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Systematic Structure Modifications of Imidazo[1,2-*a*]pyrimidine to Reduce Metabolism Mediated by Aldehyde Oxidase (AO)

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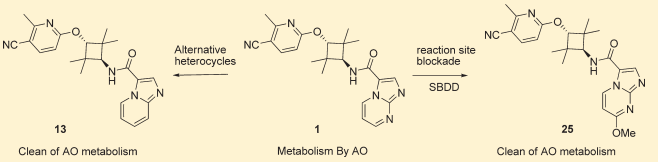
Supporting Information

ABSTRACT: *N*-{*trans*-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}imidazo[1,2-*a*]pyrimidine-3-carboxamide (**1**) was recently identified as a full antagonist of the androgen receptor, demonstrating excellent in vivo tumor growth inhibition in castration-resistant prostate cancer (CRPC). However, the imidazo[1,2-*a*]pyrimidine moiety is rapidly metabolized by aldehyde oxidase (AO). The present paper describes a number of medicinal chemistry strategies taken to avoid the AO-mediated oxidation of this particular system. Guided by an AO protein structure-based model, our investigation revealed the most probable site of AO oxidation and the observation that altering the heterocycle or blocking the reactive site are two of the more effective strategies for reducing AO metabolism. These strategies may be useful for other drug discovery programs.



INTRODUCTION

N-{*trans*-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}imidazo[1,2-*a*]pyrimidine-3-carboxamide **1** is a novel, selective pure antagonist of androgen receptor (AR), which has the potential for therapeutic value in castration-resistant prostate cancer (Figure 1).[1] Compound **1** is metabolically stable in vitro in both human liver microsome and hepatocyte systems (ER < 0.3). In a rat PK study, compound **1** displayed acceptable oral absorption (bioavailability F = 31%), low clearance (Cl_{plasma} = 11.6 mL/min/kg), and a reasonable half-life ($T_{1/2}$ = 3.6 h). The imidazo[1,2-*a*]pyrimidine-3-carboxamide moiety in compound **1** is crucial for achieving potent AR full antagonism. Heteroaryls, such as imidazopyrimidines, are versatile synthetic building blocks commonly used in medicinal chemistry because they are often capable of binding to diverse biological targets with high affinity and providing useful pharmacological activities.[2] In addition, electron-deficient heteroaryls are often resistant to cytochrome P450-mediated metabolism. However, an electron-deficient nature may also make the ring carbons susceptible to nucleophilic attack by aldehyde oxidase (AO), particularly when they are adjacent to heterocyclic nitrogen(s).[3]

The presence of a polyaza heteroaryl group in **1** suggested it could be a substrate for AO metabolism, so the compound was tested for metabolic stability in human S9 (HS9) fraction. Though only trace metabolism had been detected in the HLM assay,[4] a significant single mono-oxygenated metabolite **M1**, was observed in HS9 without NADPH, suggesting AO oxidation (Figure 1).[5] This finding is consistent with the fact that the liver microsome assay does not contain cytosolic enzymes such as AO.[6] Incubation of **1** in the presence of an AO inhibitor inhibited the formation of **M1**, consistent with the involvement of AO in the metabolism of this compound.[7] The incubation method described above indicated that the site of oxidation was somewhere on the imidazopyrimidine ring, rather than elsewhere in the molecule, but the exact position could not be determined by this assay.

Clearance mediated by cytochrome P450 enzymes is currently better understood and utilized today than in previous years. It has become a common practice in drug discovery to use the data from liver microsome assays to predict human clearance. However, in vitro HLM assays cannot accurately predict clearance for compounds for which AO plays a significant role in metabolism.[8] The present paper describes several medicinal chemistry strategies applied to mitigate the AO liability of the imidazopyrimidine ring in compound **1**. Species differences of AO activity exist among human, rat, and dog, in that rats have lower AO activity and dogs possess no AO activity. Hence human AO metabolism is generally underestimated when based on animal studies. Furthermore, multiple AO isoforms exist in rat (Aox1, AOX3, Aox4, and Aox3l1) while only one AO isoform (AOX1) is present in human, which can result in the formation of different AO metabolites between rat and human.[9] Because of the species differences, it was determined that a more appropriate approach would be to study AO metabolism of these compounds in human in vitro system (human liver S9/cytosol).

A common strategy to reduce aromatic oxidation by cytochrome P450 is to replace a carbon in the aromatic ring with a nitrogen, but doing so can increase the susceptibility of the adjacent carbons to AO oxidation.[10] The mechanism of AO oxidation is believed to involve the molybdenum pyranopterin cofactor moiety (MoCo) of the enzyme in a nucleophilic addition-like mechanism.[11] This theory is supported by the observation that

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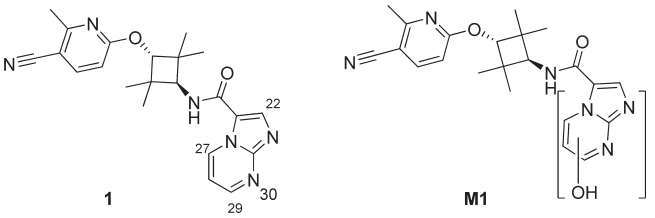


Figure 1. Androgen receptor antagonist **1** and potential metabolite **M1**.

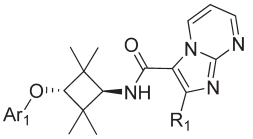
electron-deficient heteroaromatic rings are common AO substrates. In a heteroaryl system with more than one potential site for AO metabolism, such as the imidazopyrimidine ring of **1**, the susceptibility of nucleophilic attack by AO will depend not only on how electron-deficient the reactive site is but also on the shape of the substrate molecule and the spatial accessibility of the potentially reactive carbon toward the MoCo moiety. The importance of the interaction between the enzyme and its substrates has been previously addressed by Dastmalchi et al., who have constructed a homology model of human AO that can be used for computational studies.[12]

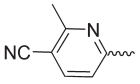
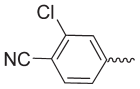
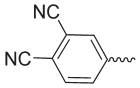
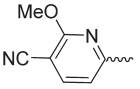
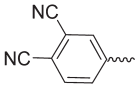
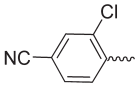
At the present, there are few publications describing methods to predict and mitigate AO metabolism, nor are there reliable computational tools to predict which molecules may be susceptible. Such tools would be useful in drug discovery.[13] It has been hypothesized that the most likely site of AO metabolism is the most positively charged aromatic carbon adjacent to an aromatic nitrogen, given that the C−H is spatially accessible to AO enzyme.[10] Compound **1** contains three possible sites for AO oxidation, C22, C27, and C29. However, only one oxidized metabolite (**M1**, retention time 12.40 min, Figure 1)[14] was observed by LC/MS when compound **1** was incubated with HS9 NADPH,[5] suggesting that only one of these carbons is sufficiently electron-deficient and/or sufficiently accessible to the enzyme.

A number of analogues of **1** were designed to avoid AO metabolism. Strategies explored to reduce AO oxidation included introducing a remote structural change in the molecule (Table 1), evaluating alternative heterocycles (ring connectivity, nitrogen position, Table 2), and incorporating hindrance either close to or directly at the suspected site of AO oxidation (Table 3). The preparation of these analogues is outlined in Scheme 1. Synthesis begins with nucleophilic displacement of various commercially available aryl fluorides **2** with the anion of Boc-protected cyclobutyl amino alcohol **3**, followed by protecting group removal under acidic conditions to yield amines **5**, which serve as versatile templates for coupling with various carboxylic acids **6** to provide amides **7** in good yields.[1]

The first strategy pursued was to introduce a structural change remote to the potential AO oxidation sites in compound **1** that would perturb binding to the AO catalytic site. With the assumption that the AR binding site and AO enzyme catalytic site are different in the region hosting the cyanopyridylether (left-hand) side of **1**, the modification could potentially result in minimal reduction to AR protein binding affinity but decreased affinity to the AO enzyme. A number of cyanoaryl ether groups have been shown to be tolerated for AR binding,[1] so analogues with different arylethers were tested for AO metabolism (Table 1). When the 2-methylnicotinonitrile in **1** was replaced with 2-chlorobenzonitrile (**8**), phthalonitrile (**9**), and 2-methoxynicotinonitrile (**10**, Table 1), the AR antagonism potency was maintained, but AO-mediated oxidation remained the main pathway for their metabolism.

Table 1. Incorporation of a Remote Change in the Molecule



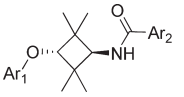
Compd	Ar_1	R1	ClogP	Oxidation by AO[a]
1		**H**	4.51	yes
8		**H**	5.28	yes
9		**H**	4.2	yes
10		**H**	4.24	yes
11		**Me**	4.13	yes
12		**Me**	5.11	yes

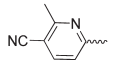
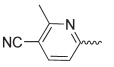
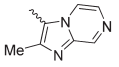
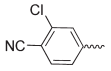
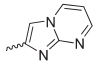
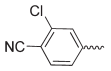
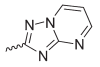
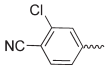
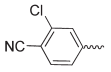
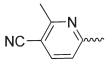
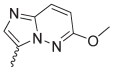
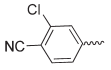
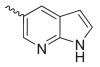
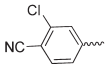
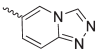
[a] "yes" indicates an oxygenated metabolite was observed in HS9 without NADHP suggesting AO oxidation. "no" means no metabolite was observed under the aforementioned conditions. The spectra for all compounds can be found in the Supporting Information.

A related strategy was to introduce a substituent on C22 that could both (1) change the dihedral angle between the amide and the azapyrimidine ring, resulting in significant change in the most stable conformation of these molecules and (2) block a potential oxidation site. Blockage of C22 by a methyl group (**11**−**12**, Table 1) had no effects on the AO metabolism, indicating that the AO active site is flexible enough to accommodate significant conformational changes in this region and that the site of oxidation in these particular molecules may be located at C27 or C29. Unlike P450-mediated metabolism, there is no apparent correlation between AO metabolism and lipophilicity, consistent with the observations reported by Pryde et al.[3]

Because remote structural changes or substitution to change the dihedral angle of the amide bond did not reduce the propensity of the molecules to be metabolized by AO, more significant structural changes were investigated. The next strategy pursued was to incorporate alternative heterocycles with different heteroatom placement, connection points, or ring systems. A similar strategy has been reported to successfully eliminate AO metabolism for a series of azetidinyl ketolide antibiotics.[13] Several compounds with significantly reduced AO liability were identified by this strategy (Table 2). Compound **13**, in which N30 of compound **1** is replaced with a carbon, was designed to eliminate the potential for AO oxidation at the adjacent C29. This compound shows no oxidation by AO, but it becomes more susceptible to

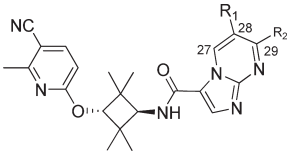
Table 2. Evaluation of Alternative Heterocycles



Compd	Ar_1	Ar_2	ClogP	Oxidation by AO[a]
13			5.51	no
14			4.43	yes
15			5.29	yes[b]
16			4.32	no
17			5.72	no
18			4.91	no
19			4.43	no
20			5.72	no
21			4.74	no

[a] "yes" indicates oxygenated metabolite(s) was qualitatively observed in HS9 without NADHP suggesting AO oxidation. "no" means no metabolite was observed under the aforementioned conditions. [b] Compound **15** indicated a very small peak corresponding to a second oxygenated species. The spectra for all compounds can be found in the Supporting Information.

Table 3. Direct Effect on C28 and C29



Compd	R1	R2	ClogP	Oxidation by AO[b]
22	Me	H	5.01	yes
23	Br	H	5.38	yes
24	F	H	4.66	yes
25	H	OMe	5.38	no
26	H		4.64	no

[a] "yes" indicates an oxygenated metabolite was observed in HS9 without NADHP suggesting AO oxidation. "no" means no metabolite was observed under the aforementioned conditions. The spectra for all compounds can be found in the Supporting Information.

P450-mediated oxidation (HLM ER 0.5) and is poorly soluble in water (kinetic solubility < 0.4uM), likely due to the increase in lipophilicity (cLogP 5.51 vs 4.51 [**1**]). Interestingly, AO oxidation remained when the heterocyclic nitrogen was moved from N30 to N29 (compound **14**, Table 2). In this case, even though there are more aromatic C−H next to a nitrogen in the 5/6 heteroaryl ring, only one oxidized metabolite was observed in the AO assay as well. Moving the amide connecting point from the 3-position (as in **1**) to the 2 position of the imidazopyrimidine (as in **15**) did not reduce AO oxidation, but replacement of C22 with a nitrogen (compound **16**) seemed to prevent the oxidation at the remaining positions (C27 and C29). Saturation of the six-membered aromatic ring (compound **18**) also eliminated AO metabolism. Compounds **17** and **19**−**21** all still contain potential AO metabolism sites as unsubstituted aromatic C−H are present adjacent to aromatic nitrogens, but these compounds were nevertheless all free of AO liability. Perhaps the significant structural changes to the right-hand side region prevent recognition by the AO enzyme. Although the right-hand side replacements resulted in loss of potency against our target of interest (AR), the alternative heterocycle strategy may be applied to other target designs for mitigating AO risk.

Because drastic changes of the right-hand heterocycle had a negative effect on potency against the primary target, a final strategy was pursued in which the connection point and heteroatom arrangement of the imidazopyrimidine ring of **1** were preserved but substitution was introduced to block the reactive sites (Table 3). It has already been shown that substitution at C22 does not prevent AO oxidation, leaving C27 or C29 as the most likely oxidations sites. Thus, introduction of steric hindrance at C28 has the potential to interfere with AO metabolism. Compounds **22** and **23** (Table 3) were prepared to test the strategy. The steric bulk of a methyl (compound **22**) or bromo group (compound **23**) at C28 did not have any significant effect on the oxidation at C27 and C29, as the rate of metabolism remained high. Electronic effects (withdrawing or donating) of bromo, fluoro, and methyl substitutents at C28 (**22**−**24**) did not change the rate of oxidation either. On the other hand, substitution directly at C29 with a methoxy group (**25**) or a morpholino group (**26**) completely eliminated AO oxidation. From these observations, we can conclude that the C29 position of **1** is the most probable site of AO oxidation. As such, the structure of the single observed metabolite **M1** may be compound **1** with its C29 hydroxylated (Figure 1).

Compound **1** was submitted to a computational docking protocol described by Pryde et al.[3] in which a homology model of human AO is used to predict the site of metabolism. The 3D structure of the docked pose, and the potential sites of metabolism of compound **1** are shown in Figure 2. The docking result showed that the predicted site of metabolism C29 is at 2.1 Å away from the MoCo moiety. Taking into consideration the rigidity of compound **1**, C22 or C27 are unlikely the sites of metabolism because, according to the modeled complex, they are not accessible to the MoCo moiety. Figure 2 also shows that there is

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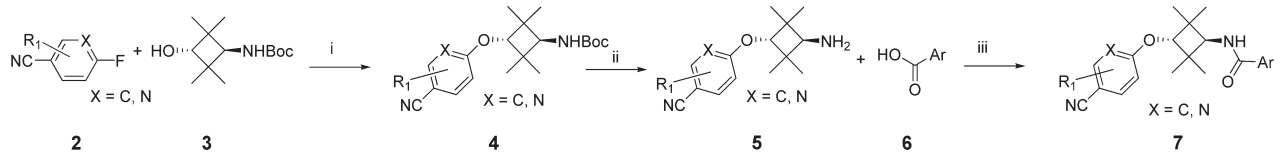
Scheme 1. Synthesis of Cyclobutyl Analogues[a]



[a] Reagents and conditions: (i) NaH, DMF; (ii) HCl, EtOAC; iii) HBTU, DMF.

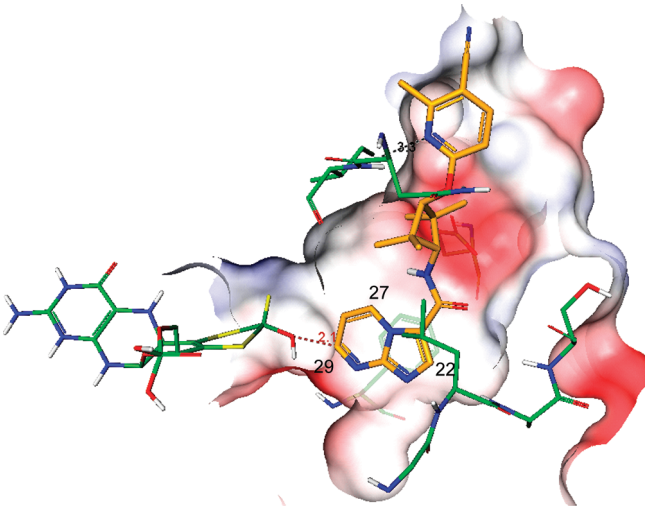


Figure 2. Docking poses for compound **1**.

potential room to accommodate small substitutions at C22, like the methyl group of compounds **11** and **12**, helping to explain why C22 substitution in compound **11** and **12** did not affect the AO metabolism.

In summary, the imidazo[1,2-*a*]pyrimidine ring system has been identified as a representative example of heteroaryl systems which are substrates of AO-mediated metabolism. Multiple medicinal chemistry approaches were employed to reduce the AO liability, and our investigation suggests that alternative heterocycles or direct blockade of reactive site are two of the more effective strategies. The potential reactive site(s) may be predicted by the AO protein structure-based model. The medicinal chemistry approaches described here may be applicable to other drug discovery programs in which similar AO metabolism problems are encountered.

EXPERIMENTAL SECTION

General. All starting materials and chemicals reagents were purchased from commercial suppliers and used without further purification unless otherwise indicated. ^{1}H NMR spectra were recorded on a Bruker instrument operating at 400 or 600 MHz. NMR spectra are obtained as $CDCl_3$, DMSO-d_6 solutions (reported in ppm), using $CDCl_3$ as the reference standard (7.25 ppm), or DMSO-d_6 (2.50 ppm). Other NMR solvents were used as needed. The mass spectra were obtained using LC/MS or HRMS. The purity of all final compounds was determined to be at least 95% pure by a combination of HPLC, LCMS, NMR (no extra peaks in the proton NMR spectrum), and combustion analysis (all final compounds have satisfying CHN results consistent with high purity).

General Procedure for the Preparation of Cyclobutyl Analogues. *Preparation of **1**: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)-oxy]-2,2,4,4-tetramethylcyclobutyl}imidazo[1,2-a]pyrimidine-3-carboxamide. Step 1: 6-((1r,3r)-3-Amino-2,2,4,4-tetramethylcyclobutoxy)-2-methylnicotinonitrile Hydrochloride.* To a cooled stirred solution of compound **3**[1] (4.8 g, 19.7 mmol) in anhydrous tetrahydrofuran (80 mL) was added sodium hydride (1.6 g, 39.4 mmol) in portions and at a temperature below 10 °C. After the addition, the mixture was stirred at room temperature for 30 min. Then the reaction mixture was cooled to 10 °C, and 6-fluoro-2-methylnicotinonitrile (Ryan Scientific, PC5881) (3.2 g, 23.6 mmol) was added in portions. After the addition, the resulting mixture was stirred at reflux for 1 h under N_2. TLC (4:1 petroleum ether/ethyl acetate) showed the reaction was complete. The reaction was cooled to room temperature and quenched with cold water (10 mL). The resulting mixture was evaporated, and the residue was diluted with ethyl acetate (100 mL) and water (100 mL). The organic layer was separated, and the aqueous layer was re-extracted with ethyl acetate (100 mL × 2). The combined organic layers were washed with brine (50 mL), dried over sodium sulfate, filtered, and evaporated to give the crude product, which was purified by column chromatography (petroleum ether/ethyl acetate = 20:1 to 10:1) to give Boc-protected intermediate: *tert*-butyl(1*r*,3*r*)-3-(5-cyano-6-methylpyridin-2-yloxy)-2,2,4,4 tetramethylcyclobutylcarbamate (4.2 g, 59%) as white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.03 (s, 6 H) 1.11 (s, 6 H) 1.40 (s, 9 H) 2.55 (s, 3 H) 3.51 (d, J = 9.60 Hz, 1 H) 4.56 (s, 1 H) 6.66 (d, J = 9.35 Hz, 1 H) 6.82 (d, J = 8.59 Hz, 1 H) 8.04 (d, J = 8.59 Hz, 1 H).

To a stirred solution of above intermediate *tert*-butyl(1*r*,3*r*)-3-(5-cyano-6-methylpyridin-2-yloxy)-2,2,4,4 tetramethylcyclobutylcarbamate (4.2 g, 11.6 mmol) in anhydrous dioxane (60 mL) was added 4 N HCl/dioxane (80 mL) dropwise at 5 °C. After the addition, the resulting mixture was stirred at room temperature for 3 h. The suspension was filtered, and the filter cake was washed with petroleum ether/ethyl acetate = 10:1 (50 mL), dried in vacuo to give the hydrochloride salt of the title compound (3.47 g, 91%) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.08 (s, 6 H) 1.30 (s, 6 H) 2.56 (s, 3 H) 3.05 (q, J = 5.56 Hz, 1 H) 4.66 (s, 1 H) 6.86 (d, J = 8.59 Hz, 1 H) 8.07 (d, J = 8.59 Hz, 1 H) 8.33 (br s, 3 H). MS (APCI): 260.2 $(M + H)^+$.

*Step 2: Coupling Reaction: N-((1r,3r)-3-(5-Cyano-6-methylpyridin-2-yloxy)-2,2,4,4-tetramethylcyclobutyl)imidazo[1,2-a]pyrimidine-3-carboxamide (**1**).* 6-((1*r*,3r)-3-Amino-2,2,4,4-tetramethylcyclobutoxy)-2-methylnicotinonitrile hydrochloride from step 1 (70.0 mg, 0.237 mmol, 1.00 equiv), imidazo[1,2-*a*]pyrimidine-3-carboxylic acid (Ryan Scientific, CA10088) (44.9 mg, 0.275 mmol, 1.16 equiv), and HBTU (*O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyl-uronium-hexafluorophosphate CAS no. 94790-37-1, 113.9 mg, 0.29 mmol, 1.2 equiv) were dissolved in 1.0 mL of dimethyl formamide (0.237 M). Triethyl amine (0.10 mL 0.72 mmol, 3.0 equiv) was added, and the reaction was stirred at room temperature for 2.5 h. LCMS after 2 h at room temperature showed clean conversion to amide coupling product (1.759 min, $(M + H)^+$ 405, R_f = 0.16 in 100% EtOAc, UV+). The reaction was partitioned between 10 mL of deionized water and 20 mL of ethyl acetate. The organic layer was washed with 5 mL of brine, dried over magnesium sulfate, filtered, and concentrated to 131.9 mg of a yellow wax, which was

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dissolved in ethyl acetate, adsorbed on silica, and purified on 12 g silica column, eluting with 100% ethyl acetate. The corresponding fractions were combined and concentrated in vacuo to afford the product as a white solid 62.8 mg (66%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.15 (s, 6 H), 1.25 (s, 6 H), 2.58 (s, 3 H), 4.12 (d, J = 9.60 Hz, 1 H), 4.79 (s, 1 H), 6.88 (d, J = 8.59 Hz, 1 H), 7.26 (dd, J = 6.95, 4.17 Hz, 1 H), 7.95 (d, J = 9.60 Hz, 1 H), 8.07 (d, J = 8.59 Hz, 1 H), 8.67−8.73 (m, 2 H), 9.70 (dd, J = 6.95, 1.89 Hz, 1 H). HRMS: $[M + H]^+$ calcd 405.203351, found 405.202098, error −3.09 ppm. Anal. Calcd For $C_{22}H_{24}N_6O_2 \cdot 0.5\ H_2O$: C, 63.91; H, 6.09; N, 20.32. Found: C, 63.85; H, 5.92; N, 20.28.

Characterization of Final Analogues. *Preparation of* ***8****: N-((1r,3r)-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl)-imidazo[1,2-a]pyrimidine-3-carboxamide.* Prepared by the method of compound 1, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.25 (s, 6 H), 1.32 (s, 6 H), 4.10 (s, 1 H), 4.18 (d, J = 8.34 Hz, 1 H), 6.09 (d, J = 8.08 Hz, 1 H), 6.83 (dd, J = 8.59, 2.53 Hz, 1 H), 6.99 (d, J = 2.53 Hz, 1 H), 7.09 (dd, J = 6.95, 4.17 Hz, 1 H), 7.59 (d, J = 8.59 Hz, 1 H), 8.22 (s, 1 H), 8.73 (dd, J = 4.29, 2.02 Hz, 1 H), 9.76 (d, J = 5.05 Hz, 1 H). MS (APCI): 424 $(M + H)^+$. Anal. Calcd For $C_{22}H_{22}ClN_5O_2 \cdot 1.15H_2O$: C, 59.43; H, 5.51; N, 15.75. Found: C, 59.19; H, 5.18; N, 15.60.

Preparation of ***9****: N-[trans-3-(3,4-Dicyanophenoxy)-2,2,4,4-tetramethylcyclobutyl]imidazo[1,2-a]pyrimidine-3-carboxamide.* Prepared by the method of compound **1**, using 4-fluorophthalonitrile (Aldrich, 47410) instead of 6-fluoro-2-methylnicotinonitrile in step 1. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 9.68 (dd, J = 6.82, 2.02 Hz, 1 H), 8.65 (dd, J = 4.04, 2.02 Hz, 1 H), 8.17 (s, 1 H), 7.66 (d, J = 8.84 Hz, 1 H), 7.17 (d, J = 2.53 Hz, 1 H), 7.08 (dd, J = 8.84, 2.53 Hz, 1 H), 7.02 (dd, J = 6.95, 4.17 Hz, 1 H), 6.06 (d, J = 8.08 Hz, 1 H), 4.13 (d, J = 8.08 Hz, 1 H), 4.07 (s, 1 H), 1.26 (s, 6 H), 1.18 (s, 6 H). MS (APCI): 415.0 $(M + H)^+$. Anal. Calcd For $C_{23}H_{22}N_6O_2 \cdot 1.0H_2O$: C, 63.88; H, 5.59; N, 19.43. Found: C, 63.73; H, 5.55; N, 19.34

Preparation of ***10****: N-{trans-3-[(5-Cyano-6-methoxypyridin-2-yl)-oxy]-2,2,4,4-tetramethylcyclobutyl}imidazo[1,2-a]pyrimidine-3-carboxamide.* Prepared by the method of compound **1**, using 6-chloro-2-methoxynicotinonitrile (ArrayChem, P21111) instead of 6-fluoro-2-methylnicotinonitrile in step 1. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.16 (s, 6 H), 1.26 (s, 6 H), 4.01 (s, 3 H), 4.15 (d, J = 9.60 Hz, 1 H), 4.81 (s, 1 H), 6.60 (d, J = 8.34 Hz, 1 H), 7.26 (dd, J = 6.82, 4.04 Hz, 1 H), 7.93 (d, J = 9.35 Hz, 1 H), 8.08 (d, J = 8.34 Hz, 1 H), 8.69 (s, 1 H), 8.71 (dd, J = 4.04, 2.02 Hz, 1 H), 9.70 (dd, J = 6.82, 2.02 Hz, 1 H). Anal. Calcd. For $C_{22}H_{24}N_6O_3 \cdot 0.1H_2O$: C, 62.58; H, 5.78; N, 19.90. Found: C, 62.89; H, 6.09; N, 19.51.

Preparation of ***11****: N-[trans-3-(3,4-Dicyanophenoxy)-2,2,4,4-tetramethylcyclobutyl]-2-methylimidazo[1,2-a]pyrimidine-3-carboxamide.* Prepared by the method of compound **1**, using 4-fluorophthalonitrile (Aldrich, 47410) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and 2-methyl-imidazo[1,2-*a*]pyrimidine-3-carboxylic acid (Ryan Scientific, CA10041) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.17 (s, 6 H), 1.28 (s, 6 H), 2.66 (s, 3 H), 3.98 (d, J = 7.83 Hz, 1 H), 4.39 (s, 1 H), 7.18 (dd, J = 6.95, 4.17 Hz, 1 H), 7.36 (dd, J = 8.84, 2.53 Hz, 1 H), 7.54 (d, J = 7.83 Hz, 1 H), 7.67 (d, J = 2.53 Hz, 1 H), 8.06 (d, J = 8.59 Hz, 1 H), 8.63 (dd, J = 4.29, 2.02 Hz, 1 H), 9.29 (dd, J = 6.82, 2.02 Hz, 1 H). HRMS: calcd For $C_{24}H_{24}N_6O_2$ $(M + H)^+$: 429.203351, found 429.201887, error −3.41 ppm. $C_{24}H_{24}N_6O_2 \cdot 0.25H_2O$: C, 66.57; H, 5.70; N, 19.41. Found: C, 66.40; H, 5.47; N, 19.35.

Preparation of ***12****: N-[trans-3-(2-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl]-2-methylimidazo[1,2-a]pyrimidine-3-carboxamide.* Prepared by the method of compound **1**, using 3-chloro-4-fluoro-benzonitrile (Aldrich, 376582) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and 2-methyl-imidazo[1,2-*a*]pyrimidine-3-carboxylic acid (Ryan Scientific, CA10041) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.61 (br s, 1 H), 7.89 (d, J = 8.78 Hz, 1 H), 7.46 (s, 1 H), 7.22 (s, 1 H), 7.17 (br s, 1 H), 7.02 (d, J = 8.78 Hz, 1 H), 4.33 (s, 1 H), 3.98 (s, 1 H), 2.66 (s, 3 H), 1.26 (s, 6 H), 1.18 (s, 6 H). MS (APCI): 438.0 $(M + H)^+$. Anal. Calcd For $C_{23}H_{24}ClN_5O_2 \cdot 2.0H_2O$: C, 58.29; H, 5.95; N, 14.78. Found: C, 57.94; H, 5.93; N, 14.47.

Preparation of ***13****: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)-oxy]-2,2,4,4-tetramethylcyclobutyl}imidazo[1,2-a]pyridine-3-carboxamide.* Prepared by the method of compound **1**, using imidazo[1,2-*a*]-pyridine-3-carboxylic acid (Ryan scientific, CC25401) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.07 (s, 6 H), 1.16 (s, 6 H), 2.49 (s, 3 H), 4.03 (d, J = 9.35 Hz, 1 H), 4.70 (d, J = 0.51 Hz, 1 H), 6.79 (d, J = 8.84 Hz, 1 H), 7.03 (td, J = 6.88, 1.14 Hz, 1 H), 7.38 (ddd, J = 8.97, 6.82, 1.39 Hz, 1 H), 7.58−7.67 (m, 1 H), 7.73 (d, J = 9.60 Hz, 1 H), 7.98 (d, J = 8.84 Hz, 1 H), 8.46 (s, 1 H), 9.25−9.39 (m, 1 H). MS (APCI): 404.0 $(M + H)^+$. Anal. Calcd For $C_{23}H_{25}N_5O_2 \cdot 1.0H_2O$: C, 65.54, H, 6.46; N, 16.62. Found: C, 65.63; H, 6.40; N, 16.55.

Preparation of ***14****: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}-2-methylimidazo[1,2-a]pyrazine-3-carboxamide.* Prepared by the method of compound **1**, using 2-methyl-imidazo[1,2-*a*]pyrazine-3-carboxylic acid (ChemICHIBA, COL-00748) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.09 (s, 6 H), 1.18 (s, 6 H), 2.48 (s, 3 H), 2.60 (s, 3 H), 3.88 (d, J = 8.08 Hz, 1 H), 4.64 (s, 1 H), 6.79 (d, J = 8.34 Hz, 1 H), 7.68 (d, J = 8.34 Hz, 1 H), 7.94 (d, J = 4.55 Hz, 1 H), 7.99 (d, J = 8.59 Hz, 1 H), 8.75 (dd, J = 4.55, 1.26 Hz, 1 H), 9.01 (d, J = 1.26 Hz, 1 H). MS (APCI): 419.0 $(M + H)^+$. Anal. Calcd For $C_{23}H_{26}N_6O_2$: C, 66.01; H, 6.26; N, 20.08. Found: C, 65.87; H, 6.29; N, 19.85.

Preparation of ***15****: N-[trans-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl]imidazo[1,2-a]pyrimidine-2-carboxamide.* Prepared by the method of compound **1**, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and imidazo[1,2-*a*]pyrimidine-2-carboxylic acid (Oakwood Products, 043463) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.24 (s, 6 H) 1.33 (s, 6 H) 4.10 (s, 1 H) 4.21 (d, J = 8.84 Hz, 1 H) 6.83 (dd, J = 8.72, 2.40 Hz, 1 H) 6.94−7.04 (m, 1 H) 7.58 (d, J = 8.84 Hz, 1 H) 7.74 (d, J = 9.09 Hz, 1 H) 8.11 (s, 1 H) 8.51 (dd, J = 6.82, 2.02 Hz, 1 H) 8.67 (dd, J = 4.04, 2.02 Hz, 1 H). MS (APCI): 424.0 $(M + H)^+$. Anal. Calcd For $C_{22}H_{22}ClN_5O_2 \cdot 0.1H_2O$: C, 62.07; H, 5.26; N, 16.45. Found: C, 61.88; H, 5.30; N, 16.21.

Preparation of ***16****: N-[trans-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl][1,2,4]triazolo[1,5-a]pyrimidine-2-carboxamide.* Prepared by the method of compound **1**, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and [1,2,4]triazolo[1,5-*a*]pyrimidine-2-carboxylic acid (Ryan Scientific, B001275) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.25 (s, 6 H), 1.34 (s, 6 H), 4.11 (s, 1 H), 4.27 (d, J = 8.84 Hz, 1 H), 6.83 (dd, J = 8.72, 2.40 Hz, 1 H), 6.99 (d, J = 2.53 Hz, 1 H), 7.29 (s, 1 H), 7.59 (d, J = 8.84 Hz, 1 H), 7.74 (d, J = 9.35 Hz, 1 H), 8.91−9.04 (m, 2 H). MS (APCI): 425.0 $(M + H)^+$. Anal. Calcd For $C_{21}H_{21}ClN_6O_2 \cdot 0.1H_2O$: C, 59.11; H, 5.01; N, 19.70. Found: C, 59.05; H, 4.98; N, 19.66.

Preparation of ***17****: N-[trans-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl]imidazo[1,5-a]pyridine-8-carboxamide.* Prepared by the method of compound **1**, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and imidazo[1,5-*a*]pyridine-8-carboxylic acid (Best PharmaTec, BP26399) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (500 MHz, DMSO-d_6) δ ppm 8.48 (d, J = 6.83 Hz, 1 H), 8.45 (s, 1 H), 7.84−7.92 (m, 2 H), 7.55 (s, 1 H), 7.27 (d, J = 6.34 Hz, 1 H), 7.21 (d, J = 2.44 Hz, 1 H), 7.01 (dd, J = 8.78, 1.95 Hz, 1 H), 6.76 (t, J = 6.83 Hz, 1 H), 4.33 (s, 1 H), 4.07 (d, J = 8.78 Hz, 1 H), 1.24 (s, 6 H), 1.16 (s, 6 H). MS (APCI): 423.2 $(M + H)^+$. Anal. Calcd For

$C_{23}H_{23}ClN_4O_2 \cdot 0.75H_2O$: C, 63.30; H, 5.66; N, 12.84. Found: C, 63.22; H, 5.50; N, 12.65.

Preparation of ***18****: N-((1r,3r)-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl)-5,6,7,8-tetrahydro-[1,2,4]triazolo[4,3-a]pyridine-3-carboxamide.* Prepared by the method of compound **1**, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and 5,6,7,8-tetrahydro-[1,2,4]triazolo-[4,3-*a*]pyridine-3-carboxylic acid (Anichem H10612) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 8.04 (d, J = 9.35 Hz, 1 H), 7.91 (d, J = 8.84 Hz, 1 H), 7.24 (d, J = 2.27 Hz, 1 H), 7.03 (dd, J = 8.72, 2.40 Hz, 1 H), 4.45 (s, 1 H), 4.25 (t, J = 5.94 Hz, 2 H), 3.99 (d, J = 9.09 Hz, 1 H), 2.91 (t, J = 6.32 Hz, 2 H), 2.68 (dt, J = 3.73, 1.80 Hz, 1 H), 2.28−2.38 (m, 1 H), 1.75−1.97 (m, 2 H), 1.24 (s, 6 H), 1.15 (s, 6 H). MS (APCI): 428.2 $(M + H)^+$. HRMS: calcd For $C_{22}H_{26}ClN_5O_2$ $(M + H)^+$: 427.177506, found 427.1769798. Anal. Calcd For $C_{22}H_{26}ClN_5O_2 \cdot 0.5H_2O$: C, 60.48; H, 6.23; N, 16.03. Found: C, 60.30; H, 6.15; N, 15.93.

Preparation of ***19****: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}-6-methoxyimidazo[1,2-b]pyridazine-3-carboxamide.* Prepared by the method of compound **1**, 6-methoxyimidazo[1,2-*b*]pyridazine-3-carboxylic acid (preparation reported in Tetrahedron 1967, 23, 2739−2746) instead of imidazo[1,2-*a*]-pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (acetonitrile-d_3, 400 MHz): δ (ppm) 8.31 (d, J = 8.6 Hz, 1H), 8.14 (s, 1H), 7.97 (d, J = 9.6 Hz, 1H), 7.86 (d, J = 8.6 Hz, 1H), 6.96 (d, J = 9.6 Hz, 1H), 6.77 (d, J = 8.6 Hz, 1H), 4.76 (s, 1H), 4.27 (d, J = 8.1 Hz, 1H), 4.12 (s, 3H), 2.59 (s, 3H), 1.31 (s, 6H), 1.16 (s, 6H). MS (APCI): 435.2 $(M + H)^+$. Anal. Calcd For $C_{23}H_{26}N_6O_3$: C, 63.58; H, 6.03; N, 19.34. Found: C, 63.32; H, 5.99; N, 19.14.

Preparation of ***20****: N-[trans-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl]-1H-pyrrolo[2,3-b]pyridine-5-carboxamide.* Prepared by the method of compound **1**, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and 1H-pyrrolo[2,3-*b*]pyridine-5-carboxylic acid (Anichem T12723) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 1.16 (s, 6 H), 1.26 (s, 6 H), 4.13 (d, J = 9.09 Hz, 1 H), 4.33 (s, 1 H), 6.58 (d, J = 3.54 Hz, 1 H), 7.02 (dd, J = 8.84, 2.53 Hz, 1 H), 7.22 (d, J = 2.27 Hz, 1 H), 7.57 (d, J = 3.28 Hz, 1 H), 7.84−7.94 (m, 2 H), 8.45 (d, J = 2.27 Hz, 1 H), 8.71 (d, J = 2.02 Hz, 1 H), 11.91 (s, 1 H). HRMS: $(M + H)^+$ calcd 423.15823, found 423.158189. Anal. Calcd For $C_{23}H_{23}ClN_4O_2 \cdot 0.5H_2O$: C, 66.82; H, 5.73; N, 10.16. Found:. C, 66.57; H, 5.64; N, 10.13.

Preparation of ***21****: N-[trans-3-(3-Chloro-4-cyanophenoxy)-2,2,4,4-tetramethylcyclobutyl][1,2,4]triazolo[4,3-a]pyridine-6-carboxamide.* Prepared by the method of compound 1, using 2-chloro-4-fluoro-benzonitrile (Aldrich, 344265) instead of 6-fluoro-2-methylnicotinonitrile in step 1 and [1,2,4]triazolo[4,3-*a*]pyridine-6-carboxylic acid (ACC, CHM0079893) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.18 (s, 6 H), 1.24 (s, 6 H), 4.01 (s, 1 H), 4.11 (d, J = 8.08 Hz, 1 H), 6.12 (d, J = 7.83 Hz, 1 H), 6.74 (dd, J = 8.72, 2.40 Hz, 1 H), 6.90 (d, J = 2.27 Hz, 1 H), 7.42 (dd, J = 9.60, 1.26 Hz, 1 H), 7.51 (d, J = 8.84 Hz, 1 H), 7.80 (d, J = 9.35 Hz, 1 H), 8.71 (s, 1 H), 8.87 (s, 1 H). MS (APCI): 424.2 $(M + H)^+$. Anal. Calcd For $C_{22}H_{22}ClN_5O_2 \cdot 0.5H_2O$: C, 61.04; H, 5.36; N, 16.18. Found: C, 60.89; H, 5.32; N, 15.80.

Preparation of ***22****: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)-oxy]-2,2,4,4-tetramethylcyclobutyl}-6-methylimidazo[1,2-a]pyrimidine-3-carboxamide. Step 1: 6-Methylimidazo[1,2-a]pyrimidine-3-carboxylic Acid.* (*E*)-Ethyl 3-ethoxyacrylate (Aldrich, 250120) (991 mg, 6.87 mmol) was dissolved in a mixture of water and dioxane 0.5:1 (10 mL) at rt, *N*-bromosuccinamide (1160 mg, 6.54 mmol) was added and the mixture was stirred at room temperature for 10 min, and 5-methylpyrimidin-2-amine (Anichem, H12650) (750 mg, 6.87 mmol) was added. The reaction was heated in a microwave to 100 °C for 10 min and then poured over saturated aqueous sodium bicarbonate (100 mL) and extracted with ethyl acetate (2 × 100 mL). The organic layer was dried over anhydrous sodium sulfate, and the filtrate was concentrated in vacuo. The resultant residue was diluted in dichloromethate and loaded into a silica column. The column was eluted with heptane/ethyl acetate 0−50%. The fractions containing the product were combined and evaporated to give ethyl 6-methylimidazo[1,2-*a*]pyrimidine-3-carboxylate as a yellow solid (2.28 g, 80%). ^{1}H NMR (acetone, 400 MHz): δ (ppm) 9.23−9.49 (m, 1H), 8.65 (d, J = 2.5 Hz, 1H), 8.31 (s, 1H), 4.41 (q, J = 7.2 Hz, 2H), 2.49 (s, 3H), 1.39 (t, J = 7.1 Hz, 3H).

To a solution of ethyl 6-methylimidazo[1,2-*a*]pyrimidine-3-carboxy late (from above) (2.28 g, 11.11 mmol) in tetrahydrofuran (20 mL) a 1N lithium hydroxide solution (16.7 mL, 16.7 mmol) was added. The mixture was stirred at room temp overnight. The reaction was taken to pH = 5 with 6 N HCl, and the volatiles were removed. The solid obtained was filtered and the cake washed with water. The solid was dried in a vacuum oven to give the titled product as a yellow solid (302 mg, 66%).

Step 2: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}-6-methylimidazo[1,2-a]pyrimidine-3-carboxamide ***22****.* Prepared by the method of compound **1**, using 6-methylimidazo[1,2-*a*]pyrimidine-3-carboxylic acid (from step 1 above) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 9.53 (s, 1H), 8.62 (s, 1H), 8.60 (d, J = 2.5 Hz, 1H), 8.07 (d, J = 8.6 Hz, 1H), 7.91 (d, J = 9.3 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 4.78 (s, 1H), 4.11 (d, J = 9.1 Hz, 1H), 2.58 (s, 3H), 2.38 (s, 3H), 1.25 (s, 6H), 1.15 (s, 6H). MS (APCI): 419.50 $(M + H)^+$. Anal. Calcd For $C_{23}H_{26}N_6O_2 \cdot 0.1H_2O$: C, 65.73; H, 6.28; N, 20.00. Found: C, 65.41; H, 6.29; N, 19.94.

Preparation of ***23****: 6-Bromo-N-{trans-3-[(5-cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}imidazo[1,2-a]pyrimidine-3-carboxamide.* Prepared by the method of compound **1**, using 6-bromoimidazo[1,2-*a*]pyrimidine-3-carboxylic acid (Anichem, H13576) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (acetonitrile-d_3, 400 MHz): δ ppm 9.84 (d, J = 2.5 Hz, 1H), 8.66 (d, J = 2.5 Hz, 1H), 8.40 (s, 1H), 7.86 (d, J = 8.6 Hz, 1H), 6.76 (d, J = 8.6 Hz, 1H), 6.73 (d, J = 8.8 Hz, 1H), 4.75 (s, 1H), 4.11 (d, J = 9.1 Hz, 1H), 2.60 (s, 3H), 1.28 (s, 6H), 1.18 (s, 6H). MS (APCI): 484.3 $(M + H)^+$. Anal. Calcd For $C_{22}H_{23}BrN_6O_2 \cdot 0.2EtOAc$: C, 54.66; H, 4.95; N, 16.77. Found: C, 54.84; H, 5.22; N, 16.43.

Preparation of ***24****: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}-6-fluoroimidazo[1,2-a]pyrimidine-3-carboxamide. Step 1: 6-Fluoroimidazo[1,2-a]pyrimidine-3-carboxylic Acid.* To a solution of (*E*)-ethyl 3-ethoxyacrylate (Aldrich, 250120) (638 mg, 4.42 mmol) in a mixture of water and 1,4-dioxane 0.5:1 (10 mL) at room temperature, *N*-Bromosuccinamide was added (750 mg, 4.21 mmol). The mixture was stirred at room temperature for 10 min, and 5-fluoropyrimidin-2-amine (Cheminstock Ltd., C4795) was added (500 mg, 4.42 mmol). The reaction was heated in a microwave to 100 °C for 10 min. The reaction was poured over saturated aqueous sodium bicarbonate, extracted with ethyl acetate, and dried over anhydrous sodium sulfate. The volatiles were removed, and the residue was diluted in dichloromethane and loaded into a silica column. The column was eluted with heptane/ethyl acetate 0−50%. The fractions containing the product were combined and evaporated to give ethyl 6-fluoro-imidazo[1,2-*a*]pyrimidine-3-carboxylateas a yellow solid (529 mg, 57%). ^{1}H NMR (acetonitrile-d_3, 400 MHz): δ ppm 9.50 (dd, J = 4.3, 3.0 Hz, 1H), 8.75 (d, J = 3.0 Hz, 1H), 8.40 (s, 1H), 4.42 (q, J = 7.1 Hz, 2H), 1.39 (t, J = 7.1 Hz, 3H). MS (APCI): 210 $(M + H)^+$.

To a solution of ethyl 6-fluoroimidazo[1,2-*a*]pyrimidine-3-carboxylate (described above) (529 mg, 2.53 mmol) in tetrahydrofuran (10 mL) a 1N lithium hydroxyde solution (3.79 mL, 3.79 mmol) was added and 10 mL of water. The mixture was stirred at room temp overnight. The reaction was taken to pH = 5 with 6 N HCl (0.650 mL, 4 mmol), and

the volatiles were removed. The solid obtained was filtered and the cake washed with water. The solid was dried in a vacuum oven to give the product as a yellow solid (302 mg, 66%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 9.48 (dd, J = 4.3, 3.0 Hz, 1H), 8.89 (d, J = 3.0 Hz, 1H), 8.35 (s, 1H). MS (APCI): 182 (M + H)$^+$.

*Step **2**: N-((1r,3r)-3-(5-Cyano-6-methylpyridin-2-yloxy)-2,2,4,4-tetramethylcyclobutyl)-6-fluoroimidazo[1,2-a]pyrimidine-3-carboxamide **24***. Prepared by the method of compound **1**, using 6-fluoroimidazo[1,2-*a*]pyrimidine-3-carboxylic acid (from step 1 above) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (acetonitrile-d_3, 400 MHz): δ ppm 9.71 (dd, J = 4.5, 3.0 Hz, 1H), 8.70 (d, J = 3.0 Hz, 1H), 8.46 (s, 1H), 7.86 (d, J = 8.6 Hz, 1H), 6.76 (d, J = 8.6 Hz, 1H), 6.73 (d, J = 8.8 Hz, 1H), 4.75 (s, 1H), 4.12 (d, J = 9.1 Hz, 1H), 2.60 (s, 3H), 1.28 (s, 6H), 1.13−1.19 (m, 6H). MS (APCI): 423.4 (M + H)$^+$. Anal. Calcd For $C_{22}H_{23}FN_6O_2$: C, 62.55; H, 5.49; N, 19.89. Found: C, 62.32; H, 5.56; N, 19.66.

*Preparation of **25**: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)-oxy]-2,2,4,4-tetramethylcyclobutyl}-7-methoxyimidazo[1,2-a]pyrimidine-3-carboxamide. Step **1**: 4-Methoxypyrimidin-2-amine.* To a solution of 4-chloropyrimidin-2-amine (Aldrich, 661325) (1.5 g, 11.58 mmol) in methanol (100 mL), a 25% w/w solution of sodium methoxide/methanol (6 mL, 30 mmol) was added portionwise. The reaction was stirred at room temp for 24 h. To the reaction 1.93 mL of 6 N HCl were added and the volatiles were removed. The residue was dissolved in water, and the pH was adjusted to 7 using more HCl and sodium bicarbonate. The aqueous mixture was extracted with ethyl acetate (4 × 20 mL), dried over anhydrous sodium sulfate, and evaporated to dryness to give the crude product as a yellow solid (1.34 g, 92%). ^{1}H NMR (acetone, 400 MHz): δ ppm 7.95 (d, J = 5.6 Hz, 1H), 6.00 (d, J = 5.6 Hz, 1H), 5.91 (br s, 2H), 3.81 (s, 3H). MS (APCI): 126 (M + H)$^+$.

*Step **2**: 7-Methoxyimidazo[1,2-a]pyrimidine-3-carboxylic Acid.* To a solution of (*E*)-ethyl 3-ethoxyacrylate (Aldrich, 250120) (691 mg, 4.8 mmol) in a mixture of water and 1,4-dioxane 0.5:1 (10 mL) at room temperature, *N*-bromosuccinamide (813 mg, 4.57 mmol) was added. The mixture was stirred at room temperature until the yellow color disappeared (10 min). To this solution, 4-methoxypyrimidin-2-amine (from step 1 above) (600 mg, 4.8 mmol) was added and the reaction was heated in a microwave to 100 °C for 15 min. The reaction was poured into an aqueous saturated sodium bicarbonate solution, and the aqueous solution was extracted with ethyl acetate (3 × 50 mL). The organics were combined and dried over anhydrous sodium sulfate. The volatiles were removed, and the residue was loaded into a silica column and eluted with heptane/ethyl acetate 0−50%. The fractions containing the ethyl 7-methoxyimidazo[1,2-*a*]pyrimidine-3-carboxylate as a yellow solid (627 mg, 29.5%).

To a solution of ethyl 7-methoxyimidazo[1,2-*a*]pyrimidine-3-carboxylate (described above) (627 mg, 2.83 mmol) in methanol (20 mL), a 1N lithium hydroxide solution (4.25 mL, 4.25 mmol) was added. The mixture was stirred at room temp overnight. The solution was adjusted to pH = 5 with 6 N HCl, the volatiles removed, and the solid obtained filtered and washed with water. The solid was dried in a vacuum oven to give 7-methoxyimidazo[1,2-*a*]pyrimidine-3-carboxylic acid as a white solid (373 mg, 68%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 9.31 (d, J = 7.3 Hz, 1H), 8.31 (s, 1H), 6.95 (d, J = 7.3 Hz, 1H), 4.02 (s, 3H). MS (APCI): 194 (M + H)$^+$.

*Step **3**: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}-6-methoxyimidazo[1,2-b]pyridazine-3-carboxamide **25***. Prepared by the method of compound **1**, using 7-methoxyimidazo[1,2-*a*]pyrimidine-3-carboxylic acid (prepared in step 2 above) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2 of compound **1**. ^{1}H NMR (acetone-*d*, 400 MHz): δ ppm 9.53 (d, J = 7.6 Hz, 1H), 8.27 (s, 1H), 7.97 (d, J = 8.6 Hz, 1H), 7.20 (d, J = 9.3 Hz, 1H), 6.88 (d, J = 8.6 Hz, 1H), 6.70 (d, J = 7.6 Hz, 1H), 4.82 (s, 1H), 4.19 (d, J = 8.8 Hz, 1H), 4.02 (s, 3H), 2.62 (s, 3H), 1.33 (s, 6H), 1.20 (s, 6H). MS (APCI): 435.5 (M + H)$^+$. Anal. Calcd For $C_{23}H_{26}N_6O_3 \cdot 0.1$EtOAc: C, 63.40; H, 6.09; N, 18.96. Found: C, 63.33; H, 6.08; N, 18.70.

*Preparation of **26**: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)-oxy]-2,2,4,4-tetramethylcyclobutyl}-7-(morpholin-4-yl)imidazo[1,2-a]-pyrimidine-3-carboxamide. Step **1**: 4-Morpholinopyrimidin-2-amine.* To a solution of 4-chloropyrimidin-2-amine (Aldrich, 661325) (100 mg, 0.772 mmol) in DMSO (2 mL), morpholine (0.202 mL, 1.36 mmol) and potassium carbonate (320 mg, 2.32 mmol) were added. The reaction was heated in a microwave to 100 °C for 20 min. The reaction was diluted with water, saturated with anhydrous sodium sulfate, and extracted with ethyl acetate (3 × 10 mL). The organics were dried over anhydrous sodium sulfate and evaporated to give the product as a crystalline solid (139 mg, 93%). ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 8.38 (d, J = 6.1 Hz, 1H), 6.53 (d, J = 6.1 Hz, 1H), 5.68 (br s, 2H), 4.16−4.27 (m, 4H), 3.97−4.09 (m, 4H). MS (APCI): 181.2 (M + H)$^+$.

*Step **2**: 7-Morpholinoimidazo[1,2-a]pyrimidine-3-carboxylic Acid.* To a solution of (*E*)-ethyl 3-ethoxyacrylate (111 mg, 0.772 mmol) in a mixture of water and dioxane 1:1 (1 mL) at room temperature, was added *N*-bromosuccinamide (131 mg, 0.735 mmol). The mixture was stirred at room temperature for 10 min, and 4-morpholinopyrimidin-2-amine (from step 1 above) was added (139 mg, 0.772 mmol). The reaction was heated in a microwave to 100 °C for 20 min. The reaction was neutralized with aqueous sodium bicarbonate and extracted with ethyl acetate (3 × 10 mL). The organics were dried over sodium sulfate and evaporated. The residue was diluted in dichloromethane, loaded into a 12 g silica column, and eluted with ethyl acetate. The fractions containing the product were combined to give 7-morpholinoimidazo[1,2-*a*]pyrimidine-3-carboxylate as a white solid (60 mg, 28%).

To a solution of ethyl 7-morpholinoimidazo[1,2-*a*]pyrimidine-3-carboxylate (described above) (60 mg, 0.22 mmol) in tetrahydrofuran (1 mL) was added 1N lithium hydroxide solution (0.240 mL, 0.239 mmol). The mixture was stirred at room temperature for 8 h. After the hydrolysis was complete, 6 N HCl (0.040 mL) were added. The volatiles were removed, and the residue was triturated in water. The supernatant was decanted and the residue freeze-dried to give the product as a white solid (60 mg, quantitative; contained 10% LiCl). ^{1}H NMR (DMSO-d_6, 400 MHz): δ ppm 9.59 (dd, J = 4.4, 3.2 Hz, 1H), 8.82 (d, J = 3.0 Hz, 1H), 8.24 (s, 1H), 2.26−2.36 (m, 4H), 2.16−2.23 (m, 4H). MS (APCI): 249 (M + H)$^+$.

*Step **3**: N-{trans-3-[(5-Cyano-6-methylpyridin-2-yl)oxy]-2,2,4,4-tetramethylcyclobutyl}-7-(morpholin-4-yl)imidazo[1,2-a]pyrimidine-3-carboxamide **26***. Prepared by the method of compound **1**, using 7-morpholinoimidazo[1,2-*a*]pyrimidine-3-carboxylic acid (prepared in step 2 above) instead of imidazo[1,2-*a*]pyrimidine-3-carboxylic acid in step 2. ^{1}H NMR (acetonitrile-d_3, 400 MHz): shift (ppm) 9.24 (d, J = 8.1 Hz, 1H), 8.01 (s, 1H), 7.85 (d, J = 8.6 Hz, 1H), 6.75 (d, J = 8.6 Hz, 1H), 6.65 (d, J = 7.8 Hz, 1H), 6.45 (d, J = 8.6 Hz, 1H), 4.72 (s, 1H), 4.07 (d, J = 8.3 Hz, 1H), 3.71−3.78 (m, 4H), 3.61−3.70 (m, 4H), 2.59 (s, 3H), 1.26 (s, 6H), 1.15 (s, 6H). MS (APCI): 490.5 (M + H)$^+$. Anal. Calcd For $C_{26}H_{31}N_7O_3 \cdot 0.1H_2O$: C, 63.55; H, 6.40; N, 19.95. Found: C, 63.28; H, 6.36; N, 19.82.

■ ASSOCIATED CONTENT

Supporting Information. Experimental procedures and AO metabolic stability in human S9 (HS9) fraction spectra of final analogues. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ABBREVIATIONS USED

AO, aldehyde oxidase; CRPC, castration-resistant prostate cancer; AR, androgen receptor; HS9, human S9 fraction; MoCo, molybdenum pyranopterin cofactor moiety; HLM, human liver microsome

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(4) HLM corresponds to the intrinsic metabolic clearance (Cl_{int}) in microsomes, in μL/min/mg of microsomal protein. Data interpretation as following: Cl_{int} < 15 μL/min/mg (low clearance); Cl_{int} 15−40 μL/min/mg (moderate clearance); Cl_{int} > 40 (high clearance).

(5) Determination of metabolites formed in HS9 fraction without NADPH was done by incubating the substrates (10 μM final concentration) and human liver S9 (1 mg/mL final concentration) in 100 mM potassium phosphate at pH 7.4. The reaction was initiated by addition of human liver S9 without preincubation. The mixture (1 mL) was then incubated at 37 °C for 1 h. The reaction was terminated by the addition of acetonitrile (5 mL) followed by vortexing for 1 min and then centrifuged. The supernatants were transferred into conical glass tubes for evaporation to dryness under nitrogen at 37 °C. The residues were reconstituted in 200 μL of 30:70 (v/v) acetonitrile:water (0.1% formic acid) and aliquots (20 μL) were injected for HPLC-MS analysis. The formation of AO metabolites was monitored by LCMS. Metabolite profiling and structure elucidation were performed using HPLC coupled in-line with UV and MS detection with ESI source in positive ion mode. The instrumental components for the methods are as follows: surveyor HPLC system and LTQ (Thermo Fisher Scientific, Waltham, MA), Phenomenex C8 column (100 mm × 2 mm, Phenomenex Torrance, CA). The mobile phase consisted of 0.1% formic acid in water (solvent A) and 0.1% formic acid in acetonitrile (solvent B). The flow rate was maintained at 0.4 mL/min. Parent drug and its metabolites were eluted using a linear gradient in mobile phase composition summarized as follows: equilibration for 0.75 min at 2% B, linear gradient to 25% B from 0.75 to 7.25 min, linear gradient to 55% B from 7.25 to 9 min, hold at 55% B from 9 to 10.25 min, linear gradient to 90% B from 10.25 to 15 min, hold at 90% B from 15 to 16 min, linear gradient to 2% B from 16 to 16.50 min, hold at 2% B from 16 to 20 min. The major operating parameters for the LTQ ESI-MS methods are shown below: spray voltage 3.5 kV; capillary temperature 350 °C; sheath gas flow rate 25 (arbitrary); auxiliary gas flow rate 10 (arbitrary); m/z scan range 120−1500 amu. Ion-trap LC-MSn (n = 2−4) experiments were performed to generate multistage mass spectra for selected molecular ions representing possible metabolites. At a constant pressure of 40 psi, helium was used as the damping and collision gas for all MSn experiments. Precursor isolation window, activation amplitude, activation Q and activation time were set at 1.6 amu, 40%, 0.25 and 30 ms, respectively. The Xcalibur software (Thermo Fisher Scientific, Waltham, MA) was used to control both HPLC and MS systems to acquire and process all spectral data. The UV chromatograms for each compound and its metabolite(s) can be found in the Supporting Information.

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