Available online at www.sciencedirect.com
ScienceDirect
Bioorganic & Medicinal Chemistry Letters 17 (2007) 6707–6713
Bioorganic & Medicinal Chemistry Letters

Design and synthesis of potent amido- and benzyl-substituted *cis*-3-amino-4-(2-cyanopyrrolidide)pyrrolidinyl DPP-IV inhibitors

J. W. Corbett,* K. Dirico, W. Song, B. P. Boscoe, S. D. Doran, D. Boyer, X. Qiu, M. Ammirati, M. A. VanVolkenburg, R. K. McPherson, J. C. Parker and E. D. Cox

Pfizer Global Research and Development, Eastern Point Road, Groton, CT 06340, USA

Received 21 August 2007; revised 12 October 2007; accepted 16 October 2007
Available online 22 October 2007

Abstract—The *cis*-3-amino-4-(2-cyanopyrrolidide)-pyrrolidine template has been shown to afford low nanomolar inhibitors of human DPP-IV that exhibit a robust PK/PD profile. An X-ray co-crystal structure of **5** confirmed the proposed mode of binding. The potent single digit DPP-IV inhibitor **53** exhibited a preferred PK/PD profile in preclinical animal models and was selected for additional profiling.

Type 2 diabetes mellitus (T2DM) is becoming increasingly prevalent and globally has reached epidemic proportions. It is currently estimated that more than 180 million people worldwide have the disease with an estimated 1.1 million deaths in 2005.[1] Even more disturbing are the projections that the number of affected people will double by 2030. Type 2 diabetes is a chronic disease that is exacerbated by a sedentary lifestyle and obesity, and is characterized by abnormal glucose regulation, with diabetics at increased risk of cardiovascular, eye, kidney, and nerve damage. Type 2 diabetics are unable to maintain adequate control of blood sugar through the action of endogenously generated insulin, even though they generally have functioning pancreatic islet β-cells. The incretin hormone glucagon-like peptide-1 (GLP-1) stimulates the release of insulin from β-cells. GLP-1 administration has been shown to have a beneficial effect on islet β-cell function and on maintenance of proper blood glucose levels without the induction of hypoglycemia.[2] However, GLP-1 is rapidly degraded and inactivated by the serine protease dipeptidyl peptidase IV (DPP-IV). Therefore, the discovery of safe, tolerated DPP-IV inhibitors is predicted to provide effective medicaments for the management of T2DM.

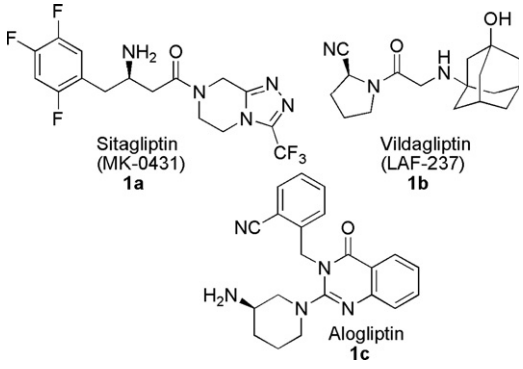


Figure 1. DPP-IV inhibitors currently in market or Phase III clinical trials.

Several DPP-IV inhibitors have been tested in the clinic, including multiple compounds that are now in Phase III clinical trials (Fig. 1).[3–5] Sitagliptin (**1a**, Januvia) has been approved for use in Mexico and has received approval from the U.S. Food and Drug Administration.[5]

Structure-based drug design was used, in conjunction with the published crystal structure of *N*-L-valylpyrrolidine, to propose the DPP-IV inhibitor scaffold *cis*-3-amino-4-pyrrolididepyrrolidine **2** (Fig. 2). The appropriately substituted pyrrolidine ring was predicted to project the amide-backbone NH and amine interactions in the same orientation as found in *N*-L-valylpyrro-

Keywords: Dipeptidyl peptidase IV; DPP-IV; Diabetes.
* Corresponding author. Tel.: +1 860 686 0252; e-mail: jeffrey.w.corbett@pfizer.com

0960-894X/$ - see front matter © 2007 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2007.10.063

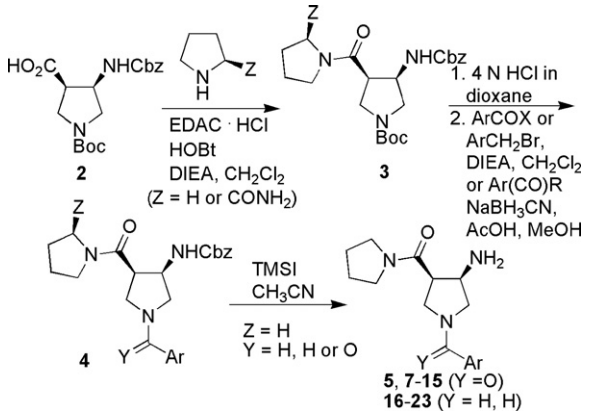


Figure 2. Synthesis of chiral *cis*-3-amino-4-(pyrrolidide)pyrrolidinyl single enantiomers.

lidine.[6,7] Modest DPP-IV inhibition (<200 nM) was achieved with the achiral *cis*-aminopyrrolidide template (data not shown), so an exhaustive SAR effort was mounted. An asymmetric synthesis of the desired template was effected using a published procedure and both enantiomers of the template were prepared (Fig. 2).[8] It was found that 3*R*,4*R*-pyrrolidinyl template (**2**) exhibited DPP-IV activity, as evidenced by the in vitro activity of enantiomers **5** and **6** (Fig. 3).[9] Diastereomeric pairs of compounds **5** and **6** were not prepared.

A representation of the single crystal X-ray structure of **5** in human DPP-IV in a 6 Å binding site surface is

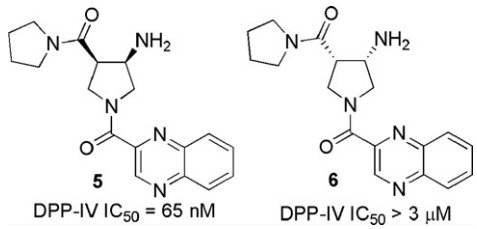


Figure 3. Structure of chiral DPP-IV inhibitors **5** and **6**.

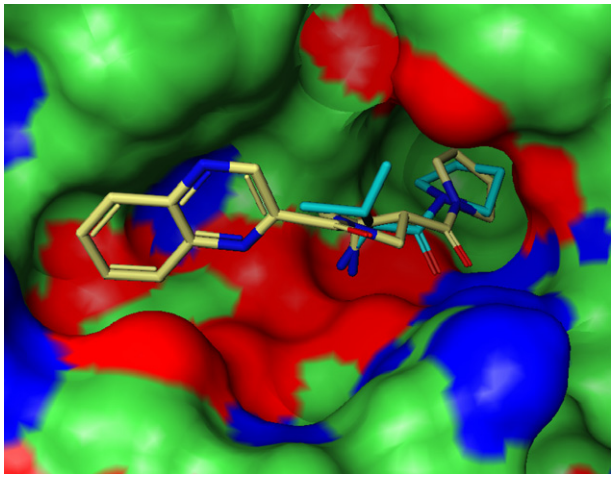


Figure 4. *N*-L-Valylpyrrolidine (blue) overlayed with compound **5** (yellow).

shown in Figure 4, where *N*-L-valylpyrrolidine is overlayed in blue onto molecule **5** (in yellow).[10] The amine and pyrrolidide groups have the anticipated orientation as was predicted by modeling. The pyrazine ring of the quinoxalinyl-group forms favorable aromatic stacking interactions with Phe357, but part of the benzene ring binds polar atoms of Glu205, Glu206, Ser209, and Arg358, suggesting further opportunities for exploring enzyme–ligand interactions.

Initial SAR work then focused on preparing amide derivatives using chiral template **2**. Several of the more potent human DPP-IV inhibitors are shown in Table 1. Good selectivity was observed against a select panel of other DPP enzymes with the most potent pyrrolidinyl-amide analog, compound **7**, having an IC_{50} against human DPP-IV of 30 nM.[11]

The pharmacokinetics of compound **5** was evaluated in Sprague–Dawley rats and had $t_{1/2}$ of 1.7 ± 0.5 h and $\%F = 90$.[9] The relatively favorable PK profile of **5** provided further impetus to continue evaluating the series. Hence, pyrrolidinyl-benzyl derivatives were evaluated and compounds with human DPP-IV IC_{50}'s < 100 nM are shown in Table 2. Cursory analysis of the benzyl derivatives indicated decreased DPP-IV inhibitory potency and increased displacement of dofetilide in a dofetilide binding assay[12] (e.g., 32% and 9% dofetilide binding inhibition for benzyl and amide analogs **16** and **12**, respectively). However, improved cell permeability of the benzyl series relative to the amides led to their continued investigation. The dofetilide binding assay was found to be a useful indicator of hERG ion channel activity and we demonstrated a correlation between the dofetilide displacement assay and the hERG patch clamp assay for a subset of compounds.[13,14] The hERG/dofetilide correlation suggested that <20% inhibition of dofetilide binding was desired in order to minimize hERG activity.

Analogs containing a 2-cyanopyrrolidide were prepared using the synthetic scheme shown in Figure 5 in an effort to obtain inhibitors with increased potency. The benzyl bromides used to synthesize final products **25**, **26**, **28**, **29**, and **31**–**34** were prepared following the method of Tanaka et al.[15]

Several of the resultant analogs exhibited a significantly improved inhibitory profile (Table 3). Analysis of the emerging SAR indicated that it was possible to obtain potent DPP-IV inhibitors with >100-fold selectivity relative to DPP-2 and -8. However, there was a trend toward increased inhibition of dofetilide binding and DPP-8 inhibition. Therefore, dofetilide binding was identified as a property that required removal from a target molecule. Compound **28** was selected for PK/PD determination since it exhibited excellent DPP-IV activity and low potential for hERG activity.[13] Unfortunately, maximal DPP-IV inhibition in Sprague–Dawley rats was only 51% between 4 and 6 h post-dose (5 mg/kg po). This pharmacodynamic effect was considered suboptimal and may be attributed to modest oral bioavailability (36 ± 15%). Therefore, a careful analysis of

Table 1. Biological activity of (3*R*,4*R*)-*cis*-3-amino-4-(pyrrolidide)pyrrolidinyl amide derivatives with human DPP-IV IC_{50} < 100 nM

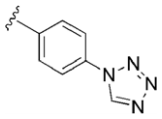
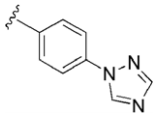
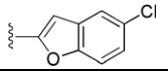
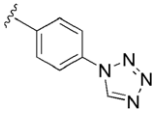
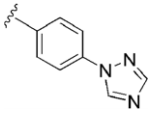
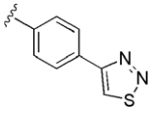
Compound	Ar	Human DPP-4 IC_{50} (nM)	Human DPP-8 IC_{50} (μM)	Percent dofetilide binding inhibition (10 μM)
7		30	27.4	15
8	Quinolin-6-yl	38	26.4	19
9		40	>30	14
10	Benzothiazol-6-yl	42	23.1	0.9
11	Isoquinolin-3-yl	47	>30	0.5
12	Quinolin-3-yl	49	>30	9
13	Benzothiaphen-2-yl	62	10.2	5
14	Naphthalen-2-yl	79	24.1	4
15		86	4.25	14

Table 2. Biological activity of (3*R*,4*R*)-*cis*-3-amino-4-pyrrolidinyl benzyl derivatives with human DPP-IV IC_{50} < 100 nM

Compound	Ar	R	Human DPP-4 IC_{50} (nM)	Human DPP-8 IC_{50} (μM)	Percent dofetilide binding inhibition[a] (10 μM)
16	Quinolin-3-yl	H	27	5.1	32
17		H	50	1.2	43
18		H	69	4.7	ND
19	3-Cyano-4-fluorophenyl	H	78	3.1	9
20	3,4-Difluorophenyl	H	80	2.5	19
21	*p*-Cyanophenyl	H	81	1.9	ND
22	*p*-Nitrophenyl	CH_3	83	1.8	13
23		H	91	1.0	84

[a] ND, no data.

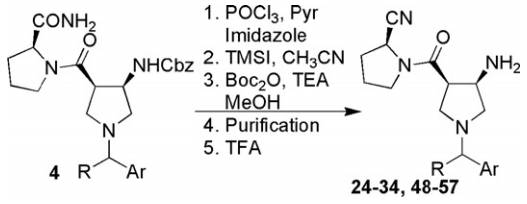
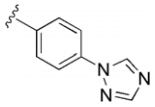
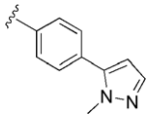
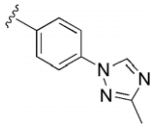
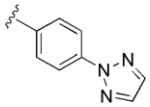
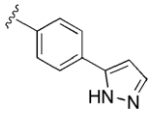
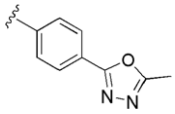
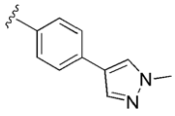
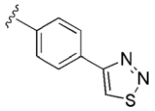


Figure 5. Synthesis of 2-cyano-pyrrolidide analogs.

extant dofetilide inhibition binding data was undertaken and multiple SAR trends were identified that correlated with decreased displacement of dofetilide binding: (1) α-methylbenzyl substitution (compound **22** had 13% inhibition of dofetilide binding, while the corresponding des-methyl analog (data not shown) had 27% inhibition of dofetilide binding); (2) heterocyclic substitution of *p*-heteroarylbenzyl compounds (e.g., triazolyl derivatives **25** and **28**); and (3) *m*-substituted benzyl analogs (e.g., *m*-cyano-derivatives **19** and **27**).

A series of compounds were subsequently synthesized to test these hypotheses in an attempt to identify low single digit nanomolar DPP-IV inhibitors possessing low inhibition of dofetilide binding. The requisite benzyl bromides were either commercially available or were prepared as shown in Figure 6. Of note, the preparation of 4-cyano-3-methylbenzyl bromide **37** required the selective reduction of methyl ester **36**, which was initially

Table 3. Biological activity of (3*R*,4*R*)-*cis*-3-amino-4-cyanopyrrolidide benzyl derivatives with human DPP-IV $IC_{50} \leq 10$ nM

Compound	Ar	R	Human DPP-4 IC_{50} (nM)	Human DPP-8 IC_{50} (nM)	Percent dofetilide binding inhibition (10 μM)
24	*p*-Cyanophenyl	H	2.4	560	47
25		H	3.2	461	40
26		H	3.3	148	36
27	3-Cyano-4-fluoro	H	3.6	922	10
28		H	5.3	858	4
29		H	5.4	247	49
30	Quinolin-3-yl	H	6.2	3290	36
31		H	6.7	326	65
32		H	8.5	704	4
33		H	10	363	71
34		H	10	190	91

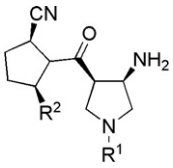
accomplished using $LiAlH_4/SiO_2$ following a published procedure. [16] Considerable caution needs to be exercised when using this protocol beyond low millimolar scale since explosive generation of hydrogen gas occurs upon mixing $LiAlH_4$ with silica. A different reductive procedure was subsequently used to prepare the desired benzyl alcohols from methyl esters **36** and **42a,b**.[17]

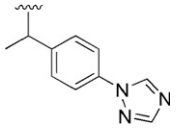
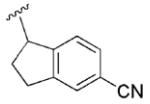
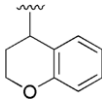
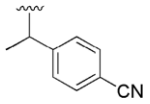
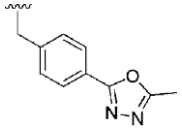
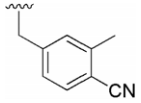
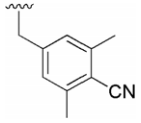
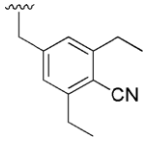
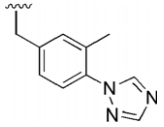
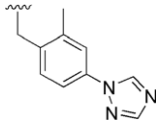
Benzyl-substituted compounds, shown in Table 4, afforded potent DPP-IV inhibitors and exhibited substantially improved dofetilide binding as was evident by comparing **48** and **51** (7% and 18% inhibition of dofetilide binding, respectively) with the corresponding desmethyl analogs **25** and **24** (40% and 47% inhibition of dofetilide binding, respectively). The 2-cyano-5-fluoropyrrolidide of **32** did not produce a substantial increase in DPP-IV potency, and actually had an adverse effect on inhibition of dofetilide binding (see compound **52** in Table 4). *m*-Substituted benzyl derivatives **53**–**56** were prepared and analogs **53** and **54** had excellent DPP-IV inhibitory potency, with **53** exhibiting a ~3.4-fold decreased dofetilide binding relative to the unsubstituted *p*-cyano derivative **24**. Interestingly, diethyl analog **55** had decreased inhibition of dofetilide binding but exhibited a >3-fold loss in DPP-IV potency relative to compound **53**.[18] In addition, **57** had ~4-fold increased inhibition of dofetilide binding compared to **56** (62% vs 17% inhibition of dofetilide binding). Other examples of minor structural changes that decreased dofetilide binding are found by comparing **49** (88%) with **51** (18%) and **57** (62%) with **25** (40%).

Compounds **48** and **53** were selected for further evaluation based upon their in vitro profiles: rat PK data are summarized in Table 5. Triazolyl analog **48** had a longer $t_{1/2}$ but a lower %*F* than **53**. Maximal DPP-IV inhibition in rats (5 mg/kg po dose) was more robust for **53** than for **48,** with **53** inhibiting rat DPP-IV >90% beyond 8 h post-dose while maximal DPP-IV inhibition with **48** began to diminish after ~4 h.

The decision was made to progress compound **53** owing to improved synthetic accessibility and since compound **48** had a more pronounced positive effect in an in vitro

Table 4. Biological activity of benzyl-substituted analogs



Compound	R^1	R^2	Human DPP-4 IC_{50} (nM)	Human DPP-8 IC_{50}[a] (nM)	Percent dofetilide binding inhibition (10 μM)
48		H	3	586	7
49		H	3.4	258	88
50		H	3.5	1150	7
51		H	5.5	1040	18
52		F	5.3	332	17
53		H	1.3	460	14
54		H	1.1	346	21
55		H	4.7	417	3
56		H	46	ND	17
57		H	3.8	203	62

[a] ND, no data.

micronucleus assay. Compound **53** subsequently tested negative in a human lymphocyte aberration (HLA) assay implying less potential for cytotoxicity. Compound instability in human and dog plasma, but not in rat, became apparent during further analysis of **53** (Table 6). Compound **53** was also unstable in fresh rat, dog, and human blood. The reasons for instability were not apparent since 2-cyanopyrrolidides are not inherently prone to human plasma instability. For example, vildagliptin (**1b**) is currently in Phase III clinical trials. The chemical reasons behind plasma instability are unknown and a decision was made to not pursue this series of DPP-IV inhibitors.

In summary, *cis*-3-amino-4-(2-cyanopyrrolidide)-pyrrolidines were shown to be a unique scaffold from which

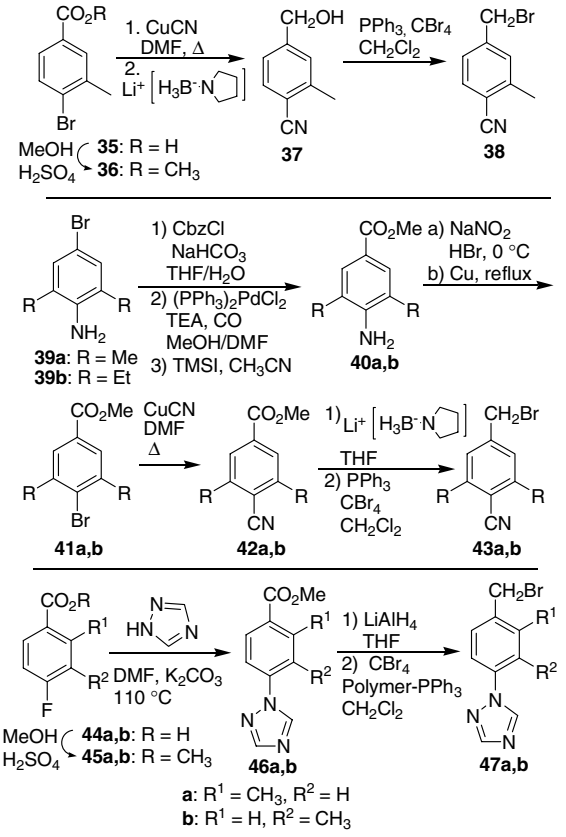


Figure 6. Synthesis of benzyl halides.

Table 5. Rat PK and PD (5 mg/kg po) for **48** and **53**

Parameter	Compound	
	48	**53**
C_{max} (ng/mL)[a]	1400 (±264)	1785 (±827)
AUC_{inf} (h ng/mL)[a]	7703 (±1452)	3130 (±1160)
CL (mL/min/kg)	2.2 (±2.6)	21.8 (±3.0)
$t_{1/2}$ (h)[a]	4.3 (±2.6)	2.3 (±0.4)
$\%F$[a]	30.6 (±5.7)	81 (±19)
Rat plasma protein binding (% free)	23 (±0.3)	34 (±0.3)
Maximal DPP-IV inhibition	86%	95%

[a] Values are means of two experiments, standard deviation is given in parentheses.

Table 6. Stability of compound **53** in rat, dog, and human plasma

	% Drug remaining			
Time (h):	0	2	4	6
Species				
Rat	100	93	98	117
Dog	100	85	84	83
Human	100	49	27	13
Control	100	99	108	127

potent DPP-IV inhibitors could be obtained. Careful analysis of the SAR enabled the design of compounds that exhibited low displacement of dofetilide in a binding assay while human DPP-IV inhibition was improved. Concomitant risk management of micronucleus positive data, via selection of a weakly positive micronucleus compound and subsequent testing in a HLA assay, enabled the selection of compound **53** having an improved cytotoxicity profile. Unexpected plasma instability led to the cessation of work with this series of DPP-IV inhibitors.

Acknowledgment

The authors thank Bernard Fermini and Shuya Wang for hERG patch clamp studies.

References and notes

1. Fact sheet No. 312. World Health Organization, Geneva, 2006.
2. Knudsen, L. B. *J. Med. Chem.* **2004**, *47*, 4128.
3. Idris, I.; Donnelly, R. *Diabetes Obes. Metab.* **2007**, *9*, 153.
4. Feng, J.; Zhang, Z.; Wallace, M. B.; Stafford, J. A.; Kaldor, S. W.; Kassel, D. B.; Navre, M.; Shi, L.; Skene, R. J.; Asakawa, T.; Takeuchi, K.; Xu, R.; Webb, D. R.; Gwaltney, S. L., II *J. Med. Chem.* **2007**, *50*, 2297.
5. Gallwitz, B. *Drugs Today* **2007**, *43*, 13.
6. Rasmussen, H. B.; Branner, S.; Wilberg, F. C.; Wagtmann, N. *Nat. Struct. Biol.* **2003**, *10*, 19.
7. Initial scaffold design was conducted by E. Cox.
8. Hanselmann, R.; Zhou, J.; Ma, P.; Confalone, P. N. *J. Org. Chem.* **2003**, *68*, 8739.
9. The dipeptidyl peptidase assays and rat pharmaco kinetic and pharmacodynamic assays are described in: Wright, S. W.; Ammirati, M. J.; Andrews, K. M.; Brodeur, A. M.; Danley, D. E.; Doran, S. D.; Lillquist, J. S.; McClure, L. D.; McPherson, R. K.; Orena, S. J.; Parker, J. C.; Polivkova, J.; Qiu, X.; Soeller, W. C.; Soglia, C. B.; Treadway, J. L.; VanVolkenberg, M. A.; Wang, H.; Wilder, D. C.; Olson, T. V. *J. Med. Chem.* **2006**, *49*, 3068. DPP-IV assay results varied by ±15% between individual runs.
10. The atomic coordinates for the crystal structure of compound **5** were deposited with the RCSB Protein Data Bank with file name 2RIP.
11. All compounds tested in Tables 1–4 had DPP-2 $IC_{50} > 10$ μM (data not shown).
12. A filter binding assay with [^{3}H]-dofetilide was used as described in: Greengrass, P. M.; Stewart, M.; Wood, C.; M. WO2003021271, 2003.
13. Dofetilide binding assays have been shown to be predictive for ranking compounds for their potential to block the hERG ion channel, as described in Diaz, G. J.; Daniell, K.; Leitza, S. T.; Martin, R. L.; Su, Z.; McDermott, J. S.; Cox, B. F.; Gintant, G. A. *J. Pharm. Toxicol. Methods* **2004**, *50*, 187.
14. A plot of log(hERG IC_{50}) versus percent dofetilide activity provides a correlation between dofetilide binding and hERG IC_{50}.

Compound	Percent dofetilide binding inhibition (10 μM)	hERG patch clamp IC_{50} (nM)
5	0.241	>300,000
32	4	165,000
53	14	19,600
51	18	10,000
54	21	28,900
25	40	1800

15. Tanaka, A.; Terasawa, T.; Hagihara, H.; Sakuma, Y.; Ishibe, N.; Sawada, M.; Takasugi, H.; Tanaka, H. *J. Med. Chem.* **1998**, *41*, 2390.

16. Kamitori, Y.; Hojo, M.; Masuda, R. *Tetrahedron Lett.* **1983**, *24*, 2575.
17. The modified reduction procedure utilized a lithium pyrrolidinoborohydride, as described in: Fisher, G. B.; Harrison, J.; Fuller, J. C.; Goralski, C. T.; Singaram, B. *Tetrahedron Lett.* **1992**, *33*, 4533.
18. Compound **53** had hERG IC_{50} ~20 μM in a hERG patch clamp assay.