

Investigation of Cardiovascular Effects of Tetrahydro- β -carboline sstr3 antagonists

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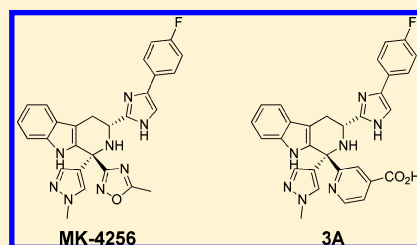
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S Supporting Information

ABSTRACT: Antagonism of somatostatin subtype receptor 3 (sstr3) has emerged as a potential treatment of Type 2 diabetes. Unfortunately, the development of our first preclinical candidate, MK-4256, was discontinued due to a dose-dependent QTc (QT interval corrected for heart rate) prolongation observed in a conscious cardiovascular (CV) dog model. As the fate of the entire program rested on resolving this issue, it was imperative to determine whether the observed QTc prolongation was associated with hERG channel (the protein encoded by the human Ether-à-go-go-Related Gene) binding or was mechanism-based as a result of antagonizing sstr3. We investigated a structural series containing carboxylic acids to reduce the putative hERG off-target activity. A key tool compound, 3A, was identified from this SAR effort. As a potent sstr3 antagonist, 3A was shown to reduce glucose excursion in a mouse oGTT assay. Consistent with its minimal hERG activity from in vitro assays, 3A elicited little to no effect in an anesthetized, vagus-intact CV dog model at high plasma drug levels. These results afforded the critical conclusion that sstr3 antagonism is not responsible for the QTc effects and therefore cleared a path for the program to progress.

KEYWORDS: sstr3, antagonist, Type-2 diabetes, β -tetrahydrocarboline, carboxylic acid, hERG channel, QTc prolongation, cardiovascular dog models



Somatostatin receptor 3 (sstr3) is a member of a group of five G-protein coupled somatostatin receptors (sstr1–sstr5).¹ Two different structural classes of selective small molecule sstr3 antagonists have been reported: imidazolyl-tetrahydro- β -carbolines derived from D-tryptophan (D-Trp) and substituted decahydroisoquinolines.^{2–6} Recently, we disclosed that antagonism of sstr3 represents a potential novel mechanism for the treatment of Type-2 diabetes mellitus (T2DM) through the evaluations of compound 1 in both in vitro assays and animal efficacy models (Figure 1).⁷ Subsequently, we reported that the optimization of this tetrahydro- β -carboline series led to the discovery of MK-4256 (Figure 1).⁸ MK-4256 possesses excellent sstr3 potency and subtype selectivity, a good pharmacokinetic (PK) profile in preclinical species, and superior efficacy in a mouse oGTT assay. Although, MK-4256 was shown to have high protein plasma binding with ~1% free fractions across several species (Table 1), only a minimal shift (~2X) was observed from in vitro assays with 20% human serum added (Figure 1).⁹ This lack of serum shift on sstr3 was attributed to a slow dissociation

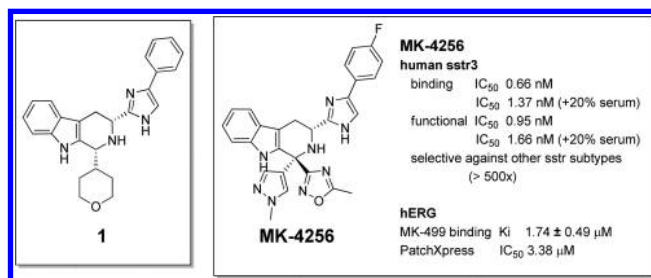


Figure 1. Tetrahydro- β -carboline sstr3 antagonists.

rate of MK-4256 from the receptor. More significantly, MK-4256 reduced glucose excursion by 86% in a mouse oGTT assay at a dose as low as 0.1 mg/kg with the maximal plasma concentration of 88 nM.⁸ On the other hand, MK-4256 had

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Table 1. In Vitro Plasma Protein Binding of MK-4256^a

MK-4256 plasma protein binding (%)				
human	monkey	dog	rat	mouse
99.2	99.4	99.2	97.4	98.2

^aDetermined by equilibrium dialysis.

modest activity on the hERG channel (the protein encoded by the human Ether-à-go-go-Related Gene), which raised some concerns from safety perspectives.^{10,11} MK-4256 inhibits radiolabeled MK-499 binding of the hERG channel with an $IC_{50} = 1.74 \mu M$.^{12,13} These results represent >2000-fold selectivity for sstr3 over hERG binding. In a functional patch clamp assay (PatchXpress), MK-4256 exhibited 50% blockade of hERG at $3.4 \mu M$ concentration.¹⁴ When MK-4256 was dosed in an anesthetized, vagotomized cardiovascular (CV) dog model, there were no cardiovascular findings at plasma levels up to $13.7 \mu M$.¹⁵ There seemed to be a shift in hERG activity of MK-4256 from in vitro assays to the CV dog models, probably due to its high plasma protein binding. Based on its highly attractive activity on sstr3 and sufficient margins expected against the hERG channel, MK-4256 was selected as a preclinical development candidate.

During the course of toxicological evaluation, MK-4256 exhibited a dose-dependent prolongation of the QTc interval (QT interval corrected for heart rate) in a conscious cardiovascular (CV) dog telemetry model, which is widely considered to be the “gold standard” for assessing the CV effects including QTc prolongation (Table 2).^{16,17} Increases in

Table 2. Effect of MK-4256 in CV Dog Models

MK-4256 Caused QTc Prolongation in a Conscious CV Dog Model		
oral dose of MK-4256 (mg/kg)	plasma C_{max} (μM) ^a	QTc increase
20	4.6	5%
100	32	10%
500	ND ^b	14%
Effect of MK-4256 in Anesthetized, Vagus-Intact CV Dog Model		
dosage of MK-4256 by iv infusion (mg/kg/30 min)	plasma C_{max} (μM) ^a	QTc increase
10	18	6%
20	45	8%
30	92	10%

^aPlasma drug levels were determined from separate PK studies in dogs. ^bNot determined.

the QTc interval of 5, 10, and 14% were noted after a single oral dosing of 20, 100, and 500 mg/kg (at plasma concentration of 4.6 and $32 \mu M$ for 20 and 100 mg/kg, respectively). Based on these observed CV adverse effects, further development of MK-4256 was discontinued.

The adverse finding of MK-4256 in a conscious CV dog telemetry model was an unexpected observation as a re-evaluation in anesthetized, vagotomized dogs at higher doses afforded no QTc interval increases after intravenous (iv) dosing at plasma concentrations up to $100 \mu M$. These results indicated that an anesthetized, vagotomized CV dog model may not be predictive. Subsequently, MK-4256 was tested in an anesthetized, vagus-intact CV dog model, since our studies with a few compounds well-documented for their QTc prolongation effects indicated there was a trend of higher sensitivity in the vagus-intact dogs to QTc prolongation.¹⁸

Indeed, in anesthetized, vagus-intact dogs, iv fusion of MK-4256 did result in a dose-dependent increased QTc interval (Table 2). This result suggested that anesthesia was not involved but that MK-4256 exerted these unwanted effects through an unknown mechanism involving vagal signaling. Nevertheless, since QTc prolongation was observed with MK-4256 in the anesthetized, vagus-intact dog model, this model was deemed to be more appropriate for assessing the CV effects of this series of compounds than the anesthetized, vagotomized dog model. Therefore, the anesthetized, vagus-intact dog model was subsequently adopted to screen later lead compounds prior to a more definitive evaluation in the more resource-intensive conscious CV dog telemetry model.

The observed QTc prolongation for MK-4256 cast a serious doubt on the entire sstr3 antagonist program. It was crucial to elucidate whether the QTc prolongation was caused by inhibition of the hERG channels (i.e., MK-4256 is deficient) or the adverse effect was due to the antagonism of sstr3. The latter scenario would lead to an immediate termination of the sstr3 program. It is well-known that somatostatin receptors are coupled to several types of potassium channels, including the delayed rectifier, inward rectifier and ATP-sensitive potassium channels.¹ It was reported that octreotide, a synthetic analogue of somatostatin, is able to improve and even normalize the QT prolongation frequently observed in acromegalic patients.¹⁹ Given that sstr agonism with octreotide was able to reduce QTc prolongation, an antagonist of sstr might have the potential to produce QTc prolongation. In order to define the path forward for sstr3 antagonists as a potential treatment for T2DM, it was critical to confirm that antagonism of sstr3 alone does not cause QTc prolongation. Therefore, further SAR exploration focused on identifying compounds with little or no inhibition of the hERG channel but still engaged sstr3.

It has been well documented in the literature that introduction of a carboxylic acid moiety into a structure can mitigate binding to the hERG channels.^{20–23} In these reported examples, some carboxylic acid compounds were able to maintain the activity on the biological targets while minimizing the interaction with the hERG channel.

Our initial introduction of a carboxylic acid group into the indole ring or the fluorophenyl imidazole moiety of the structure abolished the activity on sstr3. Our earlier experience indicated that the C-1 position of the β -carboline is flexible enough to accommodate different functional groups. Indeed, this area of the molecule tolerates an appended carboxylic acid as well as a variety of aryls and 5 or 6-membered heteroaryls. We decided that it was synthetically more expedient to focus on the compounds with a carboxylic acid group attached to a pyridyl ring at the C-1 position rather than oxadiazole-carboxylic acid analogues while keeping the *N*-methyl pyrazole moiety existing in the structure of MK-4256. Earlier SAR results indicated that phenyl derivatives at C-1 had significant problems with hERG activities. As the result, we prepared the pyridyl carboxylic acid compounds (**2A-5A** and **2B-5B**) from their corresponding ethyl ester precursors (**6A-9A** and **6B-9B**) (Figure 2). The A and B series differ by their configuration at the C-1 position.

The synthesis of the compounds followed the procedure reported previously (Scheme 1). The Pictet-Spengler reaction of fluorophenyl imidazole intermediate **10** with the corresponding ketones gave the product as a mixture of diastereomers, which were separated by chiral HPLC or SFC to give the ethyl esters as single compounds (**6A-9A** and **6B-9B**). Each ethyl

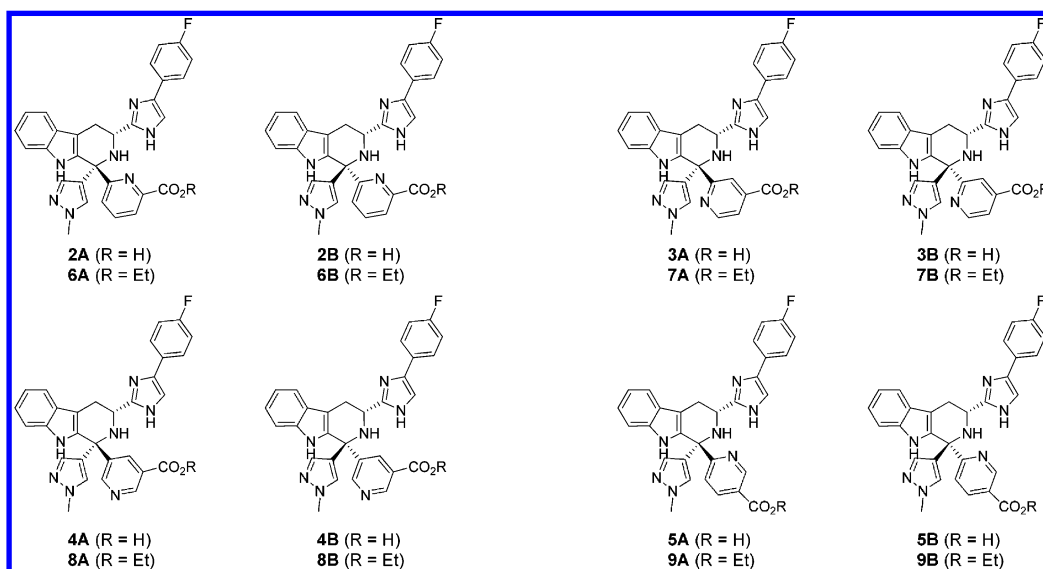
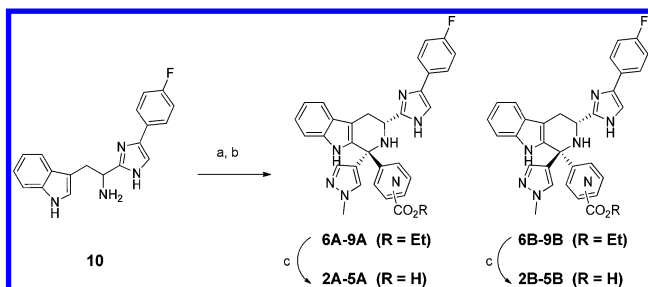


Figure 2. Imidazole tetrahydro- β -carboline incorporating a pyridyl carboxylic acid moiety.

Scheme 1. Synthesis of Acid and Ethyl Ester Compounds^a



^aExperimental details are included in the Supporting Information: a. Pictet-Spengler reaction with corresponding ketone; b. Separation of diastereomers; c. Hydrolysis of the ester group to a carboxylic acid group.

ester was then hydrolyzed to give the corresponding carboxylic acid compound (2A-5A and 2B-5B). The configuration at the C-3 position of β -carboline was set as R from the starting material **10**, which was derived from D-tryptophan. The configuration at the C-1 position relative to that of the C-3 position was established by NOE experiments. The compounds in the A series have the imidazole ring and the N-methyl pyrazole ring on the same face of the β -carboline core. The compounds in the B series have the opposite configuration at C-1 position of the β -carboline core.

The in vitro profiles of ethyl esters **6A-9A** and **6B-9B** are summarized in Table 3. In general, the compounds in the A series (**6A-9A**) are slightly more potent on sstr3 than their corresponding diastereomers in the B series (**6B-9B**) as measured in the competitive binding assay and the functional antagonism assay using recombinant human or mouse sstr3. Some compounds even achieve subnanomolar potencies (e.g., **7A**). However, all these ester compounds suffer from strong binding to the hERG channel as observed in the MK-499 binding assay, with the binding affinities comparable to that of MK-4256.

The profiles of the carboxylic acid compounds are summarized in Table 4. Following the same SAR trend as observed in the ethyl ester series, the carboxylic acid compounds in the A series (**2A-5A**) are more potent than their corresponding diastereomers **2B-5B**. However, the carboxylic acid compounds are all less potent than their corresponding ethyl ester precursors. It appears that sstr3 is less tolerant of a carboxylic acid group compared with neutral moieties such as an ethyl ester group. Fortunately, compounds **2A**, **3A** and **4A** still maintain single digit nanomolar potencies in the human sstr3 binding assay. Compound **5A** is much less potent than **2A**, **3A** and **4A**, probably due to the unfavorable positioning of the carboxylic acid group para to the β -carboline core in contrast to **2A-4A**, all of which have a carboxylic group meta to the β -carboline core. Compared with ethyl ester

Table 3. Profiles of Ethyl Ester Compounds^{a,b}

compd no.	human sstr3 binding IC ₅₀ , nM	human sstr3 functional cAMP antagonism IC ₅₀ , nM (% inh.)	mouse sstr3 binding IC ₅₀ , nM	mouse sstr3 functional cAMP antagonism IC ₅₀ , nM (% inh.)	MK-499 binding assay, IC ₅₀ , nM
6A	0.83 ± 0.19 (n = 6)	1.97 ± 0.94 (n = 3) (81%)	0.41 ± 0.00 (n = 2)	0.58 ± 0.23 (n = 2) (80%)	707 ± 27 (n = 2)
6B	1.52 ± 0.74 (n = 6)	5.36 ± 3.05 (n = 4) (81%)	0.70 ± 0.06 (n = 2)	3.48 ± 0.57 (n = 2) (87%)	625 (n = 1)
7A	0.64 ± 0.30 (n = 4)	0.78 ± 0.11 (n = 3) (101%)	0.41 ± 0.08 (n = 4)	0.36 ± 0.25 (n = 4) (91%)	1420 (n = 1)
7B	4.87 ± 0.36 (n = 2)	47.1 ± 11.1 (n = 2) (98%)	ND ^c	14.7 (n = 1) (85%)	1205 (n = 1)
8A	0.78 ± 0.00 (n = 2)	0.15 ± 0.01 (n = 2) (85%)	ND ^c	0.35 ± 0.09 (n = 2) (99%)	2257 (n = 1)
8B	3.36 ± 0.18 (n = 4)	24.0 ± 16.0 (n = 4) (134%)	ND ^c	8.37 ± 3.74 (n = 4) (107%)	2520 ± 843 (n = 2)
9A	1.72 ± 0.19 (n = 2)	5.25 ± 2.57 (n = 4) (91%)	ND ^c	1.53 ± 0.22 (n = 3) (96%)	1611 (n = 1)
9B	2.91 ± 0.40 (n = 2)	31.6 ± 18.1 (n = 4) (65%)	ND ^c	14.2 ± 2.7 (n = 3) (66%)	2845 (n = 1)

^aAssay protocols are provided in the Supporting Information. ^bThe standard deviations are included as well as the numbers of repeats. ^cNot determined.

Table 4. sstr3 in Vitro Activity of Carboxylic Acid Compounds^{a,b}

compd no.	human sstr3 binding IC ₅₀ , nM	human sstr3 functional cAMP antagonism IC ₅₀ , nM (% inh.)	mouse sstr3 binding IC ₅₀ , nM	mouse sstr3 functional cAMP antagonism IC ₅₀ , nM (% inh.)
2A	4.41 ± 1.04 (n = 8)	67.0 ± 69.0 (n = 8) (129%)	3.93 ± 0.75 (n = 2)	34.0 ± 11.2 (n = 11) (101%)
2B	74.7 ± 13.1 (n = 4)	105 ± 106 (n = 3) (83%)	ND ^c	111 ± 25 (n = 3) (69%)
3A	2.13 ± 0.37 (n = 8)	1.71 ± 0.73 (n = 4) (101%)	1.72 ± 0.34 (n = 4)	0.73 ± 0.34 (n = 3) (81%)
3B	72.7 ± 1.5 (n = 2)	52.3 ± 9.5 (n = 2) (98%)	ND ^c	17.1 (n = 1) (70%)
4A	1.22 ± 0.17 (n = 4)	0.52 ± 0.28 (n = 4) (88%)	ND ^c	0.16 ± 0.03 (n = 3) (67%)
4B	10.8 ± 1.1 (n = 2)	6.12 ± 2.17 (n = 2) (117%)	ND ^c	2.90 ± 1.71 (n = 2) (80%)
5A	23.7 ± 6.8 (n = 2)	87.2 ± 16.5 (n = 2) (88%)	ND ^c	57.1 ± 34.2 (n = 4) (87%)
5B	409 ± 21 (n = 2)	>2000 (n = 2) (27%)	ND ^c	1863 ± 58 (n = 2) (51%)

^aAssay protocols are provided in the Supporting Information. ^bThe standard deviations are included as well as the numbers of repeats.

^cNot determined.

analogues as well as MK-4256, carboxylic acid compounds **2A**, **3A** and **4A** all have a much reduced binding to hERG as observed in the MK-499 binding assay (Table 5).

Table 5. Activities of Selected Acid Compounds on the hERG Channel^{a,b}

compd no.	MK-499 binding assay, IC ₅₀ , nM	PatchXpress, hERG
2A	8703 ± 504 (n = 2)	39% inh. at 10 μM
3A	19560 ± 2913 (n = 2)	26% inh. at 30 μM
4A	11990 ± 3877 (n = 2)	70% inh. at 30 μM (IC ₅₀ = 12 μM)

^aThe standard deviations are included as well as the numbers of repeats. ^bPatchXpress assay (n = 1) is sufficient to differentiate **3A** from **2A** and **4A**.

Compound **2A**, the first carboxylic acid compound prepared in this series, had good potency in human sstr3 binding assay and some potency erosion in the human sstr3 functional assay. With MK-499 binding K_i of 8526 nM and 39% blockade of hERG at 10 μM (the highest concentration tested) in the PatchXpress assay, compound **2A** clearly demonstrated an improvement over MK-4256 (Table 5). Compound **2A** was evaluated for PK in mice. It demonstrated moderate clearance (15.6 mL min⁻¹kg⁻¹) and a reasonable half-life (2.62 h). However, it had very low oral bioavailability (1.5%).

Compound **4A** exhibited the best potency on sstr3 among all of the acid compounds prepared according to in vitro sstr3 binding and functional assays (Table 4). However, it showed ~12 μM binding in the MK-499 binding assay (Table 5). The functional blockade of the hERG channel was confirmed in the PatchXpress assay (IC₅₀ 12 μM). Compound **4A** represented some improvement over MK-4256 in term of the hERG profile.

Compound **3A** had the best overall profile. It is potent in binding and functional assays for human as well as mouse sstr3. Furthermore, **3A** is highly selective for sstr3 against other sstr subtypes with human binding IC₅₀ higher than 10 μM on sstr1, sstr2, sstr4, and sstr5, respectively (>4000x). More importantly,

compound **3A** has little interaction with the hERG channel. It has a weak binding to the hERG channel according to MK-499 binding assay. In the functional PatchXpress assay, it has minimal inhibition (26%) of hERG at the maximal dose tested (30 μM).

Due to its favorable overall profile, compound **3A** was evaluated in mouse oGTT assay to help confirm target engagement with sstr3. When acid **3A** was dosed orally at 3 and 10 mg/kg in mice, the reduction of glucose excursion was minimal (24% and 35%) compared to MK-4256, the positive control, which reduced glucose excursion by 63%. The poor efficacy was likely due to the poor oral bioavailability for **3A** in mice since the plasma drug levels of **3A** at 2.5 h post dosing were only 2 nM and 3 nM for 3 mg/kg and 10 mg/kg, respectively.²⁴ To circumvent the poor oral bioavailability, **3A** was dosed intraperitoneally (i.p.) to improve its drug exposure. When compound **3A** was dosed i.p. at 1 mg/kg, the reduction of glucose excursion was 64%, comparable to the positive control MK-4256, which reduced the glucose excursion by 74%. The plasma drug level of **3A** at 2.5 h post i.p. dosing was 74 nM, which is much higher than the drug exposures achieved after oral dosings. Given the good oGTT efficacy established together with excellent in vitro potencies in binding and functional assays, we were confident that with sufficient plasma concentrations, **3A** is capable of engaging the sstr3 receptor.

After confirming the in vivo target engagement of **3A** on sstr3, we evaluated **3A** in the anesthetized, vagus-intact CV dog model (Figure 3). Compound **3A** was intravenously infused

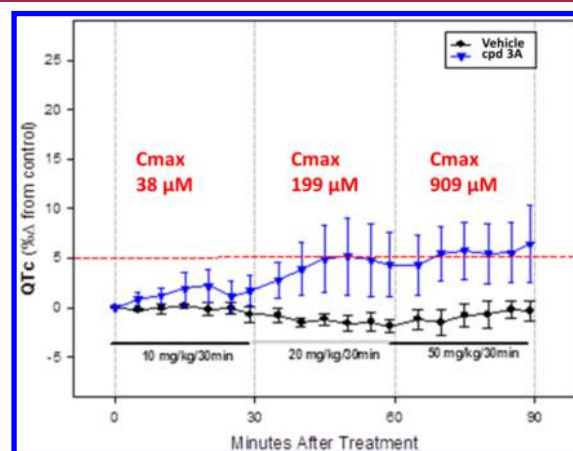


Figure 3. Effect of **3A** on QTc in an anesthetized, vagus-intact CV dog model.

continuously in dogs at increasing doses of 10, 20, and 50 mg/kg over three 30 min intervals. High plasma concentrations of **3A** were detected (38 uM, 199 uM and 909 uM, respectively). At C_{max} = 38 uM, compound **3A** did not cause QTc prolongation. At C_{max} of 199 uM, the QTc prolongation did not exceed 5% cutoff. At plasma concentration as high as 909 uM, QTc prolongation barely exceeded the 5% cutoff. In contrast to the results for MK-4256, which showed dose-dependent increases in QTc, we concluded compound **3A** caused little or no QTc prolongation at high plasma drug levels in this CV dog model.

Since MK-4256 is highly plasma protein bound (>99% in dog and human plasma), a significant serum shift on the hERG channel might be expected. However, QTc prolongation was observed with plasma concentrations in the CV dog model near

the IC_{50} obtained from in vitro assays on hERG channel. There was no observed serum shift. A thallium flux assay was used to assess the effect of added serum to the response of the hERG channel.²⁵ Table 6 shows the effect of added dog serum to the

Table 6. Effect of Added Dog Serum to the Blockade of hERG Function by MK-4256

conc of MK-4256 (μ M)	% blockade of hERG	
	0% added dog serum	50% added dog serum
3	37 \pm 10	32 \pm 8
10	61 \pm 9	62 \pm 8

ability of MK-4256 to block the response of the hERG channel. Despite being highly protein bound (dog F_u = 0.8%), MK-4256 exhibited no serum shift. This is consistent with the observed QTc increase in dogs which occurred at a plasma concentration (4.6 μ M) very near the IC_{50} in the MK-499 inhibition assay (1.7 μ M) and the patch clamp assay (3.4 μ M). Despite the lack of an expected serum shift, these results would still seem to intimate a role for the hERG channel in the observed QTc prolongation. The underlying cause(s) for a lack of a serum shift on the hERG channel remain unresolved.

In summary, we have investigated the cause of an adverse CV effect observed for MK-4256 in the CV dog model. The observed QTc prolongation with MK-4256 was dependent upon the "vagal" status of the animals: no QTc interval increase in vagotomized dogs but an observed increase in vagus-intact animals. A carboxylic acid moiety was introduced into the structure to mitigate the off-target interaction with the hERG channel. From this effort, we identified a tool compound, **3A**, which has excellent potency in sstr3 binding and functional assays. Compound **3A** demonstrated effective target engagement by suppressing glucose excursion in oGTT in mice. More significantly, compound **3A** demonstrated little to no effect in anesthetized, vagus-intact CV dog models. In contrast, MK-4256 caused a dose-dependent increase in QTc in the same model. This investigation demonstrated the QTc prolongation of MK-4256 was due to the blockade of the hERG channel and was not due to antagonism of sstr3. During this investigation, we also found that, for this class of compounds, the anesthetized, vagus-intact CV dog models appeared to be more appropriate than the anesthetized, vagotomized CV dog model for the evaluation of QTc prolongation. Compound **3A** could not be progressed due to its suboptimal PK profile in preclinical species. However, this tool compound cleared a path forward for the discovery of sstr3 antagonist devoid of the CV liability of MK-4256. Additional efforts in discovering non-carboxylic acid compounds with minimal hERG activity will be disclosed separately.²⁶

■ ASSOCIATED CONTENT

● Supporting Information

Syntheses and characterization data for compounds **2A**–**9A** and **2B**–**9B** and biological assay protocols. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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■ REFERENCES

- (1) For an excellent review on somatostatin and somatostatin receptors, see: Kumar, U.; Grant, M. Somatostatin and Somatostatin Receptors. *Results and Probl. Cell Differ* **2010**, *50*, 137–184.
- (2) Poitout, L.; Roubert, P.; Contour-Galceran, M.; Moinet, C.; Lannoy, J.; Pommier, J.; Plas, P.; Bigg, D.; Thuriel, C. Identification of Potent Non-Peptide Somatostatin Antagonists with sst3 Selectivity. *J. Med. Chem.* **2001**, *44*, 2990.
- (3) Thuriel, C.; Poitout, L.; Galceran, M.; Moinet, C.; Gordon, T. D.; Morgan, B. A.; Bigg, D.; Pommier, J. β -Carboline Compounds. WO 1999/64420, December 16, 1999.
- (4) Troxler, T.; Hurth, K.; Hoyer, D. Beta-Carboline Derivatives and Its Pharmaceutical Use Against Depression and Anxiety. WO 2002/081471, April 2, 2002.
- (5) Troxler, T.; Hurth, K.; Schuh, K.; Schoeffter, P.; Langenegger, D.; Enz, A.; Hoyer, D. Decahydroisoquinoline derivatives as novel non-peptidic, potent and subtype-selective somatostatin sst3 receptor antagonists. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 1728–1734.
- (6) Baenziger, M.; Cercus, J.; Hirt, H.; Laumen, K.; Malan, C.; Spindler, F.; Struber, F.; Troxler, T. The development of a practical synthesis of the potent and selective somatostatin sst3 receptor antagonist [4-(3,4-difluoro-phenyl)-piperazine-1-yl]-{[(4S,4aS,8aR)-2[(S)-3-(6-methoxy-pyridin-3-yl)-2-methyl-propyl]-decahydroisoquinoline-4-yl]-methanone (NVP-ACQ090)}. *Tetrahedron: Asymmetry* **2003**, *14*, 3469–3477.
- (7) Pasternak, A.; Feng, Z.; deJesus, R.; Ye, Z.; He, S.; Dobbelaar, P. H.; Bradley, S. A.; Chicchi, G. G.; Tsao, K.; Trusca, D.; Eiermann, G. J.; Feng, Y.; Wu, M.; Shao, Q.; Zhang, B.; Nargund, R. P.; Mills, S. G.; Howard, A. D.; Yang, L.; Zhou, Y. Stimulation of glucose-dependent insulin secretion by a potent, selective sstr3 antagonist. *ACS Med. Chem. Lett.* **2012**, *3*, 289–293.
- (8) He, S.; Ye, Z.; Truong, Q.; Shah, S.; Du, W.; Guo, L.; Dobbelaar, P. H.; Lai, Z.; Liu, J.; Jian, T.; Qi, H.; Bakshi, R. K.; Hong, Q.; Dellureficio, J.; Pasternak, A.; Feng, Z.; deJesus, R.; Yang, L.; Reibarkh, M.; Bradley, S. A.; Holmes, M. A.; Ball, R. G.; Ruck, R. T.; Huffman, M. A.; Wong, F.; Samuel, K.; Reddy, V. B.; Mitelman, S.; Tong, S. X.; Chicchi, G. G.; Tsao, K.-L.; Trusca, D.; Wu, M.; Shao, Q.; Trujillo, M. E.; Eiermann, G. J.; Li, C.; Zhang, B. B.; Howard, A. D.; Zhou, Y.-P.; Nargund, R. P.; Hagmann, W. K. The Discovery of MK-4256, a Potent SSTR3 Antagonist as a Potential Treatment of Type 2 Diabetes. *ACS Med. Chem. Lett.* **2012**, *3*, 484–489.
- (9) For a reference describing the measurement of plasma protein binding by equilibrium dialysis, see: Waters, N. J.; Jones, R.; Williams, G.; Sohal, B. Validation of a rapid equilibrium dialysis approach for the measurement of plasma protein binding. *J. Pharm. Sci.* **2008**, *97*, 4586–4595.
- (10) Rampe, D.; Brown, A. M. A history of the role of the hERG channel in cardiac risk assessment. *J. Pharmacol. Toxicol. Methods* **2013**, *68*, 13–22.
- (11) Jamieson, C.; Moir, E. M.; Rankovic, Z.; Wishart, G. Medicinal chemistry of hERG optimizations: Highlights and hang-ups. *J. Med. Chem.* **2006**, *49*, 5029–5046.
- (12) Radiolabeled MK-499 has been used in a hERG (potassium ion channel Kv11.1) binding assay to provide an initial screen of a compound's potential to interact with hERG. Raab, C. E.; Butcher, J. W.; Connolly, T. M.; Karczewski, J.; Yu, N. X.; Staskiewicz, S. J.; Liverton, N.; Dean, D. C.; Melillo, D. G. Synthesis of the first sulfur-35-labeled hERG radioligand. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1692–1695.

(13) For a description of the protocol of MK-499 binding assay, see: Wang, J.; Della Penna, K.; Wang, H.; Karczewski, J.; Connolly, T. M.; Koblan, K. S.; Bennett, P. B.; Salata, J. J. Functional and pharmacological properties of canine ERG potassium channels. *Am. J. Physiol. Heart Circ. Physiol.* **2003**, *284*, H256–H267.

(14) For a reference of an automated patch-clamp hERG screening method (PatchXpress assay), see: Zeng, H.; Penniman, J. R.; Kinose, F.; Kim, D.; Trepakova, E. S.; Malik, M. G.; Dech, S. J.; Balasubramanian, B.; Salata, J. J. Improved Throughput of PatchXpress hERG Assay Using Intracellular Potassium Fluoride. *Assay Drug Development Technol.* **2008**, *6*, 235–241.

(15) For a description of the anesthetized CV dog model, see: Tashibu, H.; Miyazaki, H.; Aoki, K.; Akie, Y.; Yamamoto, K. QT PRODACT: in vivo QT assay in anesthetized dog for detecting the potential for QT interval prolongation by human pharmaceuticals. *J. Pharmacol. Sci. (Tokyo, Japan)* **2005**, *99*, 473–486.

(16) For a reference on the conscious CV dog telemetry model, see: Chaves, A. A.; Zingaro, G. J.; Yordy, M. A.; Bustard, K. A.; O'Sullivan, S.; Galijatovic-Idrizbegovic, A.; Schuck, H.; Christian, D. B.; Hoe, C. M.; Briscoe, R. J. A highly sensitive canine telemetry model for detection of QT interval prolongation: Studies with moxifloxacin, haloperidol and MK-499. *J. Pharmacol. Toxicol. Methods* **2007**, *56*, 103–114.

(17) For a reference on the conscious CV dog telemetry model, see: Toyoshima, S.; Kanno, A.; Kitayama, T.; Sekiya, K.; Nakai, K.; Haruna, M.; Mino, T.; Miyazaki, H.; Yano, K.; Yamamoto, K. QT PRODACT: in vivo QT assay in the conscious dog for assessing the potential for QT interval prolongation by human pharmaceuticals. *J. Pharmacol. Sci. (Tokyo, Japan)* **2005**, *99*, 459–471.

(18) Woltmann, R.; Morissette, P. Effect of vagotomy on the cardiovascular function in pentobarbital anesthetized dogs. *J. Pharmacol. Toxicol. Methods* **2009**, *60*, 210–258, DOI: 10.1016/j.vascn.2009.04.026.

(19) Fatti, L. M.; Scacchi, M.; Lavezzi, E.; Giraldi, F. P.; De Martin, M.; Toja, P.; Michailidis, G.; Stramba-Badiale, M.; Cavagnini, F. Effects of treatment with somatostatin analogues on QT interval duration in acromegalic patients. *Clinical Endocrinol.* **2006**, *65*, 626–630.

(20) Zhu, B.-Y.; Jia, Z. J.; Zhang, P.; Su, T.; Huang, W.; Goldman, E.; Tumas, D.; Kadambi, V.; Eddy, P.; Sinha, U.; Scarborough, R. M.; Song, Y. Inhibitory effect of carboxylic acid group on hERG binding. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5507–5512.

(21) Aslanian, R.; Piwinski, J. J.; Zhu, X.; Priestley, T.; Sorota, S.; Du, X.-Y.; Zhang, X.-S.; McLeod, R. L.; West, R. E.; Williams, S. M.; Hey, J. A. Structural determinants for histamine H1 affinity, hERG affinity and QTc prolongation in a series of terfenadine analogs. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5043–5047.

(22) Meyers, M. J.; Arhancet, G. B.; Hockerman, S. L.; Chen, X.; Long, S. A.; Mahoney, M. W.; Rico, J. R.; Garland, D. J.; Blinn, J. R.; Collins, J. T.; Yang, S.; Huang, H.-C.; McGee, K. F.; Wendling, J. M.; Dietz, J. D.; Payne, M. A.; Homer, B. L.; Heron, M. I.; Reitz, D. B.; Hu, X. Discovery of (3S,3aR)-2-(3-Chloro-4-cyanophenyl)-3-cyclopentyl-3,3a,4,5-tetrahydro 2H-benzo[g]indazole-7-carboxylic Acid (PF-3882845), an Orally Efficacious Mineralocorticoid Receptor (MR) Antagonist for Hypertension and Nephropathy. *J. Med. Chem.* **2010**, *53*, 5979–6002.

(23) Mihalic, J. T.; Fan, P.; Chen, X.; Chen, X.; Fu, Y.; Motani, A.; Liang, L.; Lindstrom, M.; Tang, L.; Chen, J.-L.; Jaen, J.; Dai, K.; Li, L. Discovery of a novel melanin concentrating hormone receptor 1 (MCHR1) antagonist with reduced hERG inhibition. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 3781–3785.

(24) The PK profile of compound **3A** in rats was also not optimal with a high clearance ($119 \text{ mL min}^{-1} \text{ kg}^{-1}$).

(25) For a description of thallium flux assay, see: Schmalhofer, W. A.; Swensen, A. M.; Thomas, B. S.; Felix, J. P.; Haedo, R. J.; Solly, K.; Kiss, L.; Kaczorowski, G. J.; Garcia, M. L. A Pharmacologically Validated, High-Capacity, Functional Thallium Flux Assay for the Human Ether-à-go-go Related Gene Potassium Channel. *Assay Drug Development Technol.* **2010**, *8*, 714–726.

(26) Shah S. K.; He, S.; Guo, L.; Truong Q.; Qi H.; Wu, D.; Lai, Z.; Liu, J.; Jian, T.; Hong, Q.; Samuel, K.; Madiera, M.; Reddy, V. B.; Mitelman, S.; Tong, S. X.; Chicchi, G. G.; Tsao, K.-L.; Trusca, D.; Wu, M.; Shao, Q.; Trujillo, M. E.; Eiermann, G. J.; Li, C.; Pachanski, M.; Fernandez, G.; Nelson, D.; Bunting, P.; Morissette, P.; Volksdorf, S.; Kerr, K.; Zhang, B.; Howard, A. D.; Zhou, Y.-P.; Nargund, R. P.; Hagmann, W. K. The Discovery of MK-1421 as a Potential Treatment for Type 2 Diabetes: sstr3 Antagonists with Reduced QTc Prolongation. Manuscript in preparation.

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