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Novel 1-Methyl-1*H*-pyrazole-5-carboxamide Derivatives with Potent Anthelmintic Activity

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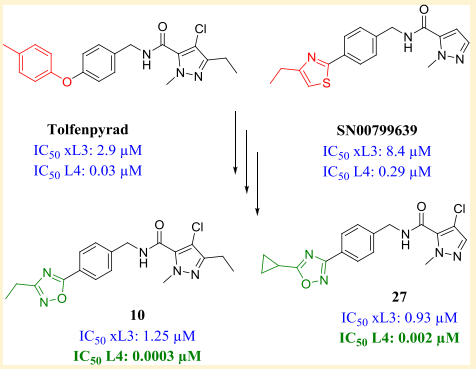
Supporting Information

ABSTRACT: A phenotypic screen of two different libraries of small molecules against the motility and development of the parasitic nematode *Haemonchus contortus* led to the identification of two 1-methyl-1*H*-pyrazole-5-carboxamide derivatives. Medicinal chemistry optimization targeted modifications of the left-hand side, middle section, and right-hand side of the hybrid structure of these two hits to elucidate the structure−activity relationship (SAR). Initial SAR around these hits allowed for the iterative and directed assembly of a focused set of 30 analogues of their hybrid structure. Compounds **10**, **17**, **20**, and **22** were identified as the most potent compounds, inhibiting the development of the fourth larval (L4) stage of *H. contortus* at sub-nanomolar potencies while displaying strong selectivity toward the parasite when tested in vitro against the human MCF10A cell line. In addition, compounds **9** and **27** showed promising activity against a panel of other parasitic nematodes, including hookworms and whipworms.



INTRODUCTION

Anthelmintic resistance imposes a huge burden on global animal healthcare, particularly livestock, with significant negative socioeconomic impacts.[1−3] This burden is caused by the excessive and uncontrolled use of marketed anthelmintics and the induced genetic change in parasite populations.[4−6] Since the ground-breaking discovery of monepantel as a novel class of anthelmintic in 2008,[7,8] there has been no new commercial chemical entity with potent anthelmintic activity approved for the control of parasitic worms in livestock. In addition, resistance has also been reported for monepantel as well as moxidectin, another anthelmintic agent for the treatment of haemonchosis.[9,10] Therefore, a sustained effort to discover and develop novel anthelmintics is an essential component of the battle against drug resistance. Our team is focused on the discovery and development of new anthelmintics for oral or percutaneous treatment of parasitic infections of livestock, preferably with a compound class that exhibits a broad spectrum of activity and may be applicable to human diseases caused by other parasitic helminths.

In a previous work, we discovered that a registered pesticide called tolfenpyrad (**TFP**)[11] and an independent chemotype, designated SN00799639 (**SN639**) (Figure 1), were potent inhibitors of the motility and development of parasitic larvae of *Haemonchus contortus*, a blood-feeding parasitic nematode of major economic importance in ruminants. **TFP** showed IC_{50}

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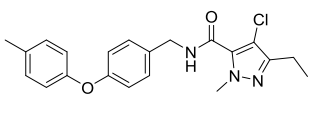
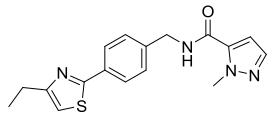


Tolfenpyrad
IC_{50} xL3: 2.9 ± 0.58 μM
IC_{50} L4: 0.03 ± 0.005 μM
MCF10A cytotoxicity IC_{50}: 37.9 μM



SN00799639
IC_{50} xL3: 8.4 ± 1.4 μM
IC_{50} L4: 0.29 ± 0.10 μM
MCF10A cytotoxicity IC_{50}: > 50 μM

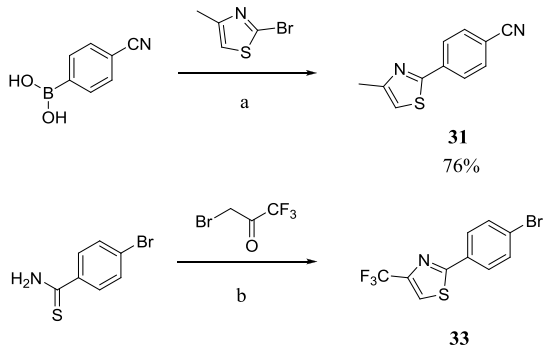
Figure 1. Structures and biological activity of the two original hits.

values of 2.9 and 0.03 μM against the motility of exsheathed third-stage larvae (xL3s) and against their development to fourth-stage larvae (L4s), respectively, whereas **SN639** displayed lower potency, with IC_{50} values of 8.4 μM (xL3 motility) and 0.29 μM (L4 development). Both hits displayed selectivity toward the parasite when tested in an assay measuring proliferation of a human breast epithelial cell line (MCF10A) in vitro, with an inhibitory IC_{50} value of 37.9 μM for **TFP** and >50 μM for **SN639**. Elaborations of the structure−activity relationships (SARs) for the two hits has been conducted by Le et al.[12,13] where potent molecules with good inherent physicochemistry were established. Importantly, both the hits share a 1-methyl-1*H*-pyrazole-5-carboxamide pharmacophore, indicating that hybrid analogues of these two hit molecules are worthy of investigation. Therefore, we herein report the synthesis and SAR exploration of a set of 30 analogues that incorporate structural elements from the two original hits. The objective of this study was to identify functional groups that lead to breakthrough activity while still maintaining drug-likeliness and selectivity toward the parasite.

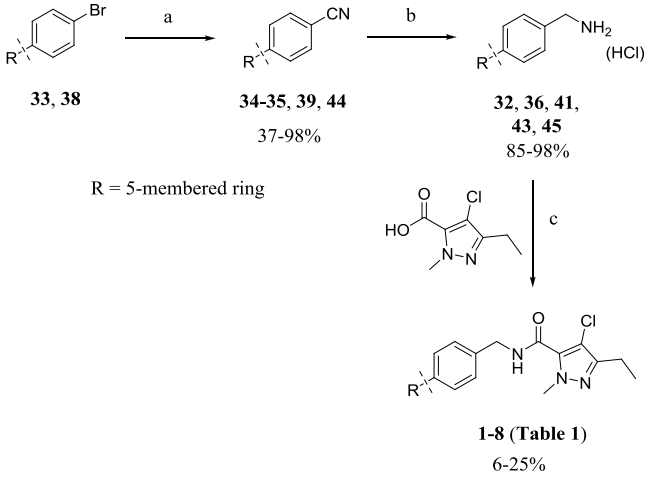
RESULTS AND DISCUSSION

Conceptually, the scaffold of the hybrid structure can be divided into three parts, the right-hand side (RHS) pyrazole, the middle section consisting of a benzyl moiety connected to the RHS pyrazole through an amide bond, and the left-hand side (LHS) consisting of a five- or six-membered ring. The construction of the LHS thiazole moiety of the hybrid scaffold is depicted in Scheme 1. In brief, the thiazole ring was installed on the LHS of the scaffold through either a Suzuki coupling between a 2-bromothiazole building block and an arylboronic acid species or a thiazole ring cyclization reaction between a thiobenzamide species and a bromoketone. Scheme 2 describes

Scheme 1. Synthetic Pathway for the Construction of the LHS Thiazole of the Hybrid Scaffold; (a) $Pd(dppf)Cl_2$, K_2CO_3, Dioxane/H_2O, 110 °C; (b) *p*-Toluenesulfonic Acid, EtOH, 60 °C

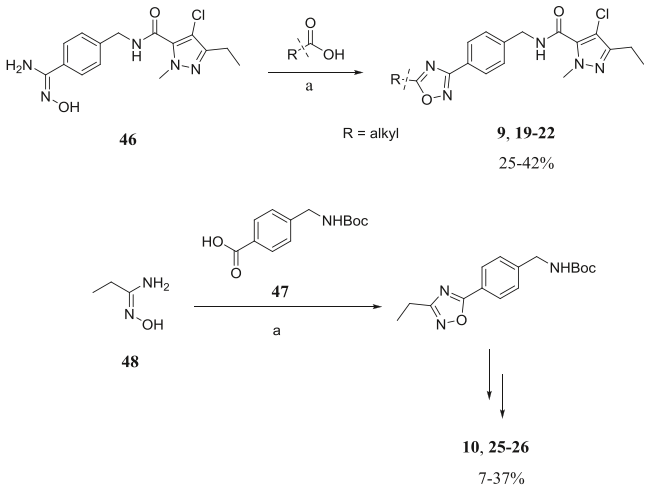


Scheme 2. Synthetic Pathway for the RHS Construction of the Hybrid Scaffold; (a) $Zn(CN)_2$, Xantphos, $Pd_2(dba)_3$, DMF, 110 °C or CuCN, DMF, 130 °C; (b) $LiAlH_4$, THF (Then Optionally: 4 M HCl in 1,4-Dioxane); (c) HOAt, EDCI, ACN, 80 °C or HATU, DIPEA, DMF or T3P, DIPEA, THF



the synthetic pathway for the construction of the RHS of the hybrid scaffold. This pathway entailed, first, a cyanation reaction that enabled a subsequent reduction to give a benzylamine intermediate that was used to couple to the RHS pyrazole through an amide coupling reaction. For the synthesis of analogues that contain the 1,2,4-oxadiazole moiety, the cyclization is described in Scheme 3.

Scheme 3. 1,2,4-Oxadiazole Ring Cyclization; (a) HOBt, EDCI, DMF, 180 °C or HATU, DIPEA, DMF, 110 °C



To examine the SAR, analogues were first subjected to a primary screen to assess their ability to inhibit the motility of *H. contortus* at the xL3 stage, using monepantel and moxidectin as positive control anthelmintics. Only compounds that resulted in ≥70% motility inhibition of xL3 larvae at 100 μM were progressed to subsequent dose−response evaluation to determine IC_{50} values and further assessment in a *H. contortus* L4 development assay. Similarly, for the cytotoxicity assay, only compounds that resulted in ≥50% inhibition of cell proliferation at 50 μM were subjected to a five-point dose−response evaluation.

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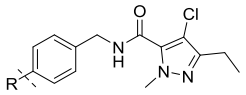
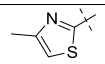
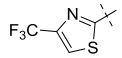
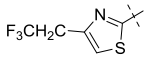
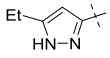
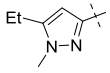
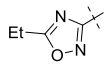
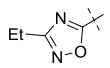
Table 1. SAR Investigation on the LHS Region of the Hybrid Scaffold



1-12

Entry	R	IC_{50} (μM) ± SD in xL3 motility assay	IC_{50} (μM) ± SD in L4 development assay	MCF10A Cytotoxicity IC_{50} (μM)
1		> 100		
2		> 100		
3		23 ± 14	0.08 ± 0.04	> 50
4		29 ± 18	0.82 ± 0.44	> 50
5		> 100		
6		> 100		
7		> 100		
8		6.0 ± 2.4	0.09 ± 0.001	> 50
9		0.19 ± 0.16	0.0025 ± 0.0007	> 50
10		1.25 ± 0.35	0.0003 ± 0.0003	> 50
11		> 100		
12		> 100		
Monepantel[14]		0.16 ± 0.008	0.075 ± 0.04	
Moxidectin[14]		0.08 ± 0.04	3.45 ± 0.75	

First, we aimed to explore the LHS chemical space of **SN639** hybridized with the RHS pyrazole of **TFP** while maintaining the middle section that was common between both the original hits. The results are summarized in Table 1. Interestingly, compound **1**, which contains the thiazole LHS of **SN639** (with a methyl substituent on the thiazole instead of the ethyl) and the pyrazole RHS of **TFP**, completely lost activity against xL3. Replacing the methyl group on the thiazole with a trifluoromethyl group, as seen for **2**, also led to an inactive molecule. However, activity against xL3 was moderately regained when the $-CF_3$ group was installed on a longer aliphatic chain on the thiazole, as seen for **3**, with activity against L4 development (IC_{50} of 0.08 μM) comparable to that of **TFP** (Table 1).

Subsequently, we evaluated the LHS of the scaffold using other five-membered ring isosteres, such as substituted pyrazoles and imidazole (**4**–**7**). Pyrazole **4** displayed a slight loss of activity against L4 development compared with **SN639**, whereas **5**–**7** did not exert any detectable activity (Table 1). Replacing the thiazole moiety with an oxazole, in the case of **8**, led to a modest improvement in activity against both xL3 motility and L4 development compared with **SN639**. The replacement of the thiazole with the 1,2,4-oxadiazole isostere (**9** and **10**) displayed a sub-μM activity against both xL3 motility and L4 development (IC_{50} 0.19 μM and 2.5 nM, respectively, exerted by oxadiazole **9**; Table 1). In addition to this encouraging result, compared with **TFP**, compound **10** not only displayed a 2-fold improvement in activity against xL3 motility but also decreased the IC_{50} value against the L4 development into the sub-nM range (0.3 nM). However, expanding the LHS from five-membered to six-membered rings led to a loss of activity, as seen for **11** and **12** (Table 1). Notably, all active compounds from this SAR evaluation of the LHS displayed a great level of selectivity toward the parasite, with cytotoxicity IC_{50} values against human MCF10A cells of >50 μM.

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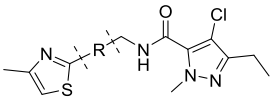
Table 2. SAR Investigation on the Middle Section of the Hybrid Scaffold



13-16

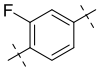
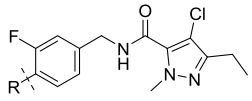
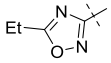
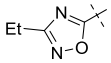
Entry	R	IC_{50} (μM) ± SD in xL3 motility assay	IC_{50} (μM) ± SD in L4 development assay	MCF10A Cytotoxicity IC_{50} (μM)
13		>100		
14		6.15 ± 0.64	0.12 ± 0.04	> 50
15		>100		
16		4.3 ± 3.15	0.002 ± 0.002	> 50
Monepantel[14]		0.16 ± 0.008	0.075 ± 0.04	
Moxidectin[14]		0.08 ± 0.04	3.45 ± 0.75	

Table 3. Next Generation SAR Investigation on the 1,2,4-Oxadiazole LHS and the 3-F Substituted Middle Region of the Hybrid Scaffold



17-18

Entry	R	IC_{50} (μM) ± SD in xL3 motility assay	IC_{50} (μM) ± SD in L4 development assay	MCF10A Cytotoxicity IC_{50} (μM)
17		3.5 ± 2.1	0.0007 ± 0.001	> 50
18		4.6 ± 4.1	0.01 ± 0.01	> 50
Monepantel[14]		0.16 ± 0.008	0.075 ± 0.04	
Moxidectin[14]		0.08 ± 0.04	3.45 ± 0.75	

After probing the SAR on the LHS of the hybrid scaffold with the 12 analogues in Table 1, we then investigated the phenyl ring in the middle section of the hit compounds, maintaining the RHS pyrazole of **TFP** and the LHS thiazole of **SN639**. The results are summarized in Table 2. The two groups selected to explore the SAR on the phenyl ring were fluorine substituents and pyridinyl moieties; both introduced changes at the 2- and 3-positions of the ring. It can be seen that the nitrogen atom in the pyridinyl group did not maintain any activity on either the 2- or 3-position (**13** and **15**), whereas a fluorine substituent at either position (**14** and **16**) modestly improved the activity of the **SN639** scaffold against xL3. Compound **16**, in particular, displayed a single-digit nM IC_{50} (2 nM) in the L4 development assay, indicating that the 3-F substituent on the phenyl ring was the best group of those assessed. Once again, the two active compounds **14** and **16** have been shown to be noncytotoxic in in vitro cell culture.

Combining the earlier discovered 1,2,4-oxadiazole moiety with the 3-F group on the phenyl ring in the middle section gave **17** and **18** (Table 3). Interestingly, compound **17** improved the activity against xL3 motility when compared with **16**, but exhibited a loss in activity when compared with **9**. However, **17** exhibited a very promising activity against L4 development, bringing the IC_{50} value down to the sub-nM range (0.7 nM), which is a significant improvement compared with both **16** and **9**. Compound **18**, although exhibiting a substantial loss in activity when compared with both **16** and **10** (its parent oxadiazole compound), still displayed a sub-μM activity against L4 development. Selectivity toward the parasite was also maintained for these two analogues.

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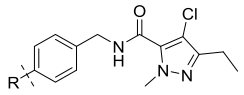
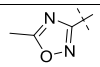
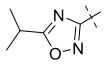
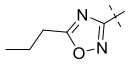
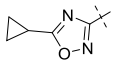
Table 4. 1,2,4-Oxadiazole Refinement SAR Investigation on the Hybrid Scaffold

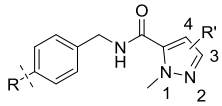


19-22

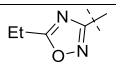
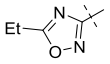
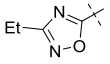
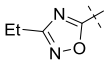
Entry	R	IC_{50} (μM) ± SD in xL3 motility assay	IC_{50} (μM) ± SD in L4 development assay	MCF10A Cytotoxicity IC_{50} (μM)
19		3.2 ± 0.56	0.02 ± 0.01	> 50
20		0.22 ± 0.05	0.0007 ± 0	> 50
21		0.39 ± 0.01	0.001 ± 0	N/A[a]
22		0.80 ± 0.22	0.0007 ± 0	> 50
Monepantel[14]		0.16 ± 0.008	0.075 ± 0.04	
Moxidectin[14]		0.08 ± 0.04	3.45 ± 0.75	

[a]Not assessed.

Table 5. SAR Investigation on the RHS Pyrazole Region of the Hybrid Scaffold with the 1,2,4-Oxadiazole LHS



23-26

Entry	R	R'	IC_{50} (μM) ± SD in xL3 motility assay	IC_{50} (μM) ± SD in L4 development assay	MCF10A Cytotoxicity IC_{50} (μM)
23		3-Et	0.79 ± 0.29	0.015 ± 0.005	> 50
24		4-Cl	0.65 ± 0.21	0.007 ± 0.002	> 50
25		3-Et	4.8 ± 3.3	0.075 ± 0.005	> 50
26		4-Cl	2.3 ± 1.1	0.003 ± 0.004	> 50
Monepantel[14]			0.16 ± 0.008	0.075 ± 0.04	
Moxidectin[14]			0.08 ± 0.04	3.45 ± 0.75	

Following our discovery of the impact of the LHS 1,2,4-oxadiazole moiety on the SAR, we analyzed further the oxadiazole ring by making a small set of second-generation analogues bearing different substituents in place of the original ethyl group on the oxadiazole. The results are summarized in Table 4. The tested substituents included methyl (**19**), *iso*-propyl (**20**), *n*-propyl (**21**), and *cyclo*-propyl (**22**). Overall, none of the compounds **20**, **21**, or **22** lost significant activity against xL3 when compared with the parent oxadiazole compound **9**. Compounds **20** and **22**, in particular, retained the IC_{50} value of 0.7 nM against L4 development while still maintaining the selectivity.

Our next aim was to explore the chemical space around the RHS of the scaffold, fixing the 1,2,4-oxadiazole groups from **9** and **10** at the LHS and keeping the middle phenyl ring unsubstituted. The results from this exploration are summarized in Table 5. Removing the 4-Cl, while keeping the 3-ethyl group on the pyrazole, gave **23** and **25**. Even though these analogues were not as potent as the original oxadiazoles **9** and **10** against xL3 motility and L4 development, they displayed encouraging sub-μM activity against L4 development. Removing the 3-ethyl group while keeping the 4-Cl substituent gave more potent compounds (**24** and **26**) than **23** and **25** against both xL3 motility and L4 development. Particularly, regarding the inhibition of L4 development, analogues **24** and **26** both produced single digit nanomolar activity of 7 and 3 nM, respectively, and were not cytotoxic. These results suggested that the 4-chloropyrazole in the RHS of the hybrid scaffold had the largest impact on the inhibition of L4 development.

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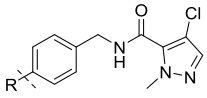
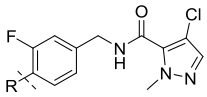
J. Med. Chem. 2019, 62, 3367−3380

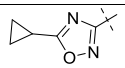
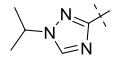
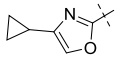
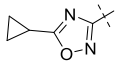
Table 6. SAR Investigation on the LHS Region of the Hybrid Scaffold with the 4-Chloropyrazole RHS



27-29



30

Entry	R	IC_{50} (μM) ± SD in xL3 motility assay	IC_{50} (μM) ± SD in L4 development assay	MCF10A Cytotoxicity IC_{50} (μM)
27		0.93 ± 0.62	0.002 ± 0.002	> 50
28		25 ± 9.3	0.56 ± 0.17	N/A[a]
29		3.0 ± 0.80	0.02 ± 0.001	N/A[a]
30		0.49 ± 0.21	0.007 ± 0.002	N/A[a]
Monepantel[14]		0.16 ± 0.008	0.075 ± 0.04	
Moxidectin[14]		0.08 ± 0.04	3.45 ± 0.75	

[a]Not assessed.

Table 7. Biological Activity Profile of Selected Compounds against a Panel of Parasitic Nematodes

	H. polygyrus adult viability (% inhibition)		*H. polygyrus* L3 (% inhibition)	*A. ceylanicum* L3 (% inhibition)		*T. muris* L1 (% inhibition)
entry	10 μM	1 μM	100 μM	100 μM	10 μM	100 μM
TFP	100	73.4	32.4	100	94.9	100
9	100	100	92.2	100	100	93.7
27	100	100	92.5	100	100	100

The favorable contribution of the 4-chloropyrazole RHS was then combined with different substitutions of the five-membered rings on the LHS of the scaffold (Table 6). These new rings included the *cyclo*-propyl-substituted oxadiazole from compound **22**, which exerted potent activity against L4 development (**27**), triazole (**28**), and *cyclo*-propyl-substituted oxazole (**29**). Compound **27** maintained the level of potency observed for **24** against both xL3 motility and L4 development, with a single digit 2 nM IC_{50} value in the L4 development assay. Whereas the *iso*-propyl-triazole **28** did not contribute to improving the activity in either the xL3 motility or the L4 development assays, oxazole **29** maintained the level of potency exerted by **TFP** in both assays. Adding the favorable 3-F group to the middle ring of **27** gave **30**, which exhibited a level of potency that was consistent with those of analogues **24** and **27**.

To construct a biological activity profile for the hybrid scaffold of **TFP** and **SN639**, we assessed **TFP**, together with analogues **9** and **27**, against a panel of different parasitic nematodes at various concentrations (Table 7). This panel included *Heligmosomoides polygyrus* (a rodent nematode)[15] and *Ancylostoma ceylanicum* (a hookworm), which are both related to *H. contortus*, and the more phylogenetically distant *Trichuris muris* (rodent whipworm). It can be seen from Table 7 that, at 100 μM, both compounds **9** and **27** significantly improved the activity against *H. polygyrus* L3s exerted by **TFP**, increasing inhibition to >90%. Compound **9** completely inhibited the adult stage of *H. polygyrus* and the L3 stage of *A. ceylanicum* at all concentrations tested and inhibited the first-stage larvae (L1s) of *T. muris* by 93.7%. Furthermore, compound **27** completely inhibited all three nematode species (same developmental stages) at all concentrations assessed. These results suggested that this scaffold holds promise as a medium- to broad-spectrum nematocidal or nematostatic starting point for drug development.

To assess the drug-likeliness of this series of analogues, we analyzed the two original hits **TFP** and **SN639** as well as the two analogues **9** and **27** to determine various physicochemical and metabolic parameters. The results are summarized in Table 8. It can be seen that the molecular weight (MW) and clog *P* values of compounds **9** and **27** are within the Lipinski's rule-of-5[16] for oral bioavailability. The polar surface area (PSA) increased relative to the parent structures but was within limits preferred for optimum cellular permeability and oral absorption of drug candidates.[17] The reduced microsomal

Table 8. Key Physicochemical Parameters and in Vitro Metabolic Stability of Selected Compounds

entry	MW[a]	PSA[a] (Å^2)	clog *P*[a]	$T_{1/2}$ (min)	$CL_{int,in\ vitro}$[b] (μL/min/mg)	predicted E_H[c]
TFP	383	56.2	4.6	14	121	N/A
SN639	326	59.8	2.6	22	80	0.63
9	373	85.8	3.4	21	85	0.65
27	357	85.8	2.6	40	43	0.48

[a]Calculated using ChemAxon JChem software. [b]In vitro intrinsic clearance determined in mouse liver microsomes. [c]Predicted hepatic extraction ratio calculated from in vitro data.

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J. Med. Chem. 2019, 62, 3367−3380

degradation relative to the original hit compounds was reflected in a longer microsomal half-life ranging from 22 to 40 min. **SN639**, **9**, and **27** had an intermediate predicted hepatic clearance.

CONCLUSIONS

We report on a series of 1-methyl-1*H*-pyrazole-5-carboxamide derivatives based on a hybrid structure derived from the two original screening hits, **TFP** and **SN639**, which had potent inhibitory activity on worm motility and an even higher potency blocking the development of the parasitic nematode *H. contortus* in vitro. This compound series resulted from a variety of modifications made to the hybrid scaffold, focusing on understanding the SAR and in obtaining more potent compounds without increasing the lipophilicity. We were successful in this endeavor: whereas ligand lipophilicity efficiency (LLE) of **TFP** was 0.6 (L3) and 2.8 (L4) and that of **SN639** was 2.2 (L3) and 3.7 (L4), for **9**, **10**, **17**, **20**, **22**, and **27**, the corresponding L3 values were all much larger at 4.4, 3.6, 2.9, 4.0, 3.8, and 4.6, and for L4, they were also much larger at 6.3, 7.2, 6.7, 6.5, 6.9, and 6.7, respectively. Compounds **10**, **17**, **20**, and **22** not only exhibited a remarkable improvement in the inhibition of L4 development, achieving sub-nM IC_{50} values, but also improved the cytotoxicity observed for the original hit **TFP**. In addition, compounds **9** and **27** also displayed promising activity against other parasitic nematodes such as whipworms and hookworms. The hybridization of the two hits has resulted in highly potent and noncytotoxic molecules that maintain the good physical properties and can serve as good starting points for further optimization. However, some pyrazole carboxamides have been recently reported to inhibit respiration in *H. contortus*,[18] and that is a biological activity that, if shared with our compounds, may not be detected in a standard cytotoxicity assay using cell cultures grown in glucose. A respiratory inhibition study is being undertaken, and the results will be published when they become available. The comprehensive antiparasitic SAR established in this study indicates a potential for 1-methyl-1*H*-pyrazole-5-carboxamides to become a novel class of anthelmintics because their potency is already on the level of commercial anthelmintics, with a significant cytotoxicity window and evidence that a broad anthelmintic spectrum is achievable. Current efforts are directed toward progressing the best compounds and assessing their efficacy and toxicity in vivo.

EXPERIMENTAL SECTION

The nematode assays and cytotoxicity assays are as described by Le et al.[12]

Physicochemical Experimental Section. The physicochemical experimental is as described by Le et al.[12]

Chemistry Experimental Section. All of the final compounds reported had purities greater than 95% based on analytical high performance liquid chromatography (HPLC), ^{1}H nuclear magnetic resonance (NMR), and liquid chromatography−mass spectrometry (LC−MS). All solvents and reagents were used directly from commercial suppliers unless otherwise stated. General chemistry experimental conditions were as reported by Le et al.[12]

General Procedure A1: Suzuki Coupling Reactions. To a microwave tube equipped with a magnetic stirring bar, halide (1.0 equiv), boronic acid or boronic ester (1.0 equiv), and K_2CO_3 (2 equiv) were dissolved in a 1:4 mixture of H_2O/1,4-dioxane. The mixture was degassed for at least 0.5 h before $Pd(dppf)Cl_2$ (0.05 equiv) was added. The reaction tube was sealed with a cap and heated in a microwave reactor at 110 °C for 2 h. Upon completion [confirmed by thin-layer chromatography (TLC) and/or LC−MS], EtOAc was added to the reaction tube, and the mixture was filtered through a pad of celite and washed with excess EtOAc. The filtrate was then washed with water and then with brine. The organic layer was then dried ($MgSO_4$), and the solvent was removed in vacuo to afford a crude product, which was purified by column chromatography to yield the desired product.

General Procedure A2: Suzuki Coupling Reactions. To a stirred solution of halide (1.0 equiv) in acetonitrile (ACN) (15 mL) was added boronic acid (1.0 equiv), followed by aqueous Na_2CO_3 (2.5 equiv) solution. The mixture was degassed for 15 min with N_2 and then $Pd(PPh_3)_4$ (0.05 equiv) was added. The reaction mixture was heated at 100 °C for 20 h and then cooled to room temperature, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated. The crude mass obtained was purified by column chromatography to yield the desired product.

General Procedure A3: Suzuki Coupling Reactions. To a mixture of halide (2.29 mmol) and boronic acid (3.43 mmol) in EtOH (1 mL)/toluene (10 mL)/H_2O (1 mL) was added Na_2CO_3 (4.57 mmol). The reaction mixture was degassed by sparging with argon for 15 min before $Pd(dppf)Cl_2{\cdot}CH_2Cl_2$ (0.22 mmol) was added. The reaction mixture was heated at 100 °C for 16 h under argon. The reaction mixture was cooled to room temperature and concentrated in vacuo. The residue was diluted with water, and the aqueous layer was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude was purified by column chromatography to yield the desired product.

General Procedure A4: Suzuki Coupling Reactions. In a sealed tube, a solution of aryl halide (10.93 mmol) and bis(pinacolato)-diboron (12.02 mmol) in dimethyl sulfoxide (DMSO, 15 mL) was degassed with argon for 10 min. $Pd(dppf)Cl_2$·dichloromethane (DCM) (1.09 mmol) and KOAc (37.16 mmol) were then added and again degassed for 10 min. The reaction mixture was stirred at 100 °C for 12 h. Upon completion, the reaction mixture was cooled and then extracted with EtOAc. The combined organic layer was washed with water and brine, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography to give the desired boronic ester. The resulting boronic ester (0.62 mmol) and halothiazole (0.56 mmol) in 1,4-dioxane (5 mL) and water (0.5 mL) was degassed with argon for 10 min. KOAc (0.56 mmol) and $PdCl_2(dppf)$·DCM (0.057 mmol) were then added to the reaction mixture. The reaction mixture was heated at 100 °C for 16 h, then extracted with EtOAC, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography to yield the desired product.

General Procedure B1: Nitrile Reduction. To a solution of benzonitrile (1.0 equiv) in anhydrous tetrahydrofuran (THF) (10 mL), $LiAlH_4$ (3.0 equiv) was slowly added. The reaction was stirred at room temperature for 2 h. Upon completion (confirmed by TLC and/or LC−MS), the reaction mixture was cooled on ice before a solution of 1 M NaOH was added. The slurry mixture was then filtered through a pad of celite. The filtrate was extracted with EtOAc (3 × 30 mL), and the combined organic layer was dried ($MgSO_4$) and concentrated in vacuo. To the residue after removal of the solvent, 15 mL of 4 M HCl in 1,4-dioxane was added. The reaction mixture was stirred at room temperature overnight. The precipitate of the resulting hydrochloride salt was then filtered, washed with diethyl ether, and then dried in a vacuum oven to yield the desired product as a hydrochloride salt.

General Procedure B2: Nitrile Reduction. To a stirred solution of benzonitrile (1.0 equiv) in THF (5.0 mL) was added $LiAlH_4$ solution (2 M in THF, 2.5 equiv) dropwise at 0 °C. The reaction mixture was stirred at room temperature for 3 h. After that, the reaction mixture was quenched with saturated Na_2SO_4 solution (2.0 mL) at 0 °C and diluted with EtOAc (20 mL). The reaction mixture was filtered through a celite bed and washed thoroughly with EtOAc. The

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J. Med. Chem. 2019, 62, 3367−3380

combined filtrate was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure to yield the desired product as a free base.

General Procedure B3: Nitrile Reduction. To a stirred solution of benzonitrile (1.0 equiv) in mEtOH (40 mL) at 0 °C was added boc anhydride (2.0 equiv) and $NiCl_2{\cdot}6H_2O$ (0.1 equiv). $NaBH_4$ (7.0 equiv) was then added in small portions over a period of 30 min at 0 °C. The resulting reaction mixture containing a black precipitate was allowed to warm to room temperature and left to stir for 12 h. The reaction mixture was diluted with ice water, and an aqueous layer was extracted with EtOAc. The combined organic layer was washed with aqueous $NaHCO_3$ solution, water, and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. To the residue after removal of the solvent, 5 mL of 4 M HCl in 1,4-dioxane was added. The reaction mixture was stirred at room temperature overnight. The precipitate of the resulting hydrochloride salt was then filtered, washed with diethyl ether, and then dried in a vacuum oven to yield the desired product as a hydrochloride salt.

General Procedure B4: Nitrile Reduction. To a stirred solution of benzonitrile (6.06 mmol) in MeOH (10 mL), $Pd(OH)_2/C$ (20%) (0.73 mmol) was added, and the mixture was stirred under a H_2 balloon for 18 h. Upon completion, the reaction mixture was filtered through a bed of celite and washed thoroughly with MeOH. The combined filtrate was concentrated in vacuo to afford the desired product, which was taken to the next step without any further purification.

General Procedure C1: Cyanation. A solution of aryl halide (1.0 equiv) in dimethylformamide (DMF, 15 mL) was degassed with argon for 10 min. After that $Zn(CN)_2$ (1.5 equiv), Xantphos (0.2 equiv), and $Pd_2(dba)_3$ (0.1 equiv) were added. Then, the reaction mixture was stirred at 110 °C for 16 h. The reaction mixture was cooled to room temperature and diluted with water. The aqueous layer was extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated. The crude compound was purified by column chromatography to yield the desired product.

General Procedure C2: Cyanation. In a sealed tube, a solution of aryl halide (5.88 mmol) in DMF (15 mL) was degassed for 10 min before CuCN (17.65 mmol) was added. The resulting reaction mixture was stirred at 130 °C for 48 h, cooled to room temperature, diluted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by column chromatography yield the desired product.

General Procedure D1: Thiazole Ring Cyclization. To a stirred solution of haloketone (1.0 equiv) in EtOH (30 mL), thiourea or thioamide (1.0−1.5 equiv) was added. The reaction mixture was stirred at reflux until completion (confirmed by TLC). Upon completion, the reaction mixture was cooled to room temperature and diluted with water. The aqueous layer was extracted with EtOAc, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated to afford the desired product, which was used in the next step without purification.

General Procedure D2: Thiazole Ring Cyclization. A mixture of thiobenzamide (4.63 mmol) and haloketone (4.63 mmol) in EtOH (10 mL) was stirred at 60 °C for 3 h. The reaction mixture was concentrated, and the residue obtained was washed with acetone and *n*-pentane to afford an orange solid mass, which was mixed with *p*-toluenesulfonic acid (0.46 mmol) in toluene (30 mL) and heated at reflux for 20 h in a Dean-stark apparatus. After removal of the solvent, the residue was diluted with diethyl ether. The resulting solution was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude was washed with *n*-pentane to afford the desired product.

General Procedure D3: Thiazole Ring Cyclization. A solution of haloketone (2.78 mmol) and thioamide (2.31 mmol) in ACN (7 mL) was heated at 80 °C overnight in a sealed tube. Upon completion, the reaction mixture was concentrated in vacuo and filtered through a pad of silica gel to give the desired product.

General Procedure E: Oxazole Ring Cyclization. To a stirred solution of bromoketone (1.5 equiv) and 4-bromobenzamide (1.0 equiv) in toluene (50 mL), $CaCO_3$ (2.0 equiv) was added, and the reaction mixture was stirred at 130 °C overnight. Upon completion, the reaction mixture was cooled, quenched with 10% NaOH solution, extracted with EtOAc, washed with water and brine, dried over Na_2SO_4, and concentrated in vacuo to give the crude product, which was purified by column chromatography to yield the desired product.

General Procedure F1: Amide Coupling. Amine (either as free base or HCl salt, 1.0 equiv), HOAt (2.0 equiv), Et_3N (2.0 equiv), EDCI·HCl (2.0 equiv), and carboxylic acid (2.0 equiv) were dissolved in 3 mL of DMF. The reaction mixture was heated at 80 °C until completion (confirmed by TLC and/or LC−MS). Upon completion, EtOAc was added to the reaction mixture. The organic layer was washed with water and dried ($MgSO_4$), and the solvent was removed in vacuo to give the crude product, which was purified by column chromatography to yield the desired product.

General Procedure F2: Amide Coupling. To a stirred solution of carboxylic acid (0.54 mmol) in DMF (5.0 mL) were added HATU (0.97 mmol) and *N*,*N*-diisopropylethylamine (DIPEA, 1.28 mmol). The solution was stirred for 5 min before amine (0.71 mmol) was added. The reaction mixture was stirred at room temperature for 16 h. After completion, the reaction was diluted with EtOAc, washed with water and brine, and then dried over anhydrous Na_2SO_4. The solvent was then removed in vacuo to give the crude product, which was purified by column chromatography to yield the desired product.

General Procedure F3: Amide Coupling. To a stirred solution of carboxylic acid (1.0 equiv) and amine (1.0 equiv) in THF (5 mL), T_3P (2.0 equiv, 50% in EtOAc) and DIPEA (3.0 equiv) were added. The reaction mixture was stirred at room temperature for 6 h. Upon completion, the reaction mixture was diluted with EtOAc, washed with saturated $NaHCO_3$ solution and brine, dried over Na_2SO_4, and concentrated in vacuo to give the crude product, which was purified by prep-HPLC to afford the desired product.

General Procedure G: Amidoxime Formation. To a stirred solution of nitrile (1.0 equiv) in EtOH (20 mL) were added K_2CO_3 (2.0 equiv) and $NH_2OH{\cdot}HCl$ (1.5 equiv). The reaction mixture was stirred at reflux for 16 h. Upon completion, the reaction was diluted with EtOAc, and the organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and then concentrated in vacuo to afford the desired amidoxime.

General Procedure H1: Boc Protection. Amine (18.54 mmol) was dissolved in a mixture of 10% aqueous NaOH (25 mL) and EtOH (50 mL). The solution was cooled to 0 °C, and boc-anhydride (20.4 mmol) was added slowly. The reaction mixture was stirred at room temperature for 18 h. Upon completion, EtOH was removed in vacuo, and water was added. The aqueous layer was acidified slowly with a saturated solution of citric acid (20 mL). The precipitate formed was filtered and dried in a vacuum oven to yield the desired Boc-protected amine.

General Procedure H2: Boc Protection. To a stirred solution of amine (1.0 equiv) in DCM (8 mL) was added di-*tert*-butyl-dicarbonate (1.0 equiv) slowly, followed by Et_3N (3.0 equiv). The reaction mixture was stirred at room temperature for 16 h. Upon completion, the reaction mixture was concentrated in vacuo, and the residue was diluted with saturated aqueous $NaHCO_3$ solution. The aqueous layer was extracted with DCM (3 × 10 mL). The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (20% EtOAc in hexane) to yield the desired Boc-protected amine.

General Procedure I1: 1,2,4-Oxadiazole Ring Cyclization. To a stirred solution of carboxylic acid (1.0 equiv) in DMF (8 mL) was added HATU (2.5 equiv) and DIPEA (3.0 equiv). The reaction mixture was stirred for 5 min before amidoxime (1.0 equiv) was added. The reaction mixture was stirred at room temperature for 16 h and then refluxed at 110 °C for 16 h. Upon completion, EtOAc was added, and the organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product, which was purified by column chromatography to yield the desired product.

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General Procedure I2: 1,2,4-Oxadiazole Ring Cyclization. Amidoxime (1.0 equiv), carboxylic acid (1.0 equiv), HOBt (1.2 equiv), and EDCI·HCl (1.3 equiv) were dissolved in 3 mL of DMF in a microwave tube. The reaction mixture was stirred at room temperature for 0.5 h and then heated in a microwave reactor at 180 °C for 20 min. EtOAc was then added, and the organic layer was washed with water and brine, dried ($MgSO_4$), and concentrated in vacuo. The crude product was purified by column chromatography to yield the desired product.

General Procedure J: Boc Deprotection. To a stirred solution of Boc-protected amine (1.65 mmol) in 1,4-dioxane (5 mL), 4 M HCl in 1,4-dioxane (5 mL) was added, and the reaction mixture was stirred at room temperature for 1 h. Upon completion, the resulting HCl salt precipitate was filtered, washed with diethyl ether, dried in a vacuum oven, and directly taken for the next step.

General Procedure K: Radical Reactions. A stirred solution of methylbenzonitrile (1.0 equiv) in 1,2-dichloroethane (240 mL) was degassed under N_2 for 20 min before *N*-bromosuccinimide (1.0 equiv) and azobisisobutyronitrile (0.1 equiv) were added. The reaction mixture was stirred at 80 °C for 6 h. Upon completion, the reaction mixture was cooled to room temperature and extracted with DCM. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (5% EtOAc in hexane) to yield the desired product.

General Procedure L: Amine Synthesis from Phthalimide Precursor. To a stirred solution of alkylhalide (1.0 equiv) in DMF (70 mL) were added phthalimide (1.7 equiv) and CS_2CO_3 (3.0 equiv). The reaction mixture was stirred at room temperature for 4 h. Upon completion, the reaction mixture was extracted with EtOAc. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (30% EtOAc in hexane) to afford the desired product, which was then on reacted with hydrazine hydrate (3.0 equiv) in *n*-butanol (20 mL) at 80 °C for 1 h. Upon completion, the reaction mixture was cooled to room temperature. A voluminous precipitate was formed, which was filtered off. The filtrate was concentrated in vacuo to give the crude product, which was purified by column chromatography (neutral Al_2O_3, 10% MeOH in DCM) to yield the desired product.

*4-Chloro-3-ethyl-1-methyl-N-(4-(4-methylthiazol-2-yl)benzyl)-1H-pyrazole-5-carboxamide (**1**).* The title compound was prepared according to general procedure F1, starting from **32** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (10%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.92 (d, J = 8.3 Hz, 2H), 7.41 (d, J = 8.4 Hz, 2H), 7.06 (s, br, 1H), 6.87 (d, J = 0.9 Hz, 1H), 4.67 (d, J = 5.8 Hz, 2H), 4.15 (s, 3H), 2.63 (q, J = 7.6 Hz, 2H), 2.51 (d, J = 0.9 Hz, 3H), 1.24 (t, J = 7.6 Hz, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 167.1, 158.7, 154.0, 149.7, 139.3, 133.4, 131.0, 128.2, 127.0, 113.6, 107.8, 43.3, 40.8, 19.3, 17.4, 12.9 ppm; LC−MS m/z: 375.1 $[M + H]^+$.

*4-Chloro-3-ethyl-1-methyl-N-(4-(4-(trifluoromethyl)thiazol-2-yl)-benzyl)-1H-pyrazole-5-carboxamide (**2**).* Intermediate **34** was subjected to general procedure B2 to give the corresponding benzylamine, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to afford the title compound as a white solid (54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (s, 1H), 8.54 (s, 1H), 7.96 (d, J = 8.0 Hz, 2H), 7.51 (d, J = 8.1 Hz, 2H), 4.54 (d, J = 5.9 Hz, 2H), 3.84 (s, 3H), 2.55 (q, J = 7.5 Hz, 2H), 1.16 (t, J = 7.6 Hz, 3H) ppm; LC−MS: m/z: 429.0 $[M + H]^+$.

*4-Chloro-3-ethyl-1-methyl-N-(4-(4-(2,2,2-trifluoroethyl)thiazol-2-yl)benzyl)-1H-pyrazole-5-carboxamide (**3**).* General procedure D3 was followed, starting from 1-bromo-4,4,4-trifluorobutan-2-one and 4-bromo-thiobenzamide to give 2-(4-bromophenyl)-4-(2,2,2-trifluoroethyl)-1,3-thiazole, which was subjected to cyanation and then reduction according to general procedure C1 and general procedure B2, respectively, to give the corresponding benzylamine that was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to afford the title compound as a white solid (15%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (t, J = 5.6 Hz, 1H), 7.92 (d, J = 8.0 Hz, 2H), 7.71 (s, 1H), 7.48 (d, J = 8.0 Hz, 2H), 4.53 (d, J = 5.6 Hz, 2H), 3.93−3.87 (m, 2H), 3.84 (s, 3H), 2.58−2.54 (m, 2H), 1.17 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 443.1 $[M + H]^+$.

*4-Chloro-3-ethyl-1-methyl-N-(4-(1-methyl-1H-pyrazol-3-yl)-benzyl)-1H-pyrazole-5-carboxamide (**4**).* The title compound was prepared according to general procedure F2, starting from **36** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (25%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.95 (t, J = 5.5 Hz, 1H), 7.76−7.71 (m, 3H), 7.36 (d, J = 7.7 Hz, 2H), 6.67 (d, J = 1.7 Hz, 1H), 4.48 (d, J = 5.5 Hz, 2H), 3.87 (s, 3H), 3.84 (s, 3H), 2.57−2.54 (m, 2H), 1.17 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 358.2 $[M + H]^+$.

*4-Chloro-3-ethyl-N-(4-(5-ethyl-1H-pyrazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**5**).* Intermediate **39** was subjected to general procedure B2 to give the corresponding benzylamine, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to afford the title compound as a pink solid (6%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.55 (br, 1H), 8.93 (t, J = 5.9 Hz, 1H), 7.72 (d, J = 7.8 Hz, 2H), 7.35 (d, J = 7.9 Hz, 1H), 6.45 (s, 1H), 4.48 (d, J = 5.9 Hz, 2H), 3.84 (s, 3H), 2.67−2.50 (m, 4H), 1.23−1.14 (m, 6H) ppm; LC−MS m/z: 372.3 $[M + H]^+$.

*4-Chloro-3-ethyl-N-(4-(5-ethyl-1-methyl-1H-pyrazol-3-yl)-benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**6**).* The title compound was prepared according to general procedure F3, starting from **41** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (15%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.93 (t, J = 5.7 Hz, 1H), 7.72 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 6.48 (s, 1H), 4.48 (d, J = 5.9 Hz, 2H), 3.84 (s, 3H), 3.75 (s, 3H), 2.63 (q, J = 7.4 Hz, 2H), 2.55 (q, J = 7.5 Hz, 2H), 1.23 (t, J = 7.4 Hz, 3H), 1.17 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 386.3 $[M + H]^+$.

*4-Chloro-N-(4-(1,4-dimethyl-1H-imidazol-2-yl)benzyl)-3-ethyl-1-methyl-1H-pyrazole-5-carboxamide (**7**).* The title compound was prepared according to general procedure F2, starting from **43** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (25%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.97 (br, 1H), 7.63 (d, J = 8.1 Hz, 2H), 7.42 (d, J = 7.6 Hz, 2H), 6.92 (s, 1H), 4.52 (d, J = 4.2 Hz, 2H), 3.85 (s, 3H), 3.66 (s, 3H), 2.56−2.53 (m, 2H), 2.10 (s, 3H), 1.13 (t, J = 7.3 Hz, 3H); ppm; LC−MS m/z: 372.2 $[M + H]^+$.

*4-Chloro-3-ethyl-1-methyl-N-(4-(4-methyloxazol-2-yl)benzyl)-1H-pyrazole-5-carboxamide (**8**).* The title compound was prepared according to general procedure F3, starting from **45** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (22%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.00 (t, J = 5.6 Hz, 1H), 7.93−7.89 (m, 3H), 7.48 (d, J = 7.8 Hz, 2H), 4.53 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H), 2.58−2.54 (m, 2H), 2.16 (s, 3H), 1.17 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 359.3 $[M + H]^+$.

*4-Chloro-3-ethyl-N-(4-(5-ethyl-1,2,4-oxadiazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**9**).* The title compound was prepared according to general procedure I1, starting from **46** and propionic acid to give a white solid (38%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (t, J = 5.6 Hz, 1H), 7.98 (d, J = 8.0 Hz, 2H), 7.53 (d, J = 7.9 Hz, 2H), 4.55 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H), 3.04−2.98 (q, J = 7.5 Hz, 2H), 2.58−2.54 (m, 2H), 1.34 (t, J = 7.4 Hz, 3H), 1.17 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 374.2 $[M + H]^+$.

*4-Chloro-3-ethyl-N-(4-(3-ethyl-1,2,4-oxadiazol-5-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**10**).* General procedure I1 was followed, starting from **47** and **48** to give *tert*-butyl(4-(3-ethyl-1,2,4-oxadiazol-5-yl)benzyl)carbamate, which was then subjected to general procedure J to give the corresponding HCl salt that was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to afford the title compound as an off-white solid (10%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (t, J = 5.7 Hz, 1H), 8.08 (d, J = 8.1 Hz, 2H), 7.59 (d, J = 7.9 Hz, 2H), 4.58 (d, J = 5.7 Hz, 2H), 3.85 (s, 3H), 2.79 (q, J = 7.5, 2H), 2.58−2.54 (m, 2H), 1.29 (t, J = 7.5 Hz, 3H), 1.17 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 374.2 $[M + H]^+$.

*4-Chloro-3-ethyl-N-((4′-fluoro-[1,1′-biphenyl]-4-yl)methyl)-1-methyl-1H-pyrazole-5-carboxamide (**11**).* General procedure A2 was

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J. Med. Chem. 2019, 62, 3367−3380

followed, starting from (4-bromophenyl)methanamine and (4-fluorophenyl)boronic acid to give (4′-fluoro-[1,1′-biphenyl]-4-yl)-methanamine, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to afford the title compound as an off-white solid (16%). ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.96 (t, J = 5.7 Hz, 1H), 7.72−7.68 (m, 2H), 7.63 (d, J = 8.0 Hz, 2H), 7.43 (d, J = 8.0 Hz, 2H) 7.30−7.26 (m, 2H), 4.51 (d, J = 5.7 Hz, 2H), 3.85 (s, 3H), 2.57−2.54 (m, 2H), 1.17 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 372.2 [M + H] $^+$.

*4-Chloro-3-ethyl-N-((2′-fluoro-4′-methyl-[1,1′-biphenyl]-4-yl)-methyl)-1-methyl-1H-pyrazole-5-carboxamide (**12**).* General procedure A2 was followed, starting from (4-bromophenyl)methanamine and (2-fluoro-4-methylphenyl)boronic acid to give (2′-fluoro-4′-methyl-[1,1′-biphenyl]-4-yl)methanamine, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to afford the title compound as an off-white solid (16%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.96 (t, J = 5.6 Hz, 1H), 7.51−7.41 (m, 2H), 7.44−7.38 (m, 3H), 7.15−7.10 (m, 2H), 4.52 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H), 2.58−2.54 (m, 2H), 2.35 (s, 3H), 1.17 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 386.3 [M + H] $^+$.

*4-Chloro-3-ethyl-1-methyl-N-((5-(4-methylthiazol-2-yl)pyridin-2-yl)methyl)-1H-pyrazole-5-carboxamide (**13**).* General procedure B3 was followed, starting from **49** to give (5-(4-methylthiazol-2-yl)pyridin-2-yl)methanamine HCl, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to give the title compound as a brown solid (13%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05−9.02 (m, 2H), 8.28 (dd, J = 8.0, 2.2 Hz, 1H), 7.51 (d, J = 8.2 Hz, 1H), 7.41 (s, 1H), 4.63 (d, J = 5.8 Hz, 2H), 3.89 (s, 3H), 2.59−2.54 (m, 2H), 2.45 (s, 3H), 1.18 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 376.2 [M + H]$^+$.

*4-Chloro-3-ethyl-N-(2-fluoro-4-(4-methylthiazol-2-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**14**).* The title compound was prepared according to general procedure F3, starting from **50** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (26%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.99 (t, J = 5.0 Hz, 1H), 7.75−7.69 (m, 2H), 7.54−7.50 (m, 1H), 7.38 (s, 1H), 4.54 (d, J = 5.0 Hz, 2H), 3.84 (s, 3H), 2.57−2.42 (m, 2H), 2.32 (s, 3H), 1.16 (t, J = 7.4 Hz, 1H) ppm; LC−MS m/z: 393.3 [M + H]$^+$.

*4-Chloro-3-ethyl-1-methyl-N-((6-(4-methylthiazol-2-yl)pyridin-3-yl)methyl)-1H-pyrazole-5-carboxamide (**15**).* General procedure B3 was followed, starting from **51** to give the corresponding HCl salt, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to give the title compound as a yellow solid (16%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (t, J = 5.4 Hz, 1H), 8.59 (s, 1H), 8.08 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.7 Hz, 1H), 7.41 (s, 1H), 4.55 (d, J = 5.4 Hz, 2H), 3.84 (s, 3H), 2.66−2.44 (m, 2H), 2.33 (s, 3H), 1.77 (t, J = 7.5, 3H) ppm; LC−MS m/z: 376.1 [M + H]$^+$.

*4-Chloro-3-ethyl-N-(3-fluoro-4-(4-methylthiazol-2-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**16**).* The title compound was prepared according to general procedure F3, starting from **52** and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (12% yields). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (t, J = 5.3 Hz, 1H), 8.18 (t, J = 8.2 Hz, 1H), 7.46 (s, 1H), 7.38−7.32 (m, 2H), 4.54 (d, J = 5.9 Hz, 2H), 3.85 (s, 3H), 2.58−2.54 (m, 2H), 2.45 (s, 3H), 1.17 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 393.1 [M + H]$^+$.

*4-Chloro-3-ethyl-N-(4-(5-ethyl-1,2,4-oxadiazol-3-yl)-3-fluorobenzyl)-1-methyl-1H-pyrazole-5-carboxamide (**17**).* General procedure G was followed, starting from **53** to give the desired amidoxime, which was subsequently cyclized with propionic acid according to general procedure I1 to afford the title compound as a white solid (14%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (br s, 1H), 7.98 (d, J = 7.8 Hz, 1H), 7.38−7.35 (m, 2H), 4.54 (d, J = 5.4 Hz, 2H), 3.82 (s, 3H), 3.01−2.97 (t, J = 7.6 Hz, 2H), 2.54−2.47 (m, 2H), 1.31 (t, J = 7.6 Hz, 3H), 1.15 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 392.2 [M + H]$^+$.

*4-Chloro-3-ethyl-N-(4-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-fluorobenzyl)-1-methyl-1H-pyrazole-5-carboxamide (**18**).* Intermediate **54** was subjected to a Boc-deprotection reaction according to general procedure J to give the desired deprotected benzylamine, which was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to give the title compound as a white solid (26%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.05 (t, J = 5.8 Hz, 1H), 8.11 (t, J = 7.8 Hz, 1H), 7.47−7.42 (m, 2H), 4.59 (d, J = 6.0 Hz, 2H), 3.85 (s, 3H), 2.81 (q, J = 7.5 Hz, 2H), 2.59−2.55 (m, 2H), 1.29 (t, J = 7.5 Hz, 3H), 1.18 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 390.2 [M − H]$^-$.

*4-Chloro-3-ethyl-1-methyl-N-(4-(5-methyl-1,2,4-oxadiazol-3-yl)-benzyl)-1H-pyrazole-5-carboxamide (**19**).* The title compound was prepared according to general procedure I1, starting from **46** and acetic acid to give a white solid (42%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (t, J = 5.6 Hz, 1H), 7.97 (d, J = 8.0 Hz, 2H), 7.52 (d, J = 7.8 Hz, 2H), 4.55 (d, J = 5.6 Hz, 2H), 3.85 (s, 3H), 2.66 (s, 3H), 2.58−2.54 (m, 2H), 1.17 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 360.1 [M + H]$^+$.

*4-Chloro-3-ethyl-N-(4-(5-isopropyl-1,2,4-oxadiazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**20**).* The title compound was prepared according to general procedure I2, starting from **46** and isobutyric acid to give a white solid (25%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10−8.04 (m, 2H), 7.46 (d, J = 8.5 Hz, 2H), 7.08 (s, br, 1H), 4.70 (d, J = 5.8 Hz, 2H), 4.15 (s, 3H), 3.34−3.23 (m, 1H), 2.64 (q, J = 7.6 Hz, 2H), 1.46 (d, J = 7.0 Hz, 6H), 1.24 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 387.8 [M + H]$^+$.

*4-Chloro-3-ethyl-1-methyl-N-(4-(5-propyl-1,2,4-oxadiazol-3-yl)-benzyl)-1H-pyrazole-5-carboxamide (**21**).* The title compound was prepared according to general procedure I2, starting from **46** and butyric acid to give a white solid (25%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09−8.04 (m, 2H), 7.46 (d, J = 8.4 Hz, 2H), 7.09 (s, br, 1H), 4.70 (d, J = 5.8 Hz, 2H), 4.15 (s, 3H), 2.93 (t, J = 7.5 Hz, 2H), 2.64 (q, J = 7.6 Hz, 2H), 1.97−1.85 (m, 2H), 1.24 (t, J = 7.6 Hz, 3H), 1.06 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 387.8 [M + H]$^+$.

*4-Chloro-N-(4-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)benzyl)-3-ethyl-1-methyl-1H-pyrazole-5-carboxamide (**22**).* The title compound was prepared according to general procedure I1, starting from **46** and cyclopropanecarboxylic acid to give a white solid (31%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.01 (t, J = 5.56 Hz, 1H), 7.94 (d, J = 7.8 Hz, 2H), 7.51 (d, J = 7.8 Hz, 2H), 4.55 (d, J = 5.5 Hz, 2H), 3.84 (s, 3H), 2.58−2.54 (m, 2H), 2.41−2.40 (m, 1H), 1.29−1.27 (m, 2H), 1.19−1.15 (m, 5H) ppm; LC−MS m/z: 386.1 [M + H]$^+$.

*3-Ethyl-N-(4-(5-ethyl-1,2,4-oxadiazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**23**).* General procedure F2 was followed, starting from 4-(aminomethyl)benzonitrile and 3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give 3-ethyl-*N*-(4-(*N*′-hydroxycarbamimidoyl)benzyl)-1-methyl-1*H*-pyrazole-5-carboxamide, which was subjected to general procedure G to give the desired amidoxime that was subsequently cyclized with propionic acid according to general procedure I1 to give the title compound as a white solid (21%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (t, J = 5.6 Hz, 1H), 7.97 (d, J = 7.9 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 6.73 (s, 1H), 4.49 (d, J = 5.6 Hz, 2H), 3.99 (s, 3H), 3.03−2.98 (m, 2H), 2.57−2.50 (m, 2H), 1.34 (t, J = 7.4 Hz, 3H), 1.16 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 340.1 [M + H]$^+$.

*4-Chloro-N-(4-(5-ethyl-1,2,4-oxadiazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**24**).* The title compound was prepared according to general procedure I2, starting from **56** and propionic acid to give a white solid (5%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06 (d, J = 8.1 Hz, 2H), 7.45 (d, J = 9.8 Hz, 3H), 7.07 (s, br, 1H), 4.69 (d, J = 5.7 Hz, 2H), 4.19 (s, 3H), 2.97 (q, J = 7.6 Hz, 2H), 1.44 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 345.9 [M + H]$^+$.

*3-Ethyl-N-(4-(3-ethyl-1,2,4-oxadiazol-5-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**25**).* General procedure I1 was followed, starting from **47** and **48** to give *tert*-butyl (4-(3-ethyl-1,2,4-oxadiazol-5-yl)benzyl)carbamate, which was then subjected to general procedure J to give the corresponding HCl salt that was subsequently coupled to 3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to afford the title compound as a white solid (7%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.02 (t, J = 5.6 Hz, 1H), 8.04 (d, J = 7.9 Hz, 2H), 7.51 (d, J = 7.6 Hz, 2H), 6.72 (s, 1H), 4.50 (d, J = 5.6 Hz, 2H), 3.97 (s, 3H), 2.77 (q, J = 7.5, 2H), 2.55−2.48 (m,

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2H), 1.26 (t, J = 7.5 Hz, 3H), 1.15 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 340.3 $[M + H]^+$.

*4-Chloro-N-(4-(3-ethyl-1,2,4-oxadiazol-5-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**26**).* General procedure I1 was followed, starting from **47** and **48** to give *tert*-butyl (4-(3-ethyl-1,2,4-oxadiazol-5-yl)benzyl)carbamate, which was then subjected to general procedure J to give the corresponding HCl salt that was subsequently coupled to 4-chloro-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F3 to afford the title compound as a white solid (37%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.08 (t, J = 6.0 Hz, 1H), 8.08 (d, J = 8.2 Hz, 2H), 7.64 (s, 1H), 7.59 (d, J = 8.2 Hz, 2H), 4.60 (d, J = 6.0 Hz, 2H), 3.91 (s, 3H), 2.79 (q, J = 7.5, 2H), 1.28 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 346.0 $[M + H]^+$.

*4-Chloro-N-(4-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**27**).* The title compound was prepared according to general procedure I1, starting from **56** and cyclopropanecarboxylic acid to give a white solid (32%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.07 (t, J = 5.8 Hz, 1H), 7.94 (d, J = 8.0 Hz, 2H), 7.64 (s, 1H), 7.52 (d, J = 7.8 Hz, 2H), 4.56 (d, J = 5.8 Hz, 2H), 3.90 (s, 3H), 2.41−2.40 (m, 1H), 1.29−1.27 (m, 2H), 1.20−1.05 (m, 2H) ppm; LC−MS m/z: 358.1 $[M + H]^+$.

*4-Chloro-N-(4-(1-isopropyl-1H-1,2,4-triazol-3-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**28**).* A mixture of **57** (0.39 g, 1.10 mmol) and isopropyl hydrazine HCl (0.60 g, 5.45 mmol) in pyridine (5 mL) was stirred at room temperature for 16 h. The reaction mixture was concentrated in vacuo, and the residue was washed with diethyl ether twice and dried to afford 4-chloro-*N*-(4-(imino(2-isopropylhydrazineyl)methyl)benzyl)-1-methyl-1*H*-pyrazole-5-carboxamide as a yellow gum, which was dissolved in formic acid (5 mL). The reaction mixture was then stirred at 80 °C for 16 h, cooled to room temperature, and concentrated in vacuo. The residue was diluted with water, and saturated $NaHCO_3$ was added. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give the crude product, which was purified by column chromatography (50% EtOAc in hexane) to afford the title compound as a white solid (7%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.03 (t, J = 5.6 Hz, 1H), 8.58 (s, 1H), 7.97 (d, J = 7.8 Hz, 2H), 7.63 (s, 1H), 7.43 (d, J = 7.8 Hz, 2H), 4.65−4.62 (m, 1H), 4.52 (d, J = 5.6 Hz, 2H), 3.90 (s, 3H), 1.49 (d, J = 6.5 Hz, 6H) ppm; LC−MS m/z: 359.1 $[M + H]^+$.

*4-Chloro-N-(4-(4-cyclopropyloxazol-2-yl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**29**).* Intermediate **58** was subjected to general procedure B3 to give the desired benzylamine, which was subsequently coupled to 4-chloro-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to afford the title compound as a white solid (25%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.04 (t, J = 5.6 Hz, 1H), 7.95 (s, 1H), 7.90 (d, J = 8.2 Hz, 2H), 7.63 (s, 1H), 7.48 (d, J = 8.2 Hz, 2H), 4.54 (d, J = 6.0 Hz, 2H), 3.90 (s, 3H), 1.89−1.85 (m, 1H), 0.90−0.85 (m, 2H), 0.76−0.73 (m, 2H) ppm; LC−MS m/z: 357.1 $[M + H]^+$.

*4-Chloro-N-(4-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-3-fluorobenzyl)-1-methyl-1H-pyrazole-5-carboxamide (**30**).* General procedure J was followed, starting from **60** to give the desired free base, which was subsequently coupled to 4-chloro-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to afford the title compound as an off-white solid (26%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.09 (t, J = 5.9 Hz, 1H), 7.95 (t, J = 7.7 Hz, 1H), 7.65 (s, 1H), 7.40−7.37 (m, 2H), 4.57 (d, J = 5.9 Hz, 2H), 3.90 (s, 3H), 2.50−2.42 (m, 1H), 1.30−1.28 (m, 2H), 1.23−1.18 (m, 2H) ppm; LC−MS m/z: 376.0 $[M + H]^+$.

*4-(4-Methylthiazol-2-yl)benzonitrile (**31**).* The title compound was prepared according to general procedure A1, starting from 2-bromo-4-methylthiazole and (4-cyanophenyl)boronic acid to give a yellow solid (76%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02 (d, J = 8.4 Hz, 2H), 7.69 (d, J = 8.4 Hz, 2H), 6.98 (s, 1H), 2.51 (s, 3H) ppm; ^{13}C NMR (101 MHz, $CDCl_3$): δ 164.9, 154.8, 137.6, 132.7, 126.8, 118.5, 115.4, 113.0, 17.2 ppm; LC−MS m/z: 201.1 $[M + H]^+$.

*(4-(4-Methylthiazol-2-yl)phenyl)methanamine HCl (**32**).* The title compound was prepared according to general procedure B1, starting from **31** to give a yellow solid (98%). ^{1}H NMR (400 MHz, DMSO): δ 8.65 (s, br, 3H), 7.95 (d, J = 8.3 Hz, 2H), 7.62 (d, J = 8.3 Hz, 2H), 7.37 (d, J = 0.9 Hz, 1H), 4.05 (q, J = 5.6 Hz, 2H), 2.43 (d, J = 0.7 Hz, 3H) ppm; ^{13}C NMR (101 MHz, DMSO): δ 165.7, 153.0, 136.0, 132.8, 129.8, 126.0, 115.1, 66.3, 16.7 ppm; LC−MS m/z: 205.1 $[M + H]^+$.

*2-(4-Bromophenyl)-4-(trifluoromethyl)-1,3-thiazole (**33**).* The title compound was prepared according to general procedure D2, starting from 4-bromo-thiobenzamide and 3-bromo-1,1,1-trifluoropropan-2-one to give an off-white solid (52%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (d, J = 8.3 Hz, 2H), 7.74 (s, 1H), 7.59 (d, J = 8.2 Hz, 2H) ppm; LC−MS m/z: 308 $[M]^+$, 310 $[M + H]^+$.

*4-(4-(Trifluoromethyl)thiazol-2-yl)benzonitrile (**34**).* The title compound was prepared according to general procedure C1, starting from **33** to give a yellow solid (98%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (d, J = 8.3 Hz, 2H), 7.84 (s, 1H), 7.76 (d, J = 8.3 Hz, 2H) ppm; LC−MS m/z: 255.2 $[M + H]^+$.

*4-(1-Methyl-1H-pyrazol-3-yl)benzonitrile (**35**).* The title compound was prepared according to general procedure A3, starting from 3-bromo-1-methyl-1*H*-pyrazole and (4-cyanophenyl)boronic acid to give a brown solid (88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.88 (d, J = 8.3 Hz, 2H), 7.66 (d, J = 8.4 Hz, 2H), 7.41 (d, J = 2.2 Hz, 1H), 6.59 (d, J = 2.2 Hz, 1H), 3.95 (d, J = 6.4 Hz, 3H) ppm; LC−MS m/z: 184.3 $[M + H]^+$.

*(4-(1-Methyl-1H-pyrazol-3-yl)phenyl)methanamine (**36**).* The title compound was prepared according to general procedure B2, starting from **35** to give a yellow gum (90%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.71−7.69 (m, 3H), 7.33 (d, J = 8.0 Hz, 2H), 6.64 (d, J = 2.1 Hz, 1H), 3.87 (s, 3H), 3.71 (s, 2H) ppm; LC−MS m/z: 188.1 $[M + H]^+$.

*1-(4-Bromophenyl)pentane-1,3-dione (**37**).* To a stirred solution of 1-(4-bromophenyl)ethan-1-one (5 g, 25.13 mmol) in 50 mL of anhydrous THF was added NaH (50−60%) (2.4 g, 50.25 mmol) portion wise at 0 °C under N_2. The reaction was stirred at 0 °C for 1 h before ethyl propionate (5.8 mL, 50.25 mmol) was added. The resulting reaction mixture was stirred at room temperature for 12 h. Upon completion, the reaction mixture was quenched with ice cold water and acidified by 4 N HCl. The aqueous layer was extracted with EtOAc, and the combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (30% EtOAc in hexane) to afford the title compound as a white solid (5.5 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 16.02 (s, 1H), 7.73 (d, J = 8.4 Hz, 2H), 7.57 (d, J = 8.4 Hz, 2H), 6.13 (s, 1H), 2.45 (q, J = 7.3 Hz, 2H), 1.30 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 255.2 $[M]^+$, 257.2 $[M + 2]^+$ (1:1 bromo pattern).

*3-(4-Bromophenyl)-5-ethyl-1H-pyrazole (**38**).* In a 50 mL round-bottom flask, 10 mL of THF was added under N_2; then, $N_2H_4{\cdot}H_2O$ (2.41 mL, 49.02 mmol) was added dropwise at 0 °C, and the mixture was stirred for 5 min. After that **37** (5 g, 19.61 mmol) in THF (20 mL) was added, and the reaction mixture was stirred at 75 °C for 2 h. Upon completion, the reaction mixture was cooled to room temperature and then extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (30% EtOAc in hexane) to afford the title compound as a yellow solid (2.5 g, 51%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.61 (d, J = 8.0 Hz, 2H), 7.50 (d, J = 8.2 Hz, 2H), 6.35 (s, 1H), 2.71 (q, J = 7.5 Hz, 2H), 1.30 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 251.1 $[M]^+$, 253.1 $[M + 2]^+$ (1:1 bromo pattern).

*4-(5-Ethyl-1H-pyrazol-3-yl)benzonitrile (**39**).* The title compound was prepared according to general procedure C1, starting from **38** to give a yellow gum (70%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.92−10.09 (br, 1H), 7.87 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 8.2 Hz, 2H), 6.43 (s, 1H), 2.74 (q, J = 7.5 Hz, 2H), 1.32 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 198.2 $[M + H]^+$.

*4-(5-Ethyl-1-methyl-1H-pyrazol-3-yl)benzonitrile (**40**).* To a stirred solution of **39** (600 mg, 3.05 mmol) in DCM (15 mL) was added Na_2CO_3 (646 mg, 6.09 mmol). The suspension was stirred at room temperature before Me_2SO_4 (0.29 mL, 3.05 mmol) was added

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J. Med. Chem. 2019, 62, 3367−3380

dropwise. The reaction mixture was stirred at 60 °C for 2 h, cooled to room temperature, and diluted with DCM. The precipitate was filtered and washed with DCM. The filtrate was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by column chromatography (30% EtOAc in hexane) to afford the title compound as a yellow liquid (250 mg, 39%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.85 (d, J = 8.0 Hz, 2H), 7.64 (d, J = 8.2 Hz, 2H), 6.38 (s, 1H), 3.83 (s, 3H), 2.64 (q, J = 7.5 Hz, 2H), 1.31 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 212.2 $[M + H]^+$.

*(4-(5-Ethyl-1-methyl-1H-pyrazol-3-yl)phenyl)methanamine (**41**).* The title compound was prepared according to general procedure B2, starting from **40** to give a white gum (95%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.72 (d, J = 7.8 Hz, 2H), 7.30 (d, J = 7.8 Hz, 2H), 6.32 (s, 1H), 3.91 (m, 2H), 3.86 (s, 3H), 3.67 (m, 2H), 2.63 (q, J = 7.5 Hz, 2H), 1.30 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 216.3 $[M + H]^+$.

*4-(1,4-Dimethyl-1H-imidazol-2-yl)benzonitrile (**42**).* The title compound was prepared according to general procedure A3, starting from 2-bromo-1,4-dimethyl-1*H*-imidazole and (4-cyanophenyl)-boronic acid to give a yellow solid (92%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.73 (d, J = 7.9 Hz, 2H), 7.71 (d, J = 8.3 Hz, 2H) 6.73 (s, 1H), 3.73 (s, 3H), 2.26 (s, 3H) ppm; LC−MS m/z: 198.3 $[M + H]^+$.

*(4-(1,4-Dimethyl-1H-imidazol-2-yl)phenyl)methanamine (**43**).* The title compound was prepared according to general procedure B2, starting from **42** to give a yellow solid (88%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 7.56 (d, J = 8.0 Hz, 2H), 7.39 (d, J = 8.0 Hz, 2H), 6.90 (s, 1H), 3.75 (s, 2H), 3.65 (s, 3H), 2.11 (s, 3H); ppm; LC−MS m/z: 202.2 $[M + H]^+$.

*4-(4-Methyloxazol-2-yl)benzonitrile (**44**).* General procedure E was followed, starting from bromoacetone and 4-bromobenzamide to afford 2-(4-bromophenyl)-4-methyloxazole as a yellow solid, which was subjected to a cyanation reaction according to general procedure C2 to give the title compound as a white solid (37%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10 (d, J = 8.4 Hz, 2H), 7.72 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 0.9 Hz, 1H), 2.26 (d, J = 1.0 Hz, 3H) ppm; LC−MS m/z: 185 $[M + H]^+$.

*(4-(4-Methyloxazol-2-yl)phenyl)methanamine HCl (**45**).* The title compound was prepared according to general procedure B3, starting from **44** to give a white solid (85%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.53 (br s, 3H), 7.98 (d, J = 8.2 Hz, 2H), 7.93 (d, J = 1.0 Hz, 1H), 7.64 (d, J = 8.3 Hz, 2H), 4.10−4.06 (m, 2H), 2.17 (d, J = 0.8 Hz, 3H) ppm; LC−MS m/z: 189 $[M + H]^+$.

*4-Chloro-3-ethyl-N-(4-(N′-hydroxycarbamimidoyl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**46**).* General procedure F2 was followed, starting from 4-aminomethyl-benzonitrile and 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid to give 4-chloro-*N*-(4-cyanobenzyl)-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxamide, which was subjected to general procedure G to give the title compound as a brown gum (53%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.58 (s, 1H), 8.94 (t, J = 5.9 Hz, 1H), 7.64 (d, J = 8.2 Hz, 2H), 7.34 (d, J = 8.2 Hz, 2H), 5.79 (s, 2H), 4.48 (d, J = 5.9 Hz, 1H), 3.84 (s, 3H), 2.57−2.54 (m, 2H), 1.17 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 336.1 $[M + H]^+$.

*4-(((tert-Butoxycarbonyl)amino)methyl)benzoic Acid (**47**).* The title compound was prepared according to general procedure H1, starting from 4-aminomethyl-benzoic acid to give a white solid (39%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.78 (br s, 1H), 7.89 (d, J = 7.8 Hz, 2H), 7.47 (t, J = 5.4 Hz, 1H), 7.33 (d, J = 7.7 Hz, 2H), 4.18 (d, J = 5.4 Hz, 2H), 1.39 (s, 9H) ppm; LC−MS m/z: 252 $[M + H]^+$.

*N-Hydroxypropionimidamide (**48**).* The title compound was prepared according to general procedure G, starting from propionitrile to give a pale yellow liquid (87%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.72 (s, 1H), 5.28 (s, 2H), 1.99−1.93 (m, 2H), 1.01 (t, J = 7.4 Hz, 3H) ppm; LC−MS m/z: 89 $[M + H]^+$.

*5-(4-Methylthiazol-2-yl)picolinonitrile (**49**).* The title compound was prepared according to general procedure A4, starting from 5-bromopicolinonitrile to give title compound as a white solid (88%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.22 (d, J = 1.8 Hz, 1H), 8.35 (dd, J = 8.1, 2.0 Hz, 1H), 7.75 (d, J = 8.1 Hz, 1H), 7.07 (s, 1H), 2.54 (s, 3H) ppm; LC−MS m/z: 202.3 $[M + H]^+$.

*(2-Fluoro-4-(4-methylthiazol-2-yl)phenyl)methanamine HCl (**50**).* General procedure A2 was followed, starting from 2-bromo-4-methylthiazole and (4-cyano-3-fluorophenyl)boronic acid to give 2-fluoro-4-(4-methyl-1,3-thiazol-2-yl)benzonitrile, which was subjected to general procedure B3 to give the title compound as a white solid (80%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.55 (br, 3H), 7.83−7.77 (m, 2H), 7.73−7.69 (m, 1H), 7.43 (s, 1H), 4.11−4.07 (m, 2H), 2.40 (s, 3H) ppm; LC−MS m/z: 223.1 $[M + H]^+$.

*6-(4-Methylthiazol-2-yl)nicotinonitrile (**51**).* A mixture of 5-bromopicolinonitrile (50 mg, 0.27 mmol), $(NH_4)_2S$ (0.02 mL, 0.3 mmol), and Et_3N (0.04 mL, 0.3 mmol) in pyridine (1 mL) was stirred at 50 °C for 4 h. Upon completion, the reaction mixture was cooled and extracted with EtOAc (3 × 10 mL). The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo to give 5-bromopyridine-2-carbothioamide, which was directly subjected to cyclization according to general procedure D1 to give 2-(5-bromopyridin-2-yl)-4-methylthiazole that was then subjected to general procedure C to give the title compound as a brown solid (54%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.83 (s, 1H), 8.27 (d, J = 8.2 Hz, 1H), 8.03 (dd, J = 8.3, 1.7 Hz, 1H), 7.12 (s, 1H), 2.53 (s, 3H) ppm; LC−MS m/z: 202.2 $[M + H]^+$.

*(3-Fluoro-4-(4-methylthiazol-2-yl)phenyl)methanamine (**52**).* To a stirring solution of 3-fluoro-4-iodo-benzonitrile (2.5 g, 10.12 mmol) in a mixture of THF (20 mL) and diethyl ether (20 mL), ^{i}PrMgCl (6 mL, 2 M in diethyl ether) was added dropwise at −78 °C under N_2. The mixture was stirred at −78 °C for another 1.5 h before triisopropyl borate (3.74 mL, 16.19 mmol) was added dropwise. The mixture was then stirred at −78 °C for 15 min and then allowed to warm to room temperature. After 3 h at room temperature, 2 M HCl was added, and the reaction mixture was stirred at room temperature for 20 min. The reaction mixture was diluted with water and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated to afford the 4-(dihydroxyboranyl)-3-fluorobenzonitrile, which was directly subjected to a Suzuki coupling reaction according to general procedure A2 to give 3-fluoro-4-(4-methylthiazol-2-yl)benzonitrile that was then subjected to general procedure B2 to give the title compound as a colorless oil (32%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.11 (t, J = 7.9 Hz, 1H), 7.42 (s, 1H), 7.39−7.36 (m, 1H), 7.29 (d, J = 7.9 Hz, 1H), 3.77 (s, 2H), 2.45 (s, 3H) ppm; LC−MS m/z: 223.0 $[M + H]^+$.

*4-Chloro-N-(4-cyano-3-fluorobenzyl)-3-ethyl-1-methyl-1H-pyrazole-5-carboxamide (**53**).* General procedure K was followed, starting from 2-fluoro-4-methylbenzonitrile to afford 4-(bromomethyl)-2-fluorobenzonitrile as a colorless oil, which was then converted to the desired benzylamine according to general procedure L. The resulting benzylamine was subsequently coupled to 4-chloro-3-ethyl-1-methyl-1*H*-pyrazole-5-carboxylic acid according to general procedure F2 to give the title compound as a white solid (72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.62−7.58 (m, 1H), 7.25−7.19 (m, 2H), 4.68 (d, J = 6.1 Hz, 2H), 4.11 (s, 3H), 2.63 (q, J = 7.6 Hz, 3H), 1.22 (t, J = 7.6 Hz, 3H) ppm; LC−MS m/z: 321.1 $[M + H]^+$.

*tert-Butyl(4-(3-ethyl-1,2,4-oxadiazol-5-yl)-3-fluorobenzyl)-carbamate (**54**).* General procedure B4 was followed, starting from 4-cyano-2-fluorobenzoic acid to give the desired benzylamine as a free base, which was then protected with a Boc group according to general procedure H1 and then cyclized with **48** according to general procedure I2 to give the title compound as a white solid (73%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.10−8.06 (m, 1H), 7.55 (t, J = 6.0 Hz, 1H), 7.33−7.30 (m, 2H), 4.24 (d, J = 6.0 Hz, 2H), 2.81 (q, J = 7.4 Hz, 2H), 1.40 (s, 9H), 1.27 (t, J = 7.5 Hz, 3H) ppm; LC−MS m/z: 322.2 $[M + H]^+$.

*4-Chloro-N-(4-cyanobenzyl)-1-methyl-1H-pyrazole-5-carboxamide (**55**).* The title compound was prepared according to general procedure F2, starting from 4-aminomethyl-benzonitrile and 4-chloro-1-methyl-1*H*-pyrazole-5-carboxylic acid to give a white solid (85%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.65 (d, J = 7.8 Hz, 2H), 7.50−7.40 (m, 3H), 7.12 (br s, 1H), 4.70 (d, J = 5.7 Hz, 2H), 4.18 (s, 3H) ppm; LC−MS m/z: 275 $[M + H]^+$.

*4-Chloro-N-(4-(N′-hydroxycarbamimidoyl)benzyl)-1-methyl-1H-pyrazole-5-carboxamide (**56**).* The title compound was prepared

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according to general procedure G, starting from **55** to give a white solid (60%), which was taken for the next step without purification. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.59 (s, 1H), 9.00 (s, 1H), 7.64−7.63 (m, 3H), 7.34 (d, J = 6.9 Hz, 2H), 5.78 (m, 2H), 4.49 (s, 2H), 3.89 (s, 3H) ppm; LC−MS m/z: 308.1 $[M + H]^+$.

*Ethyl 4-((4-Chloro-1-methyl-1H-pyrazole-5-carboxamido)-methyl)benzimidate HCl (**57**).* To an ice-cooled stirred solution of **55** (0.3 g, 1.10 mmol) in EtOH, HCl gas was bubbled for 1.5 h. The reaction mixture was stirred at room temperature for 30 min. Upon completion, the reaction mixture was concentrated in vacuo. The residue was then washed with diethyl ether and dried to afford the title compound as a white solid in a quantitative yield. ^{1}H NMR (400 MHz, DMSO-d_6): δ 11.6 (br s, 2H), 9.13 (t, J = 6.0 Hz, 1H), 8.08 (d, J = 8.4 Hz, 2H), 7.65 (s, 1H), 7.61 (d, J = 8.3 Hz, 2H), 4.63−4.58 (m, 4H), 3.90 (s, 3H), 1.49 (t, J = 7.0 Hz, 3H) ppm; LC−MS m/z: 321.1 $[M + H]^+$.

*4-(4-Cyclopropyloxazol-2-yl)benzonitrile (**58**).* General procedure E was followed, starting from 2-bromo-1-cyclopropylethan-1-one and 4-bromobenzamide to afford 2-(4-bromophenyl)-4-cyclopropyloxazole as a yellow solid, which was subjected to a cyanation reaction according to general procedure C2 to give the title compound as a white solid (21%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.89−8.86 (m, 3H), 8.77 (d, J = 7.8 Hz, 2H), 2.80−2.70 (m, 1H), 1.71−1.69 (m, 2H), 1.59−1.56 (m, 2H) ppm; LC−MS m/z: 211.1 $[M + H]^+$.

*tert-Butyl (4-Cyano-3-fluorobenzyl)carbamate (**59**).* General procedure K was followed, starting from 2-fluoro-4-methylbenzonitrile to afford 4-(bromomethyl)-2-fluorobenzonitrile as a colorless oil, which was then converted to the desired benzylamine according to general procedure L. The resulting benzylamine was subsequently protected according to general procedure H2 to give an off-white solid (68%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.57 (t, J = 6.8 Hz, 1H), 7.17−7.12 (m, 2H), 4.98 (br s, 1H), 4.35 (d, J = 4.8 Hz, 2H), 1.45 (s, 9H) ppm; LC−MS m/z: 251.4 $[M + H]^+$.

*tert-Butyl(4-(5-cyclopropyl-1,2,4-oxadiazol-3-yl)-3-fluorobenzyl)-carbamate (**60**).* General procedure G was followed, starting from **59** to give the desired amidoxime, which was then subjected to general procedure I1 with cyclopropanecarboxylic acid to give the title compound as a white solid (31%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.97−7.93 (t, J = 7.7 Hz, 1H), 7.17−7.12 (m, 2H), 4.93 (s, 1H), 4.35 (br, 2H), 2.30−2.20 (m, 1H), 1.46 (s, 9H), 1.30−1.24 (m, 4H) ppm; LC−MS m/z: 334.1 $[M + H]^+$.

Interference Compounds. All final compounds have been examined for the presence of substructures classified as Pan Assay Interference Compounds using a KNIME workflow.[19,20]

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.jmedchem.8b01790.

SMILES molecular formula strings (CSV)

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

ACN, acetonitrile; DCM, dichloromethane; DMF, dimethylformamide; LHS, left hand side; RHS, right hand side; **TFP**, tolfenpyrad

■ REFERENCES

(1) Van Wyk, J. A.; Malan, F. S.; Randles, J. L. How Long before Resistance Makes It Impossible to Control Some Field Strains of Haemonchus Contortus in South Africa with Any of the Modern Anthelmintics? *Vet. Parasitol.* **1997**, *70*, 111−122.

(2) Knox, M. R.; Besier, R. B.; Le Jambre, L. F.; Kaplan, R. M.; Torres-Acosta, J. F. J.; Miller, J.; Sutherland, I. Novel Approaches for the Control of Helminth Parasites of Livestock VI: Summary of Discussions and Conclusions. *Vet. Parasitol.* **2012**, *186*, 143−149.

(3) Woods, D. J.; Lauret, C.; Geary, T. G. Anthelmintic Discovery and Development in the Animal Health Industry. *Expert Opin. Drug Discovery* **2007**, *2*, S25−S33.

(4) Wolstenholme, A. J.; Kaplan, R. M. Resistance to Macrocyclic Lactones. *Curr. Pharm. Biotechnol.* **2012**, *13*, 873−887.

(5) Kaplan, R. M.; Vidyashankar, A. N. An Inconvenient Truth: Global Worming and Anthelmintic Resistance. *Vet. Parasitol.* **2012**, *186*, 70−78.

(6) Kotze, A. C.; Prichard, R. K. Chapter Nine—Anthelmintic Resistance in Haemonchus Contortus: History, Mechanisms and Diagnosis. In *Advances in Parasitology*; Gasser, R. B., Samson-

DOI: 10.1021/acs.jmedchem.8b01790
J. Med. Chem. 2019, 62, 3367−3380

Himmelstjerna, G. V. B. T.-A., Eds.; Academic Press, 2016; Vol. *93*; pp 397−428.
(7) Kaminsky, R.; Gauvry, N.; Schorderet Weber, S.; Skripsky, T.; Bouvier, J.; Wenger, A.; Schroeder, F.; Desaules, Y.; Hotz, R.; Goebel, T.; Hosking, B. C.; Pautrat, F.; Wieland-Berghausen, S.; Ducray, P. Identification of the Amino-Acetonitrile Derivative Monepantel (AAD 1566) as a New Anthelmintic Drug Development Candidate. *Parasitol. Res.* **2008**, *103*, 931−939.
(8) Prichard, R. K.; Geary, T. G. NEWS & VIEWS Fresh Hope to Can the Worms Patchy Solutions. *Nature* **2008**, *452*, 157−158.
(9) Van den Brom, R.; Moll, L.; Kappert, C.; Vellema, P. Haemonchus Contortus Resistance to Monepantel in Sheep. *Vet. Parasitol.* **2015**, *209*, 278−280.
(10) Love, S. C. J.; Neilson, F. J. A.; Biddle, A. J.; McKinnon, R. Moxidectin-Resistant Haemonchus Contortus in Sheep in Northern New South Wales. *Aust. Vet. J.* **2003**, *81*, 359−360.
(11) Preston, S.; Jiao, Y.; Jabbar, A.; McGee, S. L.; Laleu, B.; Willis, P.; Wells, T. N. C.; Gasser, R. B. Screening of the 'Pathogen Box' identifies an approved pesticide with major anthelmintic activity against the barber's pole worm. *Int. J. Parasitol.: Drugs Drug Resist.* **2016**, *6*, 329−334.
(12) Le, T. G.; Kundu, A.; Ghoshal, A.; Nguyen, N. H.; Preston, S.; Jiao, Y.; Ruan, B.; Xue, L.; Huang, F.; Keiser, J.; Hofmann, A.; Chang, B. C. H.; Garcia-Bustos, J.; Jabbar, A.; Wells, T. N. C.; Palmer, M. J.; Gasser, R. B.; Baell, J. B. Optimization of Novel 1-Methyl-1H-Pyrazole-5-carboxamides Leads to High Potency Larval Development Inhibitors of the Barber's Pole Worm. *J. Med. Chem.* **2018**, *61*, 10875−10894.
(13) Le, T. G.; Kundu, A.; Ghoshal, A.; Nguyen, N. H.; Preston, S.; Jiao, Y.; Ruan, B.; Xue, L.; Huang, F.; Keiser, J.; Hofmann, A.; Chang, B. C. H.; Garcia-Bustos, J.; Wells, T. N. C.; Palmer, M. J.; Jabbar, A.; Gasser, R. B.; Baell, J. B. Structure-Activity Relationship Studies of Tolfenpyrad Reveal Subnanomolar Inhibitors of Haemonchus contortus Development. *J. Med. Chem.* **2019**, *62*, 1036−1053.
(14) Preston, S.; Jiao, Y.; Baell, J. B.; Keiser, J.; Crawford, S.; Koehler, A. V.; Wang, T.; Simpson, M. M.; Kaplan, R. M.; Cowley, K. J.; Simpson, K. J.; Hofmann, A.; Jabbar, A.; Gasser, R. B. Screening of the 'Open Scaffolds' collection from Compounds Australia identifies a new chemical entity with anthelmintic activities against different developmental stages of the barber's pole worm and other parasitic nematodes. *Int. J. Parasitol.: Drugs Drug Resist.* **2017**, *7*, 286−294.
(15) Monroy, F. G.; Enriquez, F. J. Heligmosomoides Polygyrus: A Model for Chronic Gastrointestinal Helminthiasis. *Parasitol. Today* **1992**, *8*, 49−54.
(16) Lipinski, C. A. Lead- and Drug-like Compounds: The Rule-of-Five Revolution. *Drug Discovery Today: Technol.* **2004**, *1*, 337−341.
(17) Veber, D. F.; Johnson, S. R.; Cheng, H.-Y.; Smith, B. R.; Ward, K. W.; Kopple, K. D. Molecular Properties That Influence the Oral Bioavailability of Drug Candidates. *J. Med. Chem.* **2002**, *45*, 2615−2623.
(18) Jiao, Y.; Preston, S.; Song, H.; Jabbar, A.; Liu, Y.; Baell, J.; Hofmann, A.; Hutchinson, D.; Wang, T.; Koehler, A. V.; Fisher, G. M.; Andrews, K. T.; Laleu, B.; Palmer, M. J.; Burrows, J. N.; Wells, T. N. C.; Wang, Q.; Gasser, R. B. Assessing the Anthelmintic Activity of Pyrazole-5-Carboxamide Derivatives against Haemonchus Contortus. *Parasites Vectors* **2017**, *10*, 1−8.
(19) Baell, J. B.; Holloway, G. A. New Substructure Filters for Removal of Pan Assay Interference Compounds (PAINS) from Screening Libraries and for Their Exclusion in Bioassays. *J. Med. Chem.* **2010**, *53*, 2719−2740.
(20) Saubern, S.; Guha, R.; Baell, J. B. KNIME Workflow to Assess PAINS Filters in SMARTS Format. Comparison of RDKit and Indigo Cheminformatics Libraries. *Mol. Inf.* **2011**, *30*, 847−850.

DOI: 10.1021/acs.jmedchem.8b01790
J. Med. Chem. 2019, 62, 3367−3380