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Discovery of *Omecamtiv Mecarbil* the First, Selective, Small Molecule Activator of Cardiac Myosin

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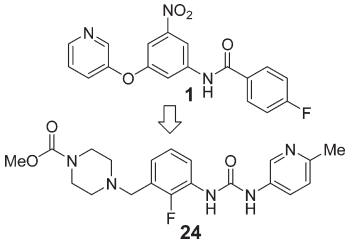
ABSTRACT We report the design, synthesis, and optimization of the first, selective activators of cardiac myosin. Starting with a poorly soluble, nitroaromatic hit compound (**1**), potent, selective, and soluble myosin activators were designed culminating in the discovery of *omecamtiv mecarbil* (**24**). Compound **24** is currently in clinical trials for the treatment of systolic heart failure.

KEYWORDS *Omecamtiv mecarbil*, small molecule enzyme activator, cardiac myosin, systolic heart failure



A strategy to treat systolic heart failure (HF) is to increase cardiac contractility. Current drugs, such as dobutamine, milrinone, and digoxin, activate second messenger pathways that raise cardiac myocyte calcium concentrations and subsequently increase cardiac contractility. Unfortunately, these drugs also increase heart rate and myocardial oxygen consumption and can produce clinically significant arrhythmias and hypotension, and their use is associated with higher mortality.[1,2]

Our approach is to selectively increase cardiac contractility by exploiting a novel mechanism of action that directly targets cardiac myosin, acting downstream of second messenger activation and calcium regulation.[3] Direct activation of myosin, the molecular motor that drives muscle contraction, may avoid the mechanism-related toxicities of current inotropes and offer a novel and potentially useful treatment for HF.[4] This report describes the discovery and optimization program that led to selective activators of the cardiac sarcomere culminating in the discovery of *omecamtiv mecarbil* (**24**), a compound with the potential to treat systolic HF in an acute situation through *iv* administration and acute and chronic systolic HF through oral administration.

In the cardiac sarcomere, force generation is directly coupled to adenosine triphosphate (ATP) hydrolysis. Compounds that activate the cardiac sarcomere were identified by measuring increases in myosin ATPase activity in a high-throughput, fully soluble, calcium-responsive, reconstituted sarcomere or myofibril assay.[5−7] Data were reported as an AC_{40}, the compound concentration resulting in a 40% increase in the cardiac sarcomere ATPase activity at the calcium concentrations that produced 25−50% of maximum calcium-dependent activation.[8]

A high-throughput screen (HTS) identified compound **1** as a sarcomere activator having an AC_{40} value in the low micromolar range. There were several interesting biological characteristics of **1** that made this series of compounds worthy of pursuit. First, **1** demonstrated excellent selectivity for the cardiac sarcomere over the skeletal sarcomere and smooth muscle myosin.[9] Second, **1** demonstrated increased contractility in freshly isolated, adult rat myocytes as measured by an increase in fractional shortening (FS).[10] Importantly, no effects on the myocyte calcium transient were observed. The degree of FS observed (ΔFS % = 135% at 2 μM) was substantial and was believed to be of sufficient potency to elicit a biological response in a whole animal at therapeutically relevant doses. However, the poor physical properties of **1** did not allow this hypothesis to be tested *in vivo*.

Although compound **1** was viewed as a promising lead based on its interesting biological properties, **1** and various analogs presented several formidable challenges to obtain agents suitable for both acute *iv* administration and chronic oral therapy. These challenges included an undesired structural moiety (nitro group), hydrolytic instability of the diaryl amide, poor aqueous solubility, low free fraction, modest intrinsic potency, off-target activities such as direct vasodilation through K_{ATP} channel activity[11] and CYP 1A2 inhibition, and high intrinsic clearance. Our goal was to address these challenges systematically while maintaining the favorable properties for compound **1** such as selectivity for cardiac

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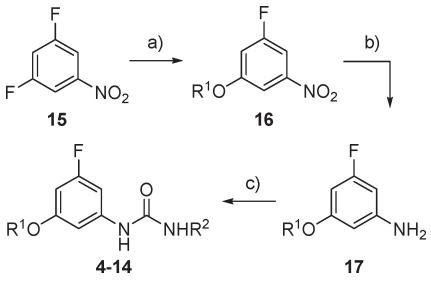
Scheme 1. General Synthesis of Aryl Ether Ureas[a]



[a] Reagents and conditions: (a) NaH or K_2CO_3, R^1OH, DMF, room temperature−70 °C, 30−85%. (b) H_2, Pd/C, MeOH, room temperature; or Pd/C, NH_4CO_3, MeOH, reflux or $SnCl_2$, EtOH, 50 → 95%. (c) Triphosgene, DIPEA, R^2NH_2, THF or CH_2Cl_2, room temperature, 60−85%.

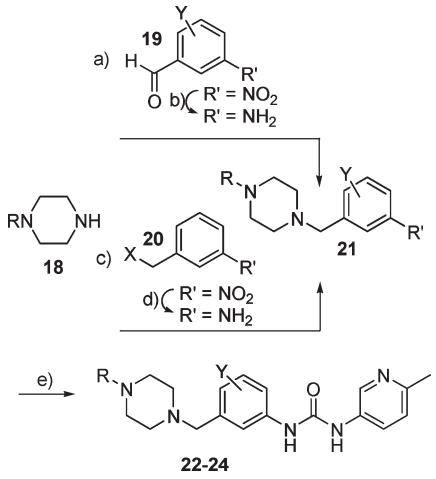
myosin over other type II myosins, lack of effect on the calcium transient or other off-target cellular effects, lack of additional off-target effects as measured by general screening,[12] and good permeability.

To achieve this goal, we designed flexible synthetic routes to allow variation at each part of the molecule as shown in Schemes 1 and 2. Nucleophilic aromatic substitution of nitroarene **15** (Scheme 1) with the appropriate deprotonated hydroxypyridine or amino alcohol provided nitro-substituted ether **16**.[13] The nitro group was reduced to aniline **17** using standard protocols (e.g., H_2, Pd/C, or Pd/C, NH_4CO_3, or $SnCl_2$). In situ generation of the isocyanate of R^2NH_2 using 0.4 equiv of triphosgene and 2.1 equiv of diisopropylethylamine (DIPEA),[14] followed by reaction with a substituted amino pyridine, afforded desired analogues **4**−**14**. Reductive amination[15] of **18** with the appropriately substituted benzaldehyde **19** (Scheme 2) yielded desired benzyl piperazines **21** (after reduction of the nitro moiety for R′ = NO_2). Alternatively, **21** was attained by alkylation of piperazine **18** with the benzyl bromide or mesylate of the nitroaromatic compound **20** in the presence of DIPEA as base followed by reduction of the nitro group as described above. Urea formation as described above or by direct addition of the isocyanatopyridine to **21** furnished the desired ureas **22**−**24** in good yield.

The first level of optimization served to remove the undesirable nitro group of **1** (Table 1). A first principle driving this structure−activity relationship (SAR) was to maintain an electron-withdrawing group at this position to reduce the potential for oxidative metabolism of the pendant aromatic ring. A fluorine atom replacement (**2**) maintained the biochemical activity of the parent nitro compound. Hydrogen (**3**) substitution dramatically attenuated the activity.

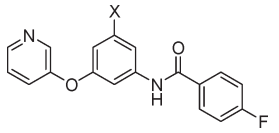
The next level of optimization addressed the low hydrolytic stability of the diaryl amide moiety and the poor aqueous solubility. Substitution of the amide of **2** with a more hydrolytically stable urea moiety[16] (**4**) maintained biochemical potency and gave measurable improvements in cardiomyocte activity (Table 2). Substitution of the 4-fluoro moiety on the terminal phenyl ring was tolerated as exemplified by compounds **4** and **5**; however, the compounds had poor

Scheme 2. General Synthesis of Benzylpiperazine Ureas[a]



[a] Reagents and conditions: X = Br or OMs; Y = F or H; R = methoxycarbonyl or *t*-butoxycarbonyl (*t*-Boc). When *t*-Boc was used as a protecting group, it was converted to the methyl carbamate in the following manner: (1) Trifluoroacetic acid, CH_2Cl_2, room temperature. (2) Methyl chloroformate, DIPEA, THF or CH_2Cl_2, 0 °C. (a) $NaHB(OAc)_3$, CH_2Cl_2, room temperature. (b) H_2, Pd/C, MeOH, room temperature. (c) DIPEA, CH_2Cl_2, room temperature, 85%. (d) H_2, Pd/C, K_2CO_3, MeOH, room temperature, 80%. (e) Triphosgene, DIPEA, 2-methyl-5-aminopyridine, THF or CH_2Cl_2, room temperature; or 2-methyl-5-isocyanatopyridine, acetone, room temperature, 65−85%.

Table 1. HTS Hit (**1**) and Replacement of the Nitro Moiety



no.	X	AC_{40} (μM)	kinetic solubility (μg/mL)
1	$-NO_2$	1.4	< 1.0
2	−F	2.5	5.3
3	−H	32	17

aqueous solubility. The addition of a nitrogen atom into the terminal phenyl ring (**6**) began to improve solubility without affecting biochemical or cellular potency. Removal of the methoxy adjacent to the pyridine nitrogen of **6** to form the more basic pyridine **7** resulted in additional modest improvements in solubility without affecting biochemical or cellular potency.

Compounds were periodically assessed for increases in FS (ΔFS %)[17] in normal rats using a bolus *iv* injection at typical screening doses of 5 and 10 mg/kg to provide a yes/no answer for *in vivo* activity. A common feature for all compounds at this stage of optimization was high clearance in rats. When high clearance is combined with a moderate affinity for the target, binding to plasma protein generally plays a role in *in vivo* potency.[18] The relationship of plasma protein binding[19−22] versus the FS (*in vivo*) observed at bolus

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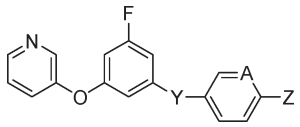
Table 2. Improved Hydrolytic Stability and Modest Improvements in Solubility[a]



no.	Y	A	Z	AC_{40} (μM)	CM (ΔFS % at μM)	KSol (μg/mL)	PB (% bound)
2	−NHCO−	CH	F	2.5	104 at 2.0	5.3	92.1
4	−NHCONH−	CH	F	1.8	121 at 2.0	1.1	NA
5	−NHCONH−	CH	OMe	0.7	139 at 2.0	< 1.0	95.1
6	−NHCONH−	N	OMe	1.4	134 at 2.0	28	92.5
7	−NHCONH−	N	H	1.1	137 at 2.0	46	92.9

[a] CM, cardiomyocytes; KSol, kinetic solubility; and PB, plasma protein binding.

doses of 5 or 10 mg/kg for 20 compounds of similar biochemical and cellular potencies is shown in Figure 1. Compounds with ΔFS % < 120% *in vivo* span a range of protein binding from approximately 80 to >95% with several compounds clustering above the 90% bound. However, compounds with ΔFS % > 120% contain only the set of molecules that have ≤90% of the drug bound to plasma proteins.

Table 3 outlines representative compounds that demonstrated reduced protein binding, removed off-target activities, improved cardiomyocyte potency, and improved intrinsic clearance. Saturation of the "left-side" aryl ring while maintaining an H-bond acceptor maintained good biochemical and cellular potencies and reduced the protein binding (**8**). CYP 1A2 inhibition was removed by sterically blocking the "right-side" pyridyl nitrogen with an adjacent methyl group (e.g., **8** versus **10**).[24,25] The vasodilatory activity mediated through K_{ATP} channel opening[26] was very sensitive to the "left-side" moiety. For example, substitution of the pendant acetyl moiety of **8** as a carbamate **11** or *N*,*N*-dimethyl sulfonyl **12** attenuated direct vasodilation, as measured by the aortic ring R_{50}, and inverting the stereochemistry of **8** to **9** removed any detectable vasodilation. Additional "left-side" modifications resulted in removal of any detectable vasodilation and improved cardiomyocyte potency (**8**, **12**, and **13**). Modest improvement in oxidative metabolism, as shown by the human extraction ratio (Re) in microsomes, was observed by shifting the connectivity of the piperidine ring to the core phenyl substituent, thus removing the stereocenter (**11** vs **14**).

With an understanding of how to remove the off-target liabilities of our compounds, additional optimization focused on further reduction in oxidative turnover to improve the *in vivo* pharmacokinetic (PK) parameters, improved solubility for the *iv* administration, and improved potency in cardiomyocytes that should result in better dose efficacy. As shown in Table 4, substitution of the O-linked left side piperidine moiety of **14** with the methylene linked piperazine ring of CK-1316189 (**22**) reduced the extraction ratio in human microsomes, dramatically improved the solubility, and improved cardiomyocyte potency. Unlike

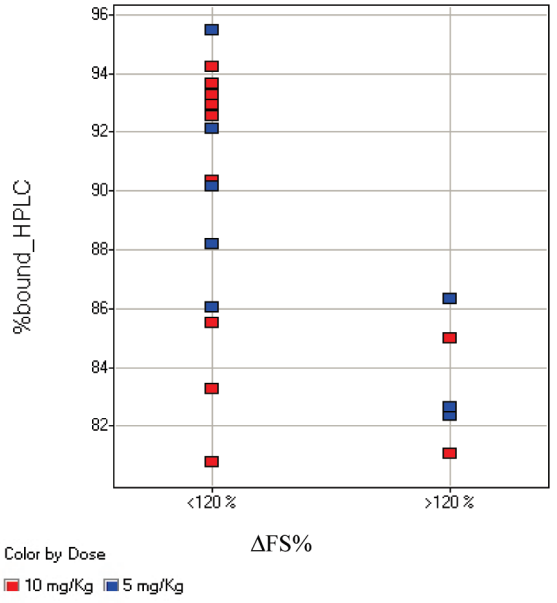


Figure 1. Plasma protein binding (% bound HPLC) vs increased FS (ΔFS %) *in vivo* (rats) from an *iv* injection of test article where measured ΔFS % > 120% is considered more active and ΔFS % < 120% is less active.

the initial SAR, removal of the fluorine from the 5-position of the central ring did not attenuate potency (**23** versus **22**). Finally, reinstallation of the fluorine in the 2-position of the central ring resulted in improved cardiomyocyte potency with no measurable off-target liabilities (**24**). Introduction of the basic nitrogen of the piperazine improved the physical properties of the molecule (*e.g.*, cLog *P* values: **14** = 3.0, and **24** = 1.4), and the weak basicity of this nitrogen (pK_a = 6.1) did not introduce additional off-target interactions.

Improved cardiac contractility as measured by increased FS in normal, Sprague−Dawley rats (Figure 2) confirmed the desired effect of cardiac sarcomere activation on systolic function.[27] Compounds **23** and **24** demonstrated concentration-dependent increases in FS in this model.

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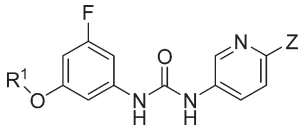
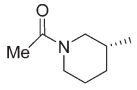
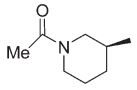
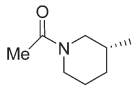
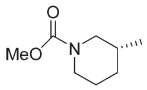
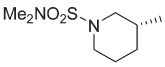
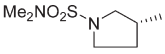
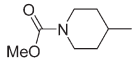
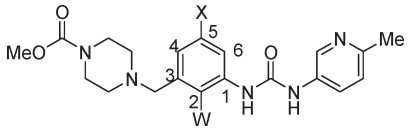
Table 3. Reduction in Protein Binding, Removal of Off-Target Activities, Improvements in Cardiomyocyte Potency, and Improvements in Intrinsic Clearance[a]



no.	R^1	Z	AC_{40} (μM)	CM (ΔFS% at μM)	Shake Sol (mg/mL at pH 5)	PB (% bound)	aortic ring R_{50} (μM)	CYP 1A2 IC_{50} (μM or (% inhib at μM))	human Re[23]
7	3-pyridyl	H	1.1	127 at 2.0	0.028	92.9	NA	NA	NA
8		H	2.5	129 at 2.0	0.39	82.5	1.7	0.08	0.5
9		H	7.3	122 at 10	0.55	82.5	> 25	(99% at 10)	NA
10		Me	2.5	140 at 2.0	0.13	NA	2.4	> 10	0.6
11		H	3.6	123 at 2.0	0.018	90.4	8.0	(97% at 10)	0.5
12		H	0.60	126 at 0.8	0.040	92.1	6.6	NA	NA
13		H	0.70	137 at 0.4	0.013	88.8	> 25	0.06	0.9
14		Me	0.80	123 at 2.0	0.017	93.5	11	> 10	0.3

[a] CM, cardiomyocytes; Shake Sol, shake flask solubility; PB, plasma protein binding; Re, ratio of extraction in microsomes; and NA, not available.

Table 4. Additional Improvements in Oxidative Turnover, Increased Solubility, and Improved Potency in Cardiomyocytes[a]



no.	X	W	AC_{40} (μM)	CM (ΔFS % at μM)	Shake Sol (mg/mL at pH 5)	PB (% bound)	aortic ring R_{50} (μM)	CYP 1A2 IC_{50} (μM)	human Re
CK-1316189 (**22**)	F	H	1.7	131 at 0.8	4.6	89.7	> 25	> 10	< 0.2
CK-1317138 (**23**)	H	H	4.5	138 at 0.8	4.0	87.2	15	> 10	< 0.2
omecamtiv mecarbil (**24**)	H	F	0.58	123 at 0.2	2.2	79.6	> 25	> 10	< 0.2

[a] CM, cardiomyocytes; Shake Sol, shake flask solubility; PB, plasma protein binding; and Re, ratio of extraction in microsomes.

As expected from its improved *in vitro* potency and greater free fraction, **24** showed greater potency *in vivo* as compared to **23**. In dog models of HF, **24** showed increases in cardiac contractility as measured by increased FS, increased stroke volume, and increased cardiac output.[28,29,30]

As predicted from *in vitro* measures of drug metabolism, **24** demonstrated good PK parameters in both rats (Sprague−Dawley) and dogs (Beagle) with clearances of 22 and 7.2 mL/min/kg, volumes of 3.5 and 3.6 L/kg, and bioavailabilities (F%) of 100 and 80%, respectively.

In conclusion, *omecamtiv mecarbil* (**24**) is a potent, selective activator of cardiac myosin that leads to increases in cardiac function in preclinical models of HF and now in patients with HF due to systolic dysfunction.[31] Its physical and PK properties make **24** suitable for either intravenous or oral administration. With the support of these results and other promising data, **24** is currently in clinical trials for the treatment of systolic HF.[32]

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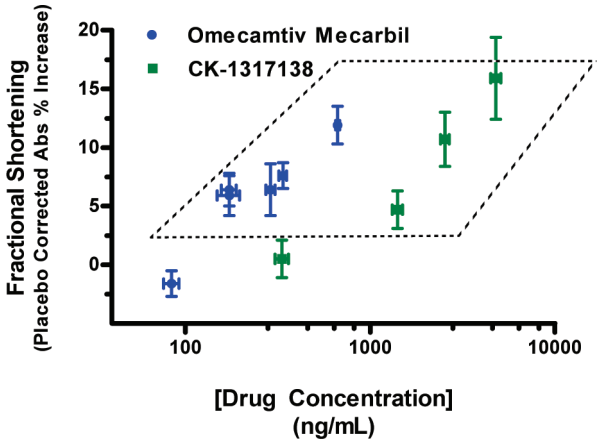

Figure 2. Concentration-dependent increase in placebo-corrected absolute fraction shortening in rats by CK-1317138 and *omecamtiv mecarbil*. $N = 5-6$ per data point. Points within the dashed lines were significantly different from placebo ($p < 0.05$).

SUPPORTING INFORMATION AVAILABLE Experimental procedures and analytical data. This material is available free of charge via the Internet at http://pubs.acs.org.

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ABBREVIATIONS ATP, adenosine triphosphate; DIPEA, diisopropylethylamine; PK, pharmacokinetic; SAR, structure–activity relationship.

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(8) Select compounds were periodically examined using bovine, rat, and human sources of an active cardiac myosin fragment to assess variability across species. No difference in activity was observed across species.

(9) Compound specificity with respect to muscle type was evaluated by comparing the effect of the compound on the actin-stimulated ATPase activity of a panel of myosin isoforms [cardiac (bovine), skeletal (rabbit), and smooth muscle (bovine uterus or chicken gizzard)] at a single dose of 40, 50, or 100 μM compound. The data for compound **1** are shown in Figure S1 in the Supporting Information.

(10) FS is the change in myocyte length divided by the resting length of the cell. ΔFS is the percent change in FS after treatment relative to FS at baseline, which is defined as 100%.

(11) Direct vasodilatation effects were measured by the ability of compounds to relax a potassium chloride (KCl)- or a phenylephrine-induced contraction of a rat aortic ring. The vasodilatation effect of **8** in rat aortic rings was shown to be independent of endothelium, protein kinase G, and protein kinase A pathways for vascular relaxation. However, a dose-related inhibition of the vasodilatation effect of **8** was observed when the aortic ring preparations were pretreated with the K_{ATP} channel inhibitor glybenclamide. Therefore, the vasodilatation effect of **8** was determined to be due to its ability to activate K_{ATP} channels.

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