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Addressing hERG activity while maintaining favorable potency, selectivity and pharmacokinetic properties of PPARδ modulators

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ABSTRACT

One of the most commonly used strategies to reduce hERG (human ether-a-go-go) activity in the drug candidates is introduction of a carboxylic acid group. During the optimization of PPARδ modulators, some of the compounds containing a carboxylic acid were found to inhibit the hERG channel in a patch clamp assay. By modifying the basicity of the imidazole core, potent and selective PPARδ modulators that do not inhibit hERG channel were identified. Some of the modulators have excellent pharmacokinetic profiles in mice.

We have recently disclosed a series ("benzamide series") of PPARδ modulators such as **1a-b** that show good selectivity for PPARδ over PPARα and PPARγ (Fig. 1).[1,2] By replacing the *cis* amide conformer found in the x-ray structure of benzamides in the ligand binding domain of PPARδ receptor, a second series ("imidazole series") of PPARδ modulators was designed (Fig. 1).[3] The compounds in the imidazole series such as **2a** were found to be more potent and selective modulators of PPARδ receptor. Modifications to the hexanoic acid moiety in both series significantly improve plasma exposures after oral dosing as compared to their unsubstituted counterparts.[1,3] We have shown that imidazole MA-0204 (**2c**), has good pharmacokinetic properties and could be an effective therapeutic for Duchene Muscular Dystrophy (DMD).[3]

Medicinal chemists routinely screen lead compounds in the discovery programs for hERG channel binding in order to assess their potential to cause QT prolongation, which could lead to Torsades de pointes arrythmias.[4] Several drugs have been withdrawn from market or given "black box" labels due to risks related to hERG channel-related QT prolongation.[5] Such measures are necessary if the concentration of drug required for its therapeutic activity approaches the concentration where the hERG channel is inhibited.[6]

In order to address this potential safety concern, medicinal chemists optimize lead compounds for minimal hERG inhibition (typically IC_{50} > 10 μM), while maintaining or improving activity for the molecular target (typically IC_{50} or EC_{50} < 100 nM). Although not an absolute requirement, a vast majority of the hERG active molecules possess basic amines in their structures. In order to understand the interaction of molecules with the channel, structural information regarding the hERG K-channel is used.[7,8] It is known that hERG channel binding pocket interactions occur *via* the hydrophobic central cavity and two amino acid residues, F656 and Y652.[9] The interactions of the hERG inhibitors with the phenylalanine moiety are more hydrophobic in nature whereas the interactions with the tyrosine moiety are hypothesized to be π-cationic in nature.[9,10] Therefore, modulating lipophilicity of compounds (TPSA, LogD or LogP) or the basicity (pKa) of nitrogens in the molecules is a commonly used tactic to reduce hERG activity.[4,11–13] A carboxyl group in the structure of a molecule can help lower the lipophilicity and minimize hydrophobic interactions. There are several examples in the literature where a carboxylic acid moiety was introduced into a compound structure to reduce the hERG activity.[14,15] In certain cases, adding a carboxylic acid moiety can result in a zwitterionic compound.[16] Zwitterionic molecules typically have low permeability, which can reduce the probability of hERG binding.[11]

When lead compounds in the benzamide series, **1a** and **1b**, were screened for hERG activity in an automated patch clamp assay, little or

Abbreviations: DMSO, Dimethyl Sulfoxide; BBr_3, boron tribromide; DCM, dichloromethane; RT, room temperature; h, hour; $Pd(PPh_3)_4$, Tetrakis(triphenylphosphine)palladium(0); Na_2CO_3, sodium carbonate; DME, 1,2-dimethoxyethane; EtOH, ethyl alcohol; $LiOH{\cdot}H_2O$, lithium hydroxide monohydrate; THF, tetrahydrofuran; EDCI·HCl, ethylcarbodiimide hydrochloride; HOBt, 1-hydroxy benzotriazole; Et_3N, triethylamine; DMF, *N*,*N*-dimethyl formamide; $Zn(OTf)_2$, zinc trifluoromethanesulfonate

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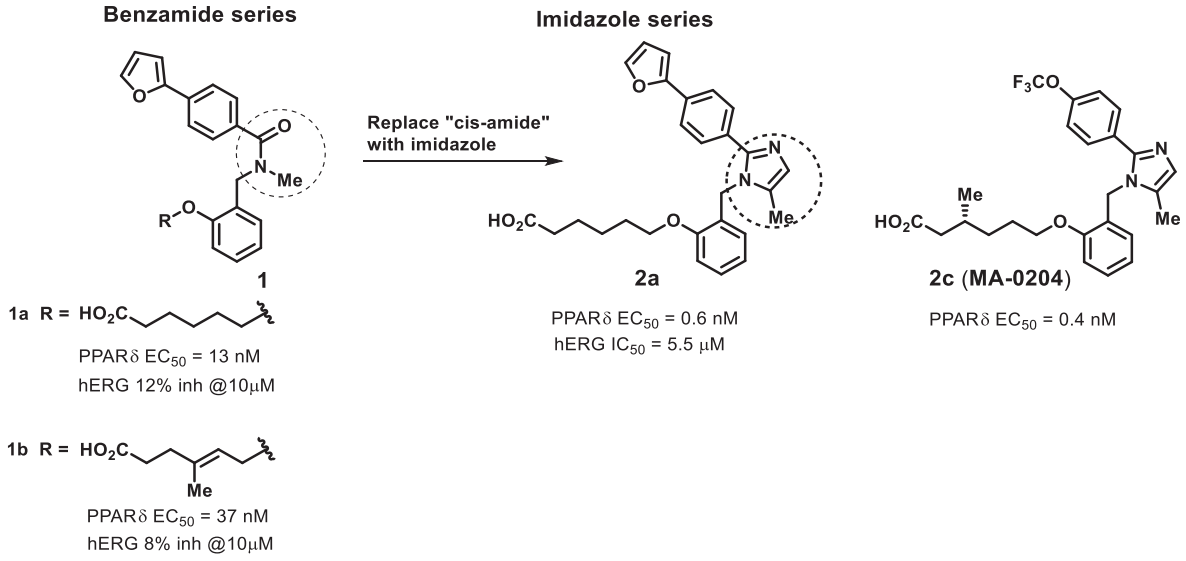


Fig. 1.

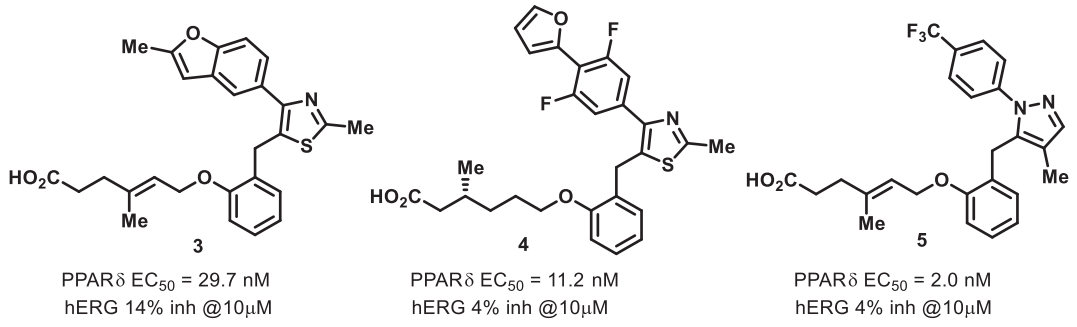


Fig. 2.

no activity (< 15% inhibition at 10 μM) was observed.[17] Such results were consistent with our expectations as **1a** and **1b** lack basic nitrogen atoms and possess a carboxylic acid. Therefore, when imidazole **2a**, was found to inhibit hERG channel activity (IC_{50} = 5 μM), the result was unexpected because: (1) the compound contains a carboxylic acid and (2) even if the imidazole nitrogen is basic, the compound still would exist in a zwitterionic form, which would lower permeability and thus not have hERG inhibition.

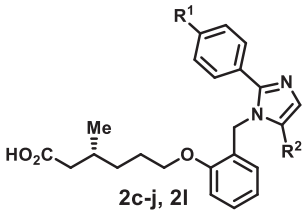
In order to further understand whether the change in the shape of the molecule resulting from replacing an amide group in **1a** and **1b** with an imidazole (**2a)** led to the hERG acivity, two thiazoles (**3** and **4**) and one pyrazole (**5**) were synthesized and screened for hERG activity (Fig. 2). All three compounds lack hERG inhibitory activity (< 15% inhibition at 10 μM).

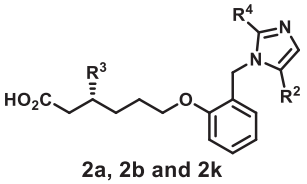
Based on these results, it is unlikely that the hERG activity observed for imidazole **2a** results from the three dimensional shape of the molecule. We assessed whether lipophilicity (as characterized by cLogP) or TPSA (topological polar surface area) impacts the hERG inhibition.[11–12] Calculated logP for **2a** (cLogP = 5.1)[18] is lower than those for **3**, **4** and **5** (cLogP = 6.1–7.5). TPSA for all four compounds are between 71A° and 78A°. Thus lipophilicity parameters do not seem to correlate with the differences in the hERG activity of these compounds. We then examined if the basicity of the nitrogen atoms in the heterocyclic rings could explain the difference in the hERG inhibitory potential of these compounds. In order to test this hypothesis, imidazoles **2b-2l** with either electron withdrawing or electron donating groups on the imidazole ring or on the phenyl ring that is directly attached to the imidazole ring (Table 1) were synthesized and tested for hERG inhibition. The imidazole compounds bearing a methyl group on the imidazole ring, were synthesized using Scheme 1.[1,3] For the synthesis of compounds **2e** and **2i-j**, the central imidazole rings bearing a trifluoromethyl group were constructed *via* 2-(4-substituted-phenyl)-4-(2,2,2-trifluoroacetyl)oxazol-5(4*H*)-one as shown in Scheme 2.[19] Compounds with a trifluoromethyl (**2f**), a chloro (**2h**) or a cyano (**2g**) substituent on the imidazole ring were synthesized *via* a intermediate **23** or **24** as shown in Scheme 3.[19].

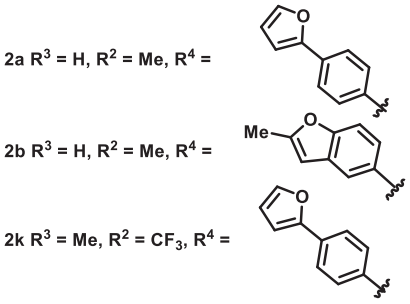
All the compounds in Table 1 show excellent PPARδ activity (EC_{50} = 0.4–30 nM) and were selctive over other PPARs (data for **2f**, **2h** and **2i** shown in Table 2). The comparison of hERG activity and cLogP or TPSA for a set of compounds (Table 1) revealed no correlation between the hERG activity and the physicochemical properties. However, a clear relationship was observed between nitrogen basicity in the heteroaromatic ring and the hERG activity with an infliction point around pKa = 6.0 (Fig. 3).[20] Decreasing electron-donation to the phenyl ring that is attached to the imidazole (**2c)** lowered hERG activity. Adding a stronger electron withdrawing (cyano) group at the same position (**2d),** reduced hERG inhibition below 50% at 10 μM. When electron withdrawing groups were placed directly on the imidazole ring (**2e**−**2 l**), hERG activity was diminished substantially, for example, when comparing **2a** (hERG IC_{50} = 5.5 μM) to **2 k** and **2 l** (< 10% inhibition of hERG @ 10 μM)or **2c**–**2e**.

The observed differences in the hERG activity of imidazole **2a**

Table 1
pKa, cLogP, TPSA and PPARδ activity for compounds **2a-2 l** and **3–5**.





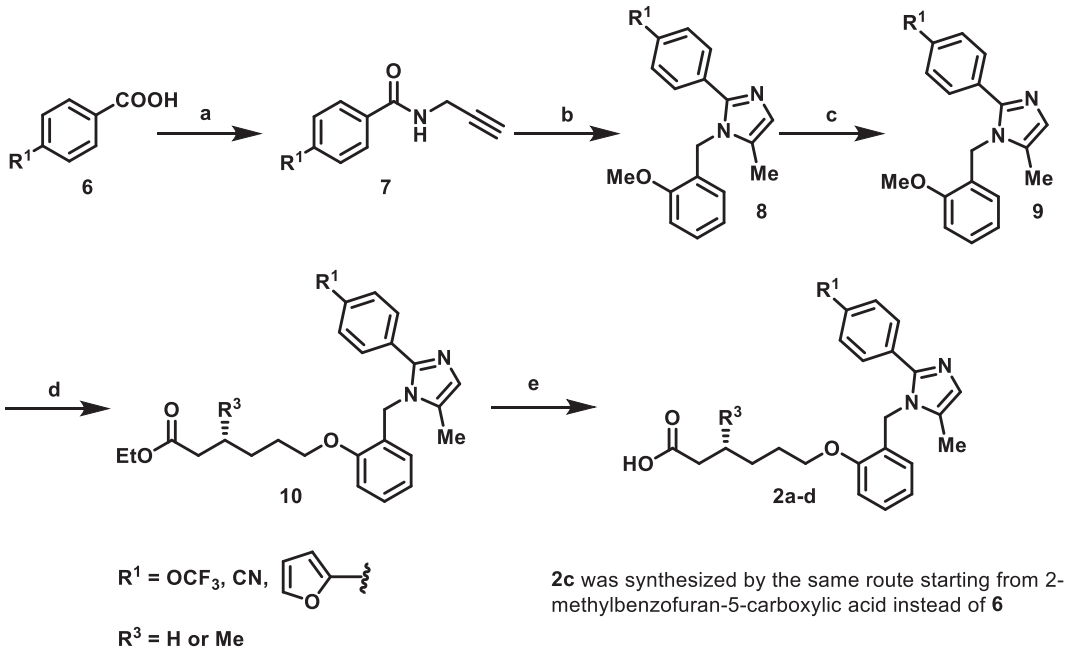


Cpd	R^1	R^2	PPARδ EC_{50} nM[a]	hERG EC_{50} μM[b] or % inh@10 μM	Calculated parameters[c]		
					pKa	cLogP	TPSA
3	–	–	29.7 ± 3.4	14	2.8	6.7	72.6
4	–	–	2.0 ± 0.1	4	1.8	6.1	64.4
5	–	–	11.2 ± 3.9	4	2.8	7.5	72.6
2a	–	–	0.6 ± 0.3	5.5 μM	6.3	5.1	77.5
2b	–	–	3.9 ± 1.3	51	6.3	5.1	77.5
2c	OCF_3	Me	0.4 ± 0.1	10 μM	6.3	5.8	73.6
2d	CN	Me	4.6	15	6.3	4.2	88.1
2e	OCF_3	CF_3	2.6 ± 1.1	2.0	4.7	5.9	73.6
2f	CF_3	CF_3	2.9 ± 0.2	5.7	4.7	5.6	64.4
2g	CF_3	CN	2.7	2.5	3.4	4.5	88.1
2h	CF_3	Cl	1.5 ± 0.8	3	4.7	5.7	64.4
2i	CN	CF_3	8.2 ± 2.6	4	4.7	4.3	88.1
2j	Cl	CF_3	8.0 ± 0.4	5.0	4.7	5.4	64.4
2k	2-Furyl	CF_3	0.7 ± 0.2	2.3	4.7	5.2	77.5
2l	–	–	1.2 ± 0.9	7.0	ND	ND	ND

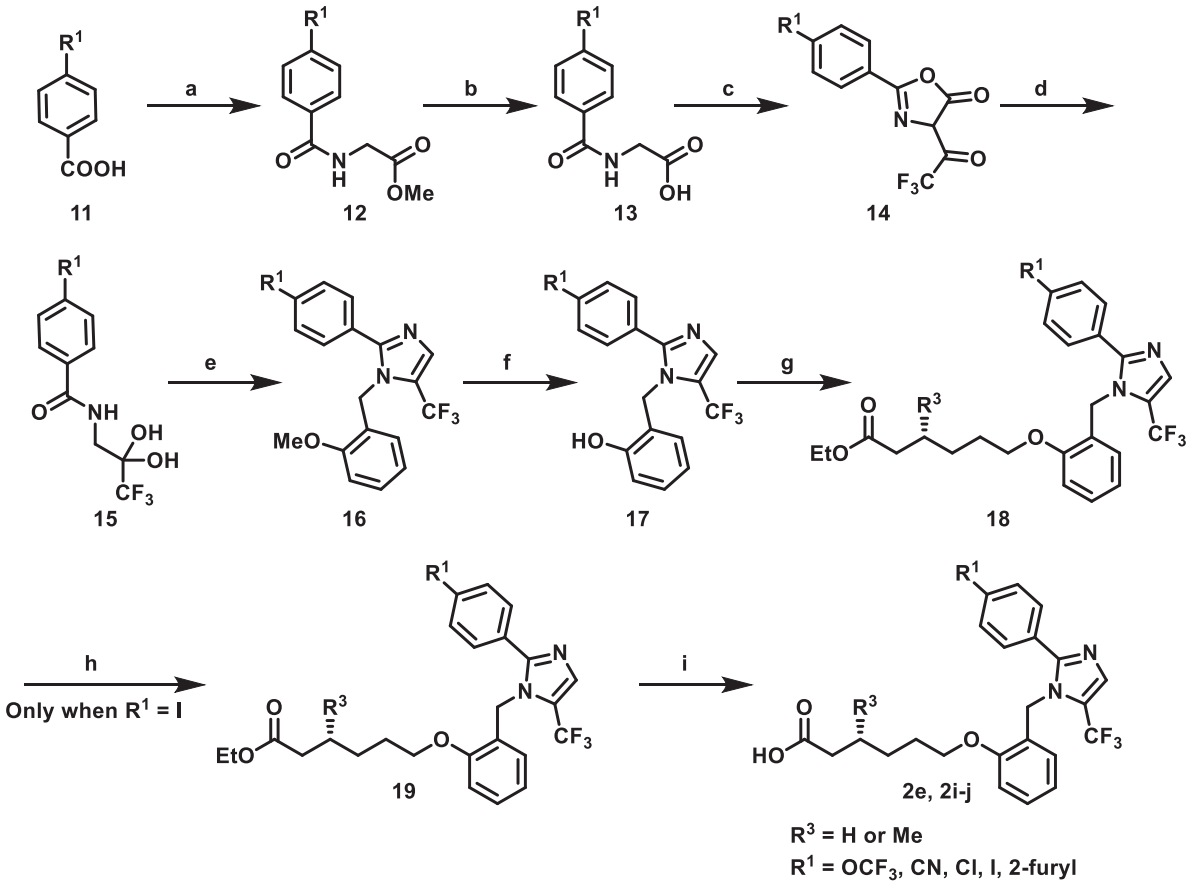
[a] Transactivation assay[24] EC_{50} values are an average of at least two experiments (SEM shown unless single determination). % Activation of compound at each concentration was calculated considering activity of GW501516 at 10 uM as 100%. The Emax was between 81 and 103% except for compound **3** (Emax = 61%). Please see reference 3 or WO2016057660 for the details of the assay system.

[b] See Ref. 17.

[c] For the calculation of cLogP, TPSA and pKa, commercially available ACD software was used.[18,21]; ND = Not Determined.



Scheme 1. Synthesis of Compounds **2a–d. Reagents and conditions:** a) Prop-2-yn-1-amine, EDCI.HCl, HOBt, Et_3N, DMF, RT, 12 h; b) 2-Methoxybenzyl amine, Zn $(OTf)_2$, toluene, 110 °C, 12 h; c) BBr_3, DCM, 0 °C-RT, 4 h; d) Ethyl (*3R*)-6-bromo-3-methylhexanoate or ethyl-6-bromohexanoate, K_2CO_3, DMF, RT, 12 h; e) $LiOH{\cdot}H_2O$, THF, EtOH, H_2O, RT, 12 h.



Scheme 2. Synthesis of Compounds **2e, 2i–j. Reagents and conditions:** a) Methyl glycinate hydrochloride, EDCI·HCl, HOBt, Et_3N, DMF, 12 h, RT; b) LiOH·H_2O, THF, EtOH, H_2O, RT, 12 h; c) 2,2,2-Trifluoroacetic anhydride, acetone, 0 °C-RT, 12 h; d) 1,4-Dioxane, H_2O, 100 °C, 3 h; e) 2-Methoxyl benzyl amine, AcOH, toluene, 120 °C, 12 h; f) BBr_3, DCM, −78 °C-RT; g) Ethyl 6-bromohexanoate, K_2CO_3, DMF, RT, 12 h; h) In the case where R^1 = I, furan-2-boronic acid, $Pd(PPh_3)_4$, Na_2CO_3, DME, EtOH, H_2O, 90 °C, 12 h; i) LiOH·H_2O, THF, EtOH, H_2O, RT, 12 h.

versus other heteroaromatic ring containing compounds (**3–5**) tracks the differences in the basicity of nitrogens (pKa = 6.3 versus 1.8–2.8). Compound **2b** where the basicity of nitrogen is similar to the nitrogen in **2a**, also has hERG activity (50% inhibition at 10 μM). With the increased basicity of nitrogen in the heteroaromatic ring, it is more likely that molecule exists in zwitterionic form, which could increase hERG activity. This may contradict some reports where zwitterionic character was introduced in the molecules as a strategy to decrease hERG activity including the well-known example of transforming terfenadine into zwitterionic fexofenadine with reduced hERG activity.[21] However, a few zwitterionic compounds that inhibit the hERG channel have been reported in literature.[22]

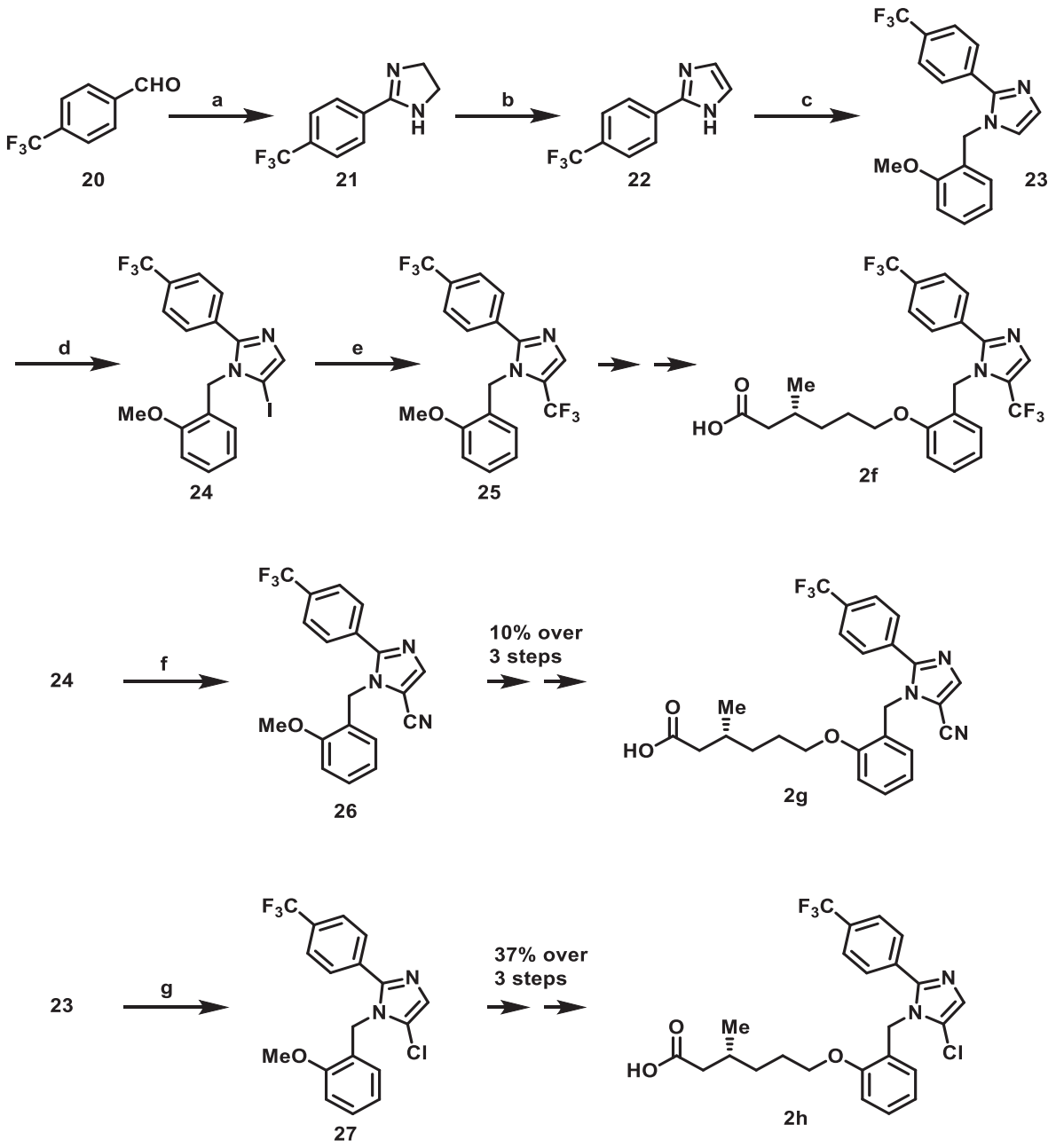
While reviewing the hERG activity of compounds, it's important to consider many points, for example, differences in the assays that were used measure hERG inhibition. The data shown here was generated using an automated patch clamp assay. For an accurate measurement of hERG activity, especially for compounds with low aqueous solubility, a manual patch clamp assay should be performed.[23] It is important to point out that most of the the imidazole compounds described in Table 1 exhibit low aqueous solubility (thermodynamic solubility < 25 μM). Compounds such as **2c** could be a viable clinical candidate because of the large window between the PPARδ potency (EC_{50} = 0.4 nM) and the observed hERG activity (IC_{50} = 10 μM, in the automated patch clamp assay).[3] Typically, cardiac safety for compounds that show hERG activity in the patch clamp assay is assessed in telemetrized animals after oral administration before a compound enters clinical trials in human. Therefore, it was necessary to detrmine oral bioavailability of these new compounds in addition to their profile for activating PPAR isoforms. Potency, selectivity and the pharmacokinetic (PK) profiles for **2f**, **2h** and **2i** are shown in Table 2.

All the compounds are potent PPARδ modulators (EC_{50} < 10 nM) and selective over PPARα and PPARγ receptors (EC_{50} > 100,000 nM)) in transactivation assays.[24] Compounds **2f**, **2h** and **2i** show good oral bioavailability (F = 70–100%) in mice, have low to moderate clearance (5–16 mL/min/kg) and reasonable elimination half-lives (3.5–4.1 h).

In summary, we have demonstrated that the hERG activity of the imidazole PPARδ modulators can be attenuated by tuning the basicity of the nitrogen in the imidazole ring. We effectively decreased the hERG activity of these potent PPARδ modulators while maintaining favorable selectivity over other PPAR receptors and oral bioavailability. This study serves as another reminder for medicinal chemists not to be overconfident that hERG activity can be ameliorated by simply introducing a carboxylic acid moiety in the structure.

Author contributions

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



Scheme 3. Synthesis of **2f, 2g** and **2h. Reagents and conditions:** a) Ethane-1,2-diamine, I_2, K_2CO_3, *t*-BuOH, 85 °C, 5 h, 85% yield; b) (Diacetoxyiodo)benzene, K_2CO_3, DMSO, RT, 12 h, 55% yield; c) 2-Methoxybenzyl bromide, NaH (60% dispersion), DMF 0 °C-RT, 4 h, 83% yield; d) NIS, DMF, 80 °C, 12 h, 36% yield; e) $TMSCF_3$, Ag_2CO_3, 1,10-phenanthroline, KF, CuI, DMF, 100 °C, 12 h, 59% yield; f) CuCN, $Pd(PPh_3)_4$, DMF, microwave, 150 °C, 2 h, 45% yield; g) NCS, CH_3CN, 70 °C, 12 h, 40% yield.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: The authors are current or former employees of Mitobridge, Inc., (an Astellas Pharma company) or Aurigene Discovery Technologies and may have or currently own shares of these companies.

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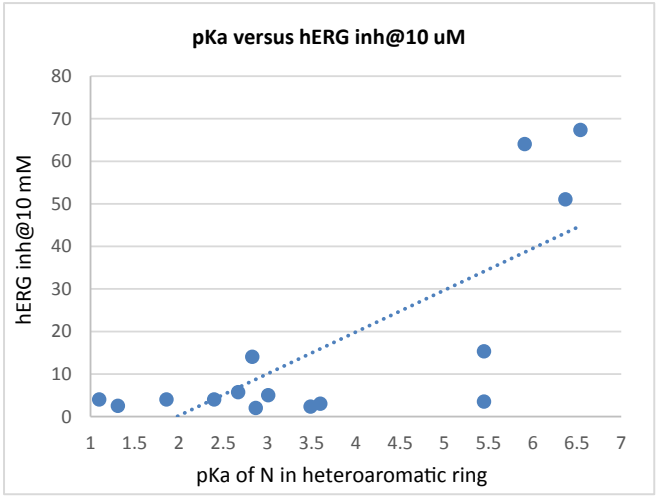

Fig. 3. Correlation between pKa and hERG activity for compounds in Table 1. The graph was generated in MS Excel. The dotted line represents a trendline generated by the software.

Table 2
PPAR isoform selectivity and mouse PK data for compounds **2f, 2h** and **2i.**

Compound	2f	2h	2i
EC_{50} PPARδ nM[a]	2.9 ± 0.2	1.5 ± 0.8	8.2 ± 2.6
EC_{50} PPARα nM[a]	> 100,000	> 100,000	> 100,000
EC_{50} PPARγ nM[a]	> 100,000	> 100,000	> 100,000
$AUC_{(0\text{-}inf)}$ (ng*h/mL) [b]	6960	3880	14,740
CL (mL/min/kg) [b]	5.0	16	2.5
$t_{1/2}$ (h)[b]	5.8	3.5	4.1
%F[b]	70	100	73

[a] Transactivation assay[24] EC_{50} values are an average of at least two experiments (SEM shown unless single determination).

[b] Exposure data for compounds dosed i.v. at 1 mg/kg and orally at 3 mg/kg in CD-1 mice.[25]

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17. Semi-automatic patch clamp method was used using Port-A- Patch (from Nanion technologies, Germany). HEK293 cells stably expressing hERG potassium channels. 0.1% DMSO in extracellular HEPES buffer was used as the vehicle. Assay conditions were room temperature and the compound addition was done manually. Aspirin at 10μM concentration was used as a negative control. Cisapride or verapamil at 1μM concentration were used as positive controls.
18. TPSA and cLogP were calculated using ACD/ToxSuite 2.95 ACD/Labs. (http://www.acdlabs.com/products/admet/tox).
19. The description of the synthesis and the spectroscopic data has been described in WO2017180818.
20. pKa were calculated using ACD/ToxSuite 2.95 ACD/Labs. Comparable pKa numbers were obtained using Marvin suite (https://chemaxon.com/products/marvin).
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24. In the transactivation assay CV-1 cells are transfected with a PPAR ligand binding domain fused to a GAL4 promoter to generate a hormone-inducible activator. A test ligand is added and activity is measured in a luciferase assay. See WO2016057660 for further details.
25. All the animal experiments were carried out as per the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India and approved by the Institutional Animal Ethics Committee (IAEC), Aurigene Discovery Technologies Ltd, Bengaluru, India.