J = 8.0 Hz, 1H), 6.56 (d, J = 8.0 Hz, 1H), 1.97 (s, 3H).

Step 2. To a solution of 2,4-dihydroxy-3-methylbenzaldehyde (2.37 g, 15.58 mmol) in EtOH (50 mL) at room temperature was added ethanolamine (1.0 mL, 16.4 mmol), and the resulting mixture was stirred at this temperature for 1 h. The mixture was concentrated in vacuo to give 4-{[(2-hydroxyethyl)imino]methyl}-2-methyl-1,3-benzenediol (3.04 g, 100%) as a yellow foam which was used in the next step without further purification. LCMS (method formate): retention time 0.39 min, $[M + H]^+$ = 196.2. ^{1}H NMR (400 MHz, MeOD-d_4) δ ppm 8.10 (s, 1H), 6.94 (d, J = 8.0 Hz, 1H), 6.24 (d, J = 8.0 Hz, 1H), 3.59−3.79 (m, 6H), 2.76−2.79 (m, 1H), 2.02 (s, 3H).

Step 3. To a solution of 4-{[(2-hydroxyethyl)imino]methyl}-2-methyl-1,3-benzenediol (3.04 g, 15.58 mmol) in THF (50 mL) at room temperature was added sodium triacetoxyborohydride (6.60 g, 31.2 mmol), and the resulting yellow suspension was stirred at this

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temperature for 1 h. Sodium triacetoxyborohydride (500 mg, 2.35 mmol) was added, and the resulting mixture was stirred at room temperature for 16 h. The mixture was then treated with 4 N HCl in 1,4-dioxane (15.6 mL, 62.3 mmol) and then was concentrated in vacuo. The residue was dissolved in MeOH and loaded on a prewet 70 g SCX column. The column was eluted with MeOH followed by a 2 N NH_3 solution in MeOH. The ammoniac fractions were combined and concentrated in vacuo to give 4-(((2-hydroxyethyl)amino)methyl)-2-methylbenzene-1,3-diol (3.07 g, 100%) which was used in the next step without further purification. The reaction was considered as quantitative at this stage. LCMS (method formate): retention time 0.46 min, $[M + H]^+ = 198.2$.

Step 4. To a solution of 4-{[(2-hydroxyethyl)amino]methyl}-2-methyl-1,3-benzenediol (3.07 g, 15.6 mmol) in MeOH (30 mL) was added triethylamine (6.51 mL, 46.7 mmol) and then di-*tert*-butyl dicarbonate (3.80 mL, 16.4 mmol), and the resulting mixture was stirred at room temperature for 1 h and then was concentrated in vacuo. The residue was purified using a 70 g SCX column (eluting first with MeOH and then with a 2 N NH_3 solution in MeOH), and the ethanolic fractions were combined and concentrated in vacuo. Purification of the residue via flash chromatography on silica gel (10−60%, EtOAc in hexanes) gave a white solid which was further purified. This residue was dissolved in EtOAc and the organic phase extracted three times with a 2 N NaOH aqueous solution. The combined aqueous phases were acidified with a 5 N HCl aqueous solution and then extracted twice with EtOAc. The combined organics were washed with brine, dried over $MgSO_4$, and concentrated in vacuo to give *tert*-butyl 2,4-dihydroxy-3-methylbenzyl(2-hydroxyethyl)-carbamate (1.61 g, 35%) as a pale pink solid. LCMS (method formate): retention time 0.93 min, $[M + H]^+ = 298.2$. ^1H NMR (400 MHz, MeOD-d_4) δ ppm 6.82 (d, J = 8.1 Hz, 1H), 6.31 (d, J = 8.1 Hz, 1H), 4.38 (br s, 2H), 3.64 (t, J = 6.30 Hz, 2H), 2.07 (s, 3H), (1.50, 9H). Some peaks were obscured by residual NMR solvent.

Step 5. To a solution of 1,1-dimethylethyl [(2,4-dihydroxy-3-methylphenyl)methyl](2-hydroxyethyl)carbamate (1.61 g, 5.41 mmol) and triphenylphosphine (1.56 g, 5.96 mmol) in THF (40 mL) at 0 °C was added DIAD (1.16 mL, 5.96 mmol) dropwise, and the resulting yellow mixture was stirred at this temperature for 30 min and then was concentrated in vacuo. The residue was dissolved in EtOAc, and the organic phase was washed twice with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (100 g silica, 10−40% EtOAc in hexanes) gave *tert*-butyl 8-hydroxy-9-methyl-2,3-dihydrobenzo[*f*]-[1,4]oxazepine-4(5*H*)-carboxylate (1.26 g, 84%) as white foam. LCMS (method formate): retention time 1.05 min, $[M + H]^+ = 280.3$. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 6.84−7.02 (m, 1H), 6.34−6.58 (m, 1H), 5.06 (s, 1H), 4.35 (s, 2H), 3.98−4.08 (m, 2H), 3.74−3.88 (m, 2H), 2.17 (s, 3H), 1.43 (s, 9H).

Step 6. To a solution of 1,1-dimethylethyl 8-hydroxy-9-methyl-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxylate (1.23 g, 4.42 mmol) in CH_2Cl_2 (40 mL) at 0 °C under nitrogen was added pyridine (0.715 mL, 8.84 mmol), then triflic anhydride (0.822 mL, 4.86 mmol) dropwise. The yellow solution was stirred at this temperature for 10 min, then was concentrated in vacuo and the residue dissolved in EtOAc. The organic phase was washed with a 1 N HCl aqueous solution, a saturated $NaHCO_3$ aqueous solution, then brine, dried over $MgSO_4$, and concentrated in vacuo to give *tert*-butyl 9-methyl-8-(((trifluoromethyl)sulfonyl)oxy)-2,3-dihydrobenzo[*f*][1,4]oxazepine-4(5*H*)-carboxylate (1.82 g, 100%) as an orange oil which was used in the next step without further purification. LCMS (method formate): retention time 1.43 min, $[M + H_2O]^+ = 429.2$. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.12−7.22 (m, 1H), 6.90−7.00 (m, 1H), 4.40−4.43 (m, 2H), 4.07−4.10 (m, 2H), 3.84−3.86 (m, 2H), 2.29 (s, 3H), 1.43 (s, 9H).

Step 7. A solution of crude 1,1-dimethylethyl 9-methyl-8-{[(trifluoromethyl)sulfonyl]oxy}-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxylate (1.82 g, 4.42 mmol) in DMF (15 mL) was degassed for 10 min under house vacuum and quenched several times with nitrogen, then was treated with tetrakis(triphenylphosphine)-palladium(0) (0.511 g, 0.442 mmol) and zinc cyanide (0.675 g, 5.75 mmol). The resulting mixture was stirred at 100 °C under nitrogen for 7 h, then was cooled to room temperature. The insoluble material was filtered off and washed with EtOAc. The combined organic phases were concentrated in vacuo. The residue was dissolved in EtOAc, and the organic phase was washed with a saturated $NaHCO_3$ aqueous solution. The aqueous phase was extracted with EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by flash chromatography on silica gel (50 g column, 5−25% EtOAc in hexanes) gave *tert*-butyl 8-cyano-9-methyl-2,3-dihydrobenzo[*f*][1,4]-oxazepine-4(5*H*)-carboxylate (1.16 g, 91%) as a white solid. LCMS (method formate): retention time 1.21 min, $[M + H]^+ = 289.2$. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 7.28−7.32 (m, 1H), 7.20−7.30 (m, 1H), 4.40−4.51 (m, 2H), 4.05−4.07 (m, 2H), 3.83−3.85 (m, 2H), 2.46 (s, 3H), 1.43 (s, 9H).

Step 8. Oxalyl chloride (6.4 mL, 73 mmol) was added to a solution of 3-cyano-4-[(1-methylethyl)oxy]benzoic acid (10.7 g, 52 mmol) in CH_2Cl_2 (100 mL) followed by the addition of DMF (0.044 mL, 0.57 mmol), and the mixture was stirred at room temperature for 4 h. The reaction mixture was filtered and concentrated in vacuo. The residue was coevaporated with cyclohexane (2 × 50 mL) to give 3-cyano-4-[(1-methylethyl)oxy]benzoyl chloride (11.7 g, 100%) as a pale yellow oil which solidified on standing. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 8.35 (d, J = 2.3 Hz, 1H), 8.26 (dd, J = 2.3, 9.1 Hz, 1H), 7.06 (d, J = 9.1 Hz, 1H), 4.81 (spt, J = 6.1 Hz, 1H), 1.47 (d, J = 6.1 Hz, 6H).

Step 9. To a stirred solution of 1,1-dimethylethyl 6-[(hydroxyamino)(imino)methyl]-5-methyl-3,4-dihydro-2(1*H*)-isoquinolinecarboxylate (4.7 g, 15.39 mmol) in toluene (35 mL) and pyridine (26 mL) at ambient temperature was slowly added 3-cyano-4-[(1-methylethyl)oxy]benzoyl chloride (3.8 g, 17.1 mmol) in toluene (35 mL). After 20 min, the mixture was warmed to 80 °C and stirred at this temperature for 15 h, then was cooled to room temperature and concentrated in vacuo. The residue was suspended in EtOAc (50 mL), and the organic phase was washed sequentially with 0.5 N HCl aqueous solution (2 × 40 mL), a saturated $NaHCO_3$ aqueous solution (2 × 40 mL), and water (2 × 40 mL). The organic layer was dried through a hydrophobic frit and the solvent removed in vacuo. Purification of the residue via flash chromatography on silica gel (330 g column, 0−60% EtOAc in cyclohexane) gave *tert*-butyl 8-(5-(3-cyano-4-isopropoxyphenyl)-1,2,4-oxadiazol-3-yl)-9-methyl-2,3-dihydrobenzo-[*f*][1,4]oxazepine-4(5*H*)-carboxylate (2.85 g, 35%) as an off white solid. LCMS (method formate): retention time 1.43 min, $[M + H]^+ = 491.0$. ^1H NMR (250 MHz, DMSO-d_6) δ ppm 8.50 (d, J = 2.1 Hz, 1H) 8.36−8.45 (m, 1H) 7.61−7.72 (m, 1H) 7.56 (d, J = 9.0 Hz, 1H) 7.28 (d, J = 7.9 Hz, 1H) 4.99 (s, 1H) 4.48 (br s, 2H) 4.07 (br s, 2H) 3.77 (br s, 2H) 2.47 (s, 3H) 1.40 (d, J = 6.1 Hz, 6H) 1.36 (s, 9H).

Step 10. A solution of 1,1-dimethylethyl 8-(5-{3-cyano-4-[(1-methylethyl)oxy]phenyl}-1,2,4-oxadiazol-3-yl)-9-methyl-2,3-dihydro-1,4-benzoxazepine-4(5*H*)-carboxylate (2.91 g, 5.93 mmol) in CH_2Cl_2 (25 mL) at room temperature was treated with trifluoroacetic acid (6 mL, 78 mmol), and the resulting mixture was stirred at this temperature for 2 h and then was concentrated in vacuo, and the residue coevaporated with toluene. Trituration of the residue with Et_2O gave 2-isopropoxy-5-(3-(9-methyl-2,3,4,5-tetrahydrobenzo[*f*]-[1,4]oxazepin-8-yl)-1,2,4-oxadiazol-5-yl)benzonitrile trifluoroacetate (2.9 g, 97%) as a white solid. LCMS (method formate): retention time 0.86 min, $[M + H]^+ = 391.1$. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 9.45 (br s, 2H), 8.49 (d, J = 2.3 Hz, 1H), 8.38 (dd, J = 9.0, 2.1 Hz, 1H), 7.73 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 9.1 Hz, 1H), 7.49 (d, J = 7.8 Hz, 1H), 4.98 (spt, J = 6.0 Hz, 1H), 4.41 (s, 2H), 4.26 (d, J = 2.3 Hz, 2H), 3.56 (br s, 2H), 2.50 (s, 3H), 1.38 (d, J = 6.1 Hz, 6H).

Step 11. A stirred mixture of 2,2-dimethyl-1,3-dioxan-5-one (0.75 g, 5.76 mmol) and 2,2-dimethyl-1,3-dioxan-5-one (0.75 g, 5.76 mmol) in CH_2Cl_2 (20 mL) at room temperature under nitrogen was treated with sodium triacetoxyborohydride (2.036 g, 9.60 mmol) added in two portions over 5 min. The mixture was stirred for 15 h, then was evaporated under vacuum and the white solid suspended in EtOAc (20 mL). The organic layer was washed sequentially with a saturated $NaHCO_3$ aqueous solution (20 mL) and then water (20 mL). The organic layer was separated, dried through a hydrophobic frit and the

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solvent removed in vacuo. Purification of the residue via flash chromatography on silica gel (100 g column, 0−100% AcOEt in CH_2Cl_2) gave 5-{3-[4-(2,2-dimethyl-1,3-dioxan-5-yl)-9-methyl-2,3,4,5-tetrahydro-1,4-benzoxazepin-8-yl]-1,2,4-oxadiazol-5-yl}-2-[(1-methylethyl)oxy]benzonitrile (0.74 g, 1.47 mmol, 76%) as a white solid. LCMS (method formate): retention time 0.99 min, $[M + H]^+$ = 505.2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.49 (d, J = 2.3 Hz, 1H), 8.39 (dd, J = 9.1, 2.3 Hz, 1H), 7.60 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 9.1 Hz, 1H), 7.32 (d, J = 7.8 Hz, 1H), 4.92−5.03 (m, 1H), 3.99−4.06 (m, 2H), 3.86−3.94 (m, 4H), 3.71 (dd, J = 11.2, 9.0 Hz, 2H), 3.10−3.17 (m, 2H), 2.64−2.74 (m, 1H), 2.46 (s, 3H), 1.38 (d, J = 6.1 Hz, 6H), 1.36 (s, 3H), 1.25 (s, 3H).

Step 12. A solution of 5-{3-[4-(2,2-dimethyl-1,3-dioxan-5-yl)-9-methyl-2,3,4,5-tetrahydro-1,4-benzoxazepin-8-yl]-1,2,4-oxadiazol-5-yl}-2-[(1-methylethyl)oxy]benzonitrile (0.72 g, 1.43 mmol) in THF (10 mL) was treated with a 2 N HCl aqueous solution (8 mL, 16.0 mmol) and allowed to stand in an open vessel at room temperature for 17 h. The reaction mixture was evaporated to dryness under vacuum. The solid residue was twice suspended in toluene and coevaporated under vacuum. The solid was then triturated with Et_2O and dried under vacuum to give **24** (400 mg, 56%) as a white solid. LCMS (method formate): retention time 0.90 min, $[M + H]^+$ = 464.9. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.50 (d, J = 2.3 Hz, 1H), 8.36−8.42 (m, 1H), 7.58 (d, J = 7.8 Hz, 1H), 7.55 (d, J = 9.1 Hz, 1H), 7.24 (d, J = 8.1 Hz, 1H), 4.92−5.03 (m, 1H), 4.36 (t, J = 5.2 Hz, 2H), 3.99−4.08 (m, 2H), 3.90 (s, 2H), 3.45−3.62 (m, 4H), 3.16 (br s, 2H), 2.69−2.80 (m, 1H), 2.45 (s, 3H), 1.38 (d, J = 6.1 Hz, 6H).

■ ASSOCIATED CONTENT

Supporting Information

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Molecular formula strings (CSV)

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Notes

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■ ABBREVIATIONS USED

AhR, arylhydrocarbon receptor; THIQ, tetrahydroisoquinoline; BZ, benzazepine; BZO, benzoxazepine; CYP, cytochrome P450

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