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In vitro evaluation of imidazo[4,5-*c*]quinolin-2-ones as gametocytocidal antimalarial agents

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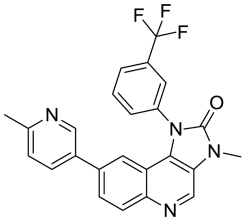
Abstract

Novel imidazo[4,5-c]quinolin-2-ones were synthesized and evaluated in asexual blood stage and late stage gametocyte assays of Plasmodium falciparum, a major causative agent of malaria. The design of these compounds is based on a recently identified lead compound from a high throughput screen. A concise synthesis was developed that allowed for generation of analogues with substitution around both the quinoline and imidazolidinone rings. Through structure-activity relationship studies, a number of potent compounds were identified that possessed excellent antimalarial activity against both the asexual and sexual stages with minimal cytotoxicity in mammalian cells. This is the first report describing SAR and gametocytocidal activity of imidazo[4,5-c]quinolin-2-ones, a new lead series for malaria treatment and prevention.

Graphical Abstract



Antimalarial activity
Asexual parasite: IC_{50} = 21 nM
Gametocytes: IC_{50} = 81 nM

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Keywords

Antimalarial; *Plasmodium falciparum*; gametocytes; gametocytocidal; imidazo[4,5-*c*]quinolin-2-one

Malaria remains a major infectious disease affecting populations primarily in underdeveloped countries. Despite the availability of improved drugs, particularly those based on the artemisinins, there were an estimated 198 million cases of malaria worldwide in 2013 with children being most susceptible to death from this disease.[1] Eradication of malaria has major challenges that include resistance to current therapies such as chloroquine, and the potential emergence of resistance to the artemisinins, the only class of drugs that can treat multi-drug resistant parasites.[2] To date, most drug development efforts, including recent high throughput screens, have focused primarily on the asexual blood stages of *Plasmodium falciparum*, the malarial parasite responsible for most deaths.

Transmission of malaria requires the intermediacy of the mosquito as a host for male and female gametocytes taken up in a blood meal to undergo fertilization, sporozoite formation, and transmission during a subsequent blood meal. *P. falciparum* gametocytes develop through five stages (I–V) over their 3 week lifespan in a human host. Since most antimalarials target the asexual parasite, gametocytes survive and are capable of being transmitted even after treatment with antimalarial drugs. Therefore, a therapeutic approach that targets the gametocyte stage is highly desirable, and would be key to any potential malaria eradication strategy by limiting transmission.

Relatively few gametocytocidal drugs have been described in the literature. Currently, the only drug used to effectively clear gametocytes is primaquine, an antimalarial not widely distributed because of its toxicity to individuals with a glucose-6-phosphate dehydrogenase deficiency (Figure 1).[3] The compound methylene blue has also been reported to possess potent gametocytocidal activity in vitro[4] and in vivo.[5,6] Most recently, the compound DDD107498 was reported to target plasmodium elongation factor 2, thereby inhibiting protein synthesis and acting against multiple lifecycle stages including gametocytes (Figure 1).[7]

Gametocytes do not replicate during development; therefore, typical in vitro assays used for monitoring DNA replication and asexual parasite growth are less useful. The development of an alamarBlue assay, recently reported by our groups, takes advantage of a fluorescent oxidation-reduction indicator as a measure of cytosolic metabolic activity and thereby gametocyte viability.[8,9] Using this assay, we recently reported the high throughput screen of 5,215 known compounds, and identified 27 gametocytocidal compounds. Among the most potent lead compounds was the mTOR inhibitor Torin 2 with an IC_{50} of 8 nM against gametocytes and possessing efficacy for blocking malaria transmission in a mouse model.[10]

Given the structural similarity of imidazo[4,5-*c*]quinolin-2-ones to Torin 2, these scaffolds were of interest as potential gametocytocidal antimalarial agents. Compounds with this structure have primarily been developed as kinase inhibitors and have become clinical candidates for anticancer indications. For example, NVP-BEZ235 and NVP-BGT226 were

identified as dual PI3K and mTOR inhibitors, and are currently in clinical trials for a number of cancers including advanced solid tumors and metastatic breast cancers.[11,12,13] A recent publication described the optimization of NVP-BEZ235 for anti-trypanosomal activity and reported promising compounds with improved selectivity over human PI3K and mTOR.[14] Given that these agents have safety profiles consistent with clinical use, we reasoned this series merited further study to optimize their antimalarial properties. Described herein is the rational design, synthesis, and establishment of structure-activity relationships that will facilitate the development of imidazo[4,5-*c*]quinolin-2-ones as gametocytocidal agents.

In order to rapidly develop SAR for the imidazo[4,5-*c*]quinolin-2-one series, a concise synthesis was developed that allows for diversification at three positions around the quinoline and imidazolidinone rings (Scheme 1). The synthesis of analogues commenced with 6-bromo-4-chloro-3-nitroquinoline (**5**). A nucleophilic aromatic substitution reaction of **5** with various alkyl or aryl amines provided substituted quinolines of the structure **6**. Reduction of the nitro group with stannous chloride followed by cyclization upon treatment with diphosgene gave the imidazolidinone ring **8**. *N*-alkylation of this ring was performed with MeI or EtI to afford analogues substituted at position R^2 (**9**). Suzuki–Miyaura coupling of bromides **8** and **9** with desired boronic acids or esters under microwave conditions enables diversification of the quinoline ring and generation of final products. All final analogues were purified by reverse phase HPLC prior to biological evaluation.

All analogues were tested initially in a blood stage assay with asexual parasites of the chloroquine-sensitive 3D7 strain of *P. falciparum*.[15] The gametocytocidal activity of compounds was determined utilizing the previously described alamarBlue high throughput assay with late stage 3D7 gametocytes (III–V).[8–10] In order to measure general cytotoxicity, compounds were tested in the presence of HepG2 cells for cell viability.[16] Biological assays were all run in 1536-well format with 11-point dose response curves. All compounds were tested in duplicate, and all assays incorporated Torin 2 (**4**) as a positive control and DMSO as a negative control.

We initiated SAR studies by synthesizing compounds **11–13** incorporating the aryl substituents of the screening hit Torin 2 (**4**). The imidazo[4,5-*c*]quinolin-2-one series allows for relatively easy access to substitution at the –NH of the imidazolidinone ring, providing a handle for further synthetic manipulations. Using this strategy, analogues were synthesized where R^1 was kept constant as a 3-trifluoromethylphenyl, and R^2 was either unsubstituted or alkyl substituted (Table 1). Compounds **11–13** incorporate the aminopyridine substituent at the R^3 position, providing a direct comparison to Torin 2. Of these analogues, compound **12** showed the best antimalarial activity in the asexual assay as well as the gametocyte assay with IC_{50} values in the low nanomolar range. One notable trend was that methyl substitution provided improved activity relative to either the proton or ethyl variants. Additionally, compound **12** displayed a 4-fold difference in activity between asexual and gametocyte IC_{50}'s, such difference was noticed with most analogues having heterocyclic substituents at R^3. While compound **12** was quite potent, it did exhibit HepG2 cytotoxicity in the low micromolar range, which we hoped to diminish through continued SAR.

Following this initial evaluation based on the structure of Torin 2 (**4**), analogues were synthesized where the aminopyridine moiety was exchanged for various heterocycles. A few pyridine and substituted pyridine analogues were examined in an effort to understand the relative role of the heterocyclic nitrogen itself in the activity of the aminopyridine substituted compound **12**. Unsubstituted 3- and 4-pyridines were examined at the R^3 position (**14–17**). Compounds **15** and **17** possessed similar biological activity, albeit with a 10-fold decrease in both asexual and gametocyte activity compared to **12**. When R^2 was unsubstituted (**14** and **16**), there was a significant decrease in activity, as was seen with compound **11**. With all of the examined heterocyclic substituents at R^3, it was observed that methyl substitution at R^2 led to a 2 to 10-fold increase in activity with both the asexual and gametocyte assays.

Substituted pyridine analogues that were examined included methylpyridine (**18**, **19**) acetylaminopyridine (**22**, **23**), and hydroxypyridine (**24**, **25**). Compounds **19** and **23** showed low nanomolar activity in the asexual assay, and good gametocytocidal activity (< 100 nM). The hydroxypyridine compound **25** was slightly less potent than other substituted pyridine analogues. Additionally, compound **21**, where R^3 is aminopyrimidine, showed low nanomolar activity similar to **12**. Notably, the presence of an amino or amide moiety at the para position of heterocycles at R^3 showed low micromolar activity in the HepG2 cytotoxicity assay. There were significant reductions in general cytotoxicity with methylpyridine or hydroxypyridine substituents, with HepG2 IC_{50}'s greater than 10 μM. From these data, compound **19** indicates that methylpyridine is a promising substituent at the R^3 position because it demonstrates excellent antimalarial activity with low HepG2 cytotoxicity. These results suggest that reductions in mammalian cytotoxicity can be obtained without sacrificing potent antimalarial activity, a critical step in pursuing this series of compounds.

Larger bicyclic heterocycles at the R^3 position were also examined. The quinoline ring at R^3 (**26**, **27**) led to a significant abrogation of antimalarial activity and was thought to be too bulky at this position. The benzimidazole compound **29**, on the other hand, showed good activity in the asexual blood stage assay but was only moderately active in the gametocyte assay. Therefore, larger heterocyclic or aryl substituents were not pursued at R^3 for this series of compounds. Additionally, compounds **30** and **31** with no substitution at R^3 which were isolated as side products in the Suzuki–Miyaura coupling, showed weak antimalarial activity.

With an understanding of heterocyclic substitutions at the R^3 position of the quinoline ring, we turned our attention to phenyl derivatives at this position (Table 2). We started by looking at analogues with an unsubstituted phenyl ring (**32**, **33**) which showed moderate activity for the asexual blood stage assay and little activity in the gametocyte assay. Substitution of the phenyl ring at the 3- or 4-position with primary amines (**34–37**) was examined to understand the importance of hydrogen bond donors or acceptors at these positions. As seen for analogues containing heterocyclic substituents at R^3 (Table 1), phenyl derivatives with methyl substitution at R^2 also led to significant improvements in potency for both antimalarial assays (Table 2). Compounds **35** and **37** did show good activity (< 100 nM) in the asexual assay and comparable activity in the gametocyte assay as well. In contrast to

heterocyclic substituents at R^3, substituted phenyl derivatives generally had less variability between asexual and gametocyte activity, typically showing a 1- to 2-fold difference.

A number of additional 4-substituted phenyl derivatives were synthesized with both electron withdrawing and donating substituents. The 4-hydroxyphenyl derivative **39** showed slightly lower activity to the 4-aminophenyl **35**, suggesting that both hydrogen atoms are beneficial for activity. In comparison, electron withdrawing groups at the 4-position such as nitrile (**46**, **47**), chloro (**50–52**), and fluoro (**53**, **54**) gave poor activity in the asexual and gametocytocidal assays. The electron donating 4-methylphenyl gave a moderate gametocytocidal IC_{50} of 524 nM. The 3-benzonitrile **45** derivative demonstrated good asexual activity (184 nM) but poor gametocytocidal activity. We next examined amides, sulfones, and sulfonamides as functional groups incorporating both hydrogen bond acceptors and donors of varying sizes. The primary amide **41** showed excellent asexual activity, but demonstrated a 10-fold lower gametocytocidal activity. The methylsulfone and sulfonamide derivatives **43** and **49**, respectively, were similar to each other in activity and had moderate asexual and gametocyte activity. Of note, the HepG2 cytotoxicity of the phenyl derivatives was significantly lower than heterocyclic substitution at R^3, with most compounds showing no cytotoxicity at the highest concentration tested (46 μM).

Having considered numerous analogues with changes to R^3, we turned our attention to variations at R^1 of the imidazolidinone ring (Table 3). Substitution at R^1 requires incorporation of the varying anilines from the beginning of the synthesis with the S_NAr reaction. In order to directly compare modifications at R^1, the aminopyridine group at R^3 was held constant, since it was found to be the most potent substituent in Table 1.

We began by making a relatively simple change from compounds **11** and **12**, by incorporating a 4-trifluoromethylphenyl group at R^1 (Table 3). One can hypothesize that modulation of R^1 may more directly impact the R^2 substituent; therefore, we tested the unsubstituted –NH and methyl substitution at position R^2. Comparison of compounds **57** and **58** clearly showed that methyl substitution provides a 3- to 5-fold increase in antimalarial activity. The more active compound **58** showed good asexual and gametocytocidal activity; however, these potencies were more than 10-fold lower than the 3-trifluoromethylphenyl derivative (**12**).

Additional 3- and 4-substituted phenyl derivatives that were examined included variations in electron withdrawing or donating properties. The 4-benzonitrile compound **60** showed good activity in both the asexual and gametocytocidal assays with no measurable HepG2 cytotoxicity. Additionally, substitution with the electron donating methoxy group at either the 4- (**61**) or 3-position (**62**) of the phenyl ring demonstrated good asexual and gametocytocidal activity. While cytotoxicity of these compounds was in the low micromolar range, modulation of the R^3 position may decrease general cytotoxicity as measured by the HepG2 assays. Interestingly, the unsubstituted phenyl derivative **63** possessed good asexual antimalarial activity and gametocyte activity that was comparable to substituted phenyl derivatives. Alkyl substitution at R^1 included both the cyclohexyl derivatives **64** and **65** as well as the cyclopropyl compound **66**. All of the alkyl compounds tested to date, have shown a significant decrease in activity compared to the phenyl derivatives. Therefore, our focus at

the R^1 position has been phenyl or substituted phenyl derivatives. With flexibility regarding the substituents at R^1, we anticipate continuing SAR studies with the aim to decrease cytotoxicity, increase solubility, and optimize physical properties of this series of compounds.

In conclusion, by designing a concise synthesis, a number of analogues incorporating changes to both the quinoline ring and imidazolidinone rings of the imidazo[4,5-*c*]quinolin-2-one series were synthesized. These analogues were tested in previously established *P. falciparum* asexual blood stage assay and the alamarBlue gametocytocidal assay recently described by our groups. Through these studies, a number of compounds were identified with potent, low nanomolar activity in both the asexual and gametocyte stages. In particular, compound **19** was found to be among the best compounds with excellent antimalarial activity in both assays and low cytotoxicity. We also demonstrated improved cytotoxicity profiles upon incorporation of phenyl derivatives at R^3 compared to heterocyclic substitution, and showed that methyl substitution at R^2 was key for improved antimalarial activity. Finally, derivatives at position R^1, while tolerated, seemed to show little improvement from the 3-trifluoromethylphenyl group (**11–12**). With these potent compounds in hand, we anticipate more significant efforts aimed at understanding the targets of these phenotypic screens. We have demonstrated key structure-activity relationships around the imidazo[4,5-*c*]quinolin-2-one series of compounds. Further lead optimization to develop this class of compounds as antimalarials with a focus on gametocytocidal activity aimed at limiting transmission of the malaria parasite is under way in our group, we will report the results in due course.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Angulo-Barturen I, Ferrer-Bazaga S, Gamo F, Wyatt P, Leroy D, Siegl P, Delves M, Kyle D, Wittlin S, Marfurt J, Price R, Sinden R, Winzeler E, Charman S, Bebrevska L, Gray D, Campbell S, Fairlamb A, Willis P, Rayner J, Fidock D, Read K, Gilbert I. Nature. 2015; 522:315. [PubMed: 26085270]

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11. Maira SM, Stauffer F, Brueggen J, Furet P, Schnell C, Fritsch C, Brachmann S, Chene P, De Pover A, Schoemaker K, Fabbro D, Gabriel D, Simonen M, Murphy L, Finan P, Sellers W, Garcia-Echeverria C. Mol. Cancer Ther. 2008; 7:1851. [PubMed: 18606717]
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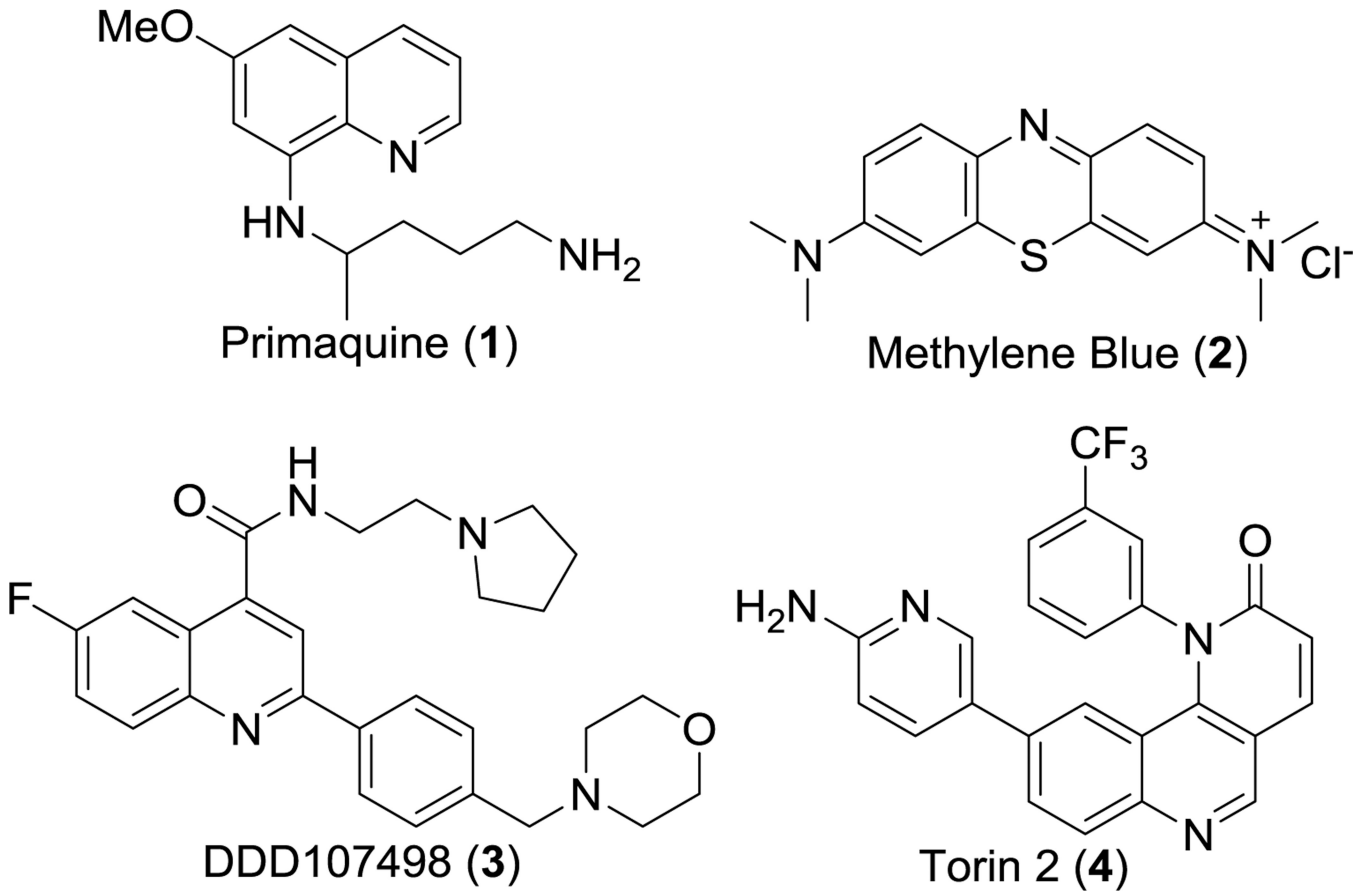
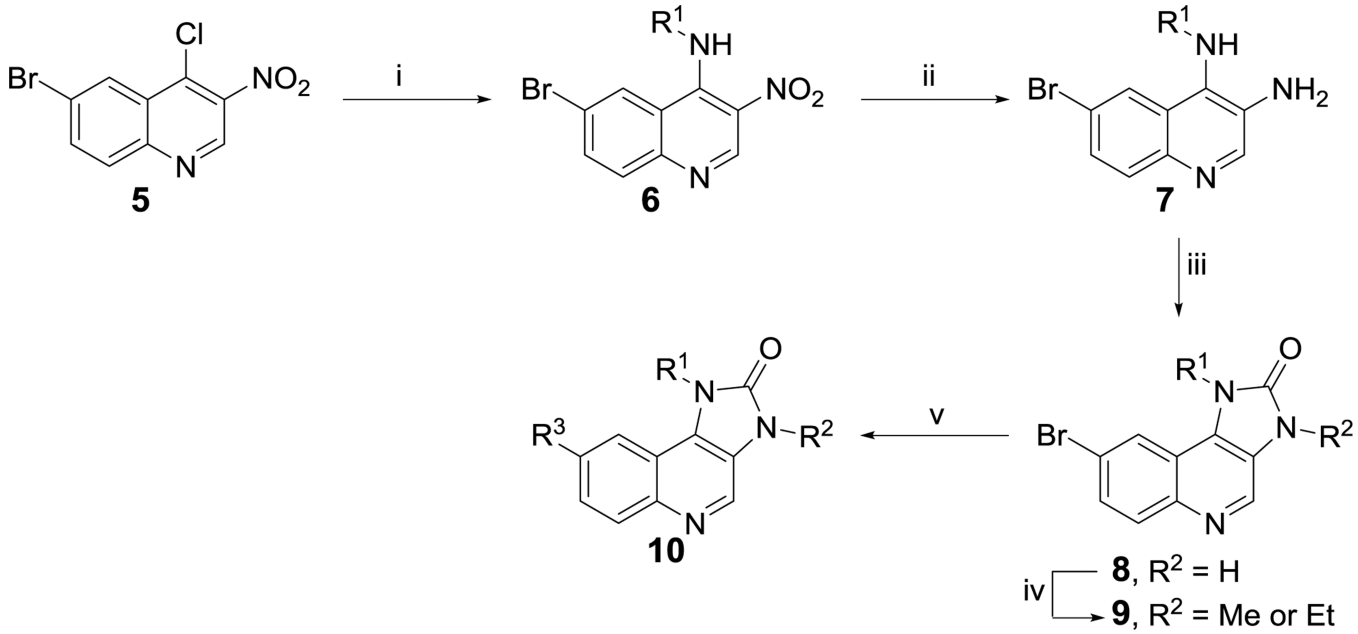


Figure 1.
Gametocytocidal antimalarial small molecules.

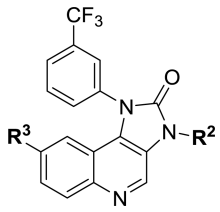


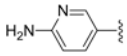
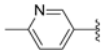
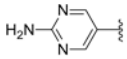
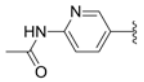
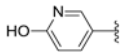
Scheme 1.

Reagents and conditions: (i) R^1NH_2, 1,4-dioxane, 150 °C MW; (ii) $SnCl_2$, EtOH, reflux; (iii) $Cl_3COCOCl$, Et_3N, DCM; (iv) MeI or EtI, NaH, DMF, rt; (v) R^3-$B(OR)_2$, $Pd(PPh_3)_4$, $NaHCO_3$, DMF, H_2O, 120 °C MW.

Table 1

IC_{50} values of heteroaromatic substitution at R^3.



Cmpd.	R^3	R^2	Asex. IC_{50} (μM)	Gamet. IC_{50} (μM)	HepG2 IC_{50} (μM)
11		H	0.091	0.097	3.11
12		Me	0.007	0.028	2.60
13		Et	0.183	0.205	3.68
14		H	2.82	11.4	2.25
15		Me	0.095	0.236	10.3
16		H	0.313	1.14	1.50
17		Me	0.093	0.359	1.73
18		H	0.18	0.32	>45.0
19		Me	0.021	0.081	14.5
20		H	0.213	0.45	2.54
21		Me	0.017	0.052	1.33
22		H	0.081	0.186	>45.0
23		Me	0.025	0.042	3.98
24		H	0.412	4.63	>45.0
25		Me	0.158	0.294	23.5

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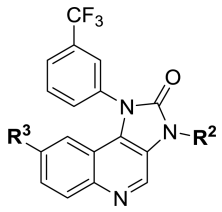
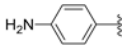
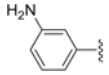
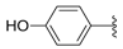
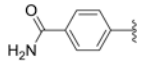
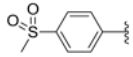
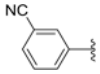
Cmpd.	R^3	R^2	Asex. IC_{50} (μM)	Gamet. IC_{50} (μM)	HepG2 IC_{50} (μM)
26		H	15.4	2.35	>45.0
27		Me	5.78	5.27	31.6
28		H	0.214	0.347	3.24
29		Me	0.065	0.157	2.63
30		H	5.03	7.46	>45.0
31		Me	17.0	3.64	>45.0

Table 2

IC_{50} values of aromatic substitution at R^3.

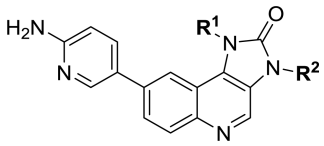


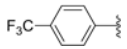
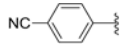
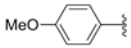
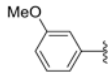
Cmpd.	R^3	R^2	Asex. IC_{50} (μM)	Gamet. IC_{50} (μM)	HepG2 IC_{50} (μM)
32		H	4.04	18.4	>45.0
33		Me	0.442	4.71	>45.0
34		H	0.283	3.27	7.23
35		Me	0.086	0.087	5.53
36		H	0.275	0.637	6.17
37		Me	0.077	0.145	13.9
38		H	0.781	1.47	4.06
39		Me	0.114	0.229	2.33
40		H	0.109	0.294	>45.0
41		Me	0.038	0.497	4.69
42		H	1.16	1.11	>45.0
43		Me	0.578	0.855	>45.0
44		H	0.874	2.79	>45.0
45		Me	0.184	2.16	>45.0

Cmpd.	R^3	R^2	Asex. IC_{50} (μM)	Gamet. IC_{50} (μM)	HepG2 IC_{50} (μM)
46		H	2.58	6.33	>45.0
47		Me	7.33	11	>45.0
48		H	0.948	2.33	>45.0
49		Me	0.452	0.816	>45.0
50		H	1.69	3.56	>45.0
51		Me	1.75	1.86	>45.0
52		Et	3.93	10.4	>45.0
53		H	1.57	1.83	>45.0
54		Me	3.18	5.59	>45.0
55		H	5.19	7.05	>45.0
56		Me	0.733	0.524	>45.0

Table 3

IC_{50} values of substitution at R^1.



Cmpd.	R^1	R^2	Asex. IC_{50} (μM)	Gamet. IC_{50} (μM)	HepG2 IC_{50} (μM)
57		H	0.530	0.576	>45.0
58		Me	0.104	0.237	>45.0
59		H	0.386	0.482	>45.0
60		Me	0.098	0.118	>45.0
61		Me	0.047	0.148	2.34
62		Me	0.088	0.126	2.53
63		Me	0.069	0.219	6.54
64		H	1.35	2.87	6.50
65		Me	0.832	1.88	3.84
66		Me	1.16	0.865	11.4