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Structural determinants for histamine H_1 affinity, hERG affinity and QTc prolongation in a series of terfenadine analogs

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ABSTRACT

In the late 1980's reports linking the non-sedating antihistamines terfenadine and astemizole with torsades de pointes, a form of ventricular tachyarrhythmia that can degenerate into ventricular fibrillation and sudden death, appeared in the clinical literature. A substantial body of evidence demonstrates that the arrhythmogenic effect of these cardiotoxic antihistamines, as well as a number of structurally related compounds, results from prolongation of the QT interval due to suppression of specific delayed rectifier ventricular K+ currents via blockade of the hERG-IKr channel. In order to better understand the structural requirements for hERG and H_1 binding for terfenadine, a series of analogs of terfenadine has been prepared and studied in both in vitro and in vivo hERG and H_1 assays.

In the late 1980's reports linking the non-sedating antihistamines terfenadine and astemizole with torsades de pointes ('twisting of the points'), a form of ventricular tachyarrhythmia that can degenerate into ventricular fibrillation and sudden death, appeared in the clinical scientific literature. This led to the eventual removal of these drugs from the market in 1997 for terfenadine and in 1999 for astemizole.[1] A substantial body of evidence indicates that the arrhythmogenic effect of these cardiotoxic antihistamines, as well as a number of structurally related compounds, results from prolongation of the QTc interval due to suppression of specific delayed rectifier ventricular K^+ currents via blockade of the hERG-IKr channel.[1–3] The question arose as to whether hERG activity could be separated from the antihistaminic activity of this structural class. In order to define, and therefore better understand, the structural requirements for hERG binding as compared to H_1 binding for terfenadine, a series of compounds that are analogs of terfenadine was prepared and studied in both in vitro and in vivo hERG and H_1 assays. The present report describes our findings.[4]

To determine the contribution of the different structural regions of terfenadine to hERG binding and antihistamine activity, the parent molecule was divided into three segments, the aromatic benzhydryl region **A**, the basic piperidine region **B** and the lipophilic tail portion **C** and analogs of each were prepared (Fig. 1). Furthermore, in order to simplify the synthesis, we converted the secondary benzylic hydroxyl and *t*-butyl groups of terfenadine to hydrogen and methyl, respectively. hERG activity was initially determined in a high throughput patch clamp screening assay (Ionworks)[5] while a human H_1 binding assay was used to determine H_1 binding affinity.[6] Selected results were confirmed in vitro using an IonWorks Quattro patch clamp assay and in vivo in the guinea pig.[7,8] Histamine H_1 activity was confirmed in vivo in the guinea pig.[7]

Most of the analogs in this study were conveniently prepared starting with an unsubstituted piperidine and the side chain was introduced via either reductive amination or alkylation. For example, commercially available amine **1** (R = Ph_2COH) was coupled with 4-(*p*-tolyl)butyric acid to give the amide **2** (*n* = 3) in 68% yield. The amide carbonyl was reduced using $LiAlH_4$ to yield amine **3** in 59% yield. Alternatively, reaction of **1** with an alkyl halide, such

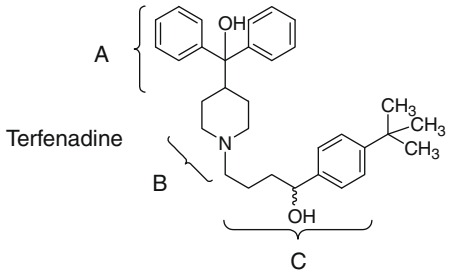

Figure 1.

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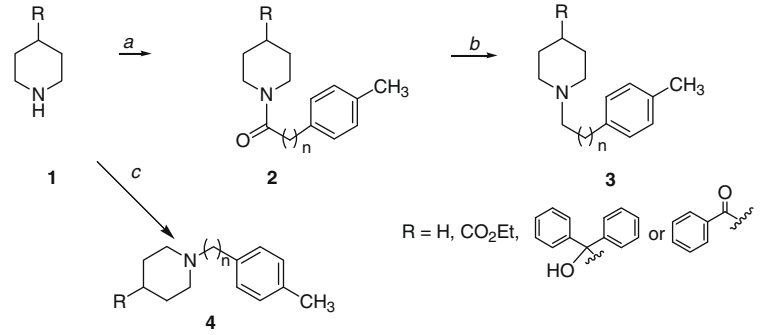


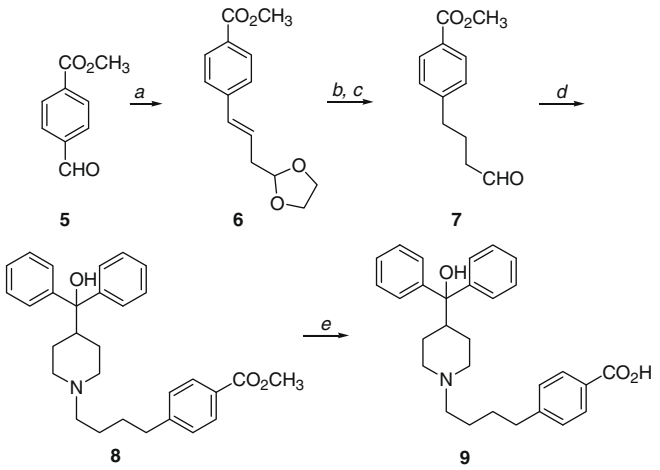
Scheme 1. Reagents and conditions: (a) (*p*-tolyl)alkyl acid, HBTA, DEC, CH_2Cl_2; (b) LAH, THF; (c) *p*-tolylalkyl bromide, Et_3N, DMF, Δ (for $n = 1$) or K_2CO_3, *i*-Pr_2NEt, room temperature (for $n = 4$).

as 4-methylbenzyl bromide, in the presence of a base such as triethylamine gave the tertiary amine **4** directly in 86% yield (see Scheme 1).

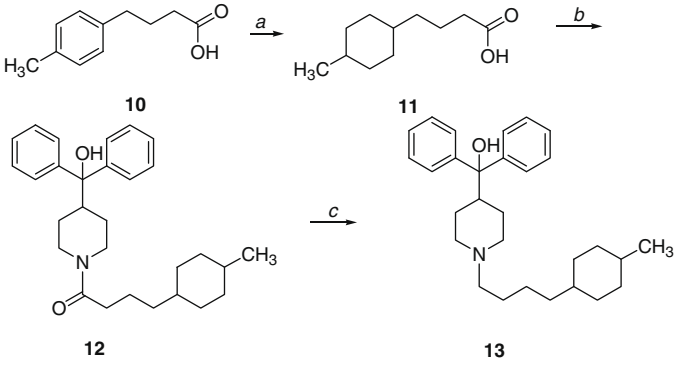
The synthesis of the simplified analog of terfenadine carboxylate, compound **9**, first required the preparation of aldehyde **7**. Wittig olefination of aldehyde **5** gave olefin **6**. The double bond in **6** was reduced using 5% Pd/C and H_2 and the aldehyde unmasked with pyridinium *p*-toluene sulfonate. Reductive amination of aldehyde **7** with amine **1** followed by saponification gave the desired product **9** (Scheme 2).

Many of the compounds that are known to interact with the hERG channel possess a lipophilic tail consisting of an aromatic moiety tethered to a basic amine via a carbon chain of various lengths (for example, ebastine, droperidol and haloperidol in addition to terfenadine). To determine if the binding interaction was the result of a specific interaction with the aromatic group or simply a lipophilic effect, the cyclohexyl analog **13** was prepared (Scheme 3). 4-(*p*-Tolyl)butyric acid (**10**) was reduced to the cyclohexane **11** using 5% Rh/C. Compound **11** was coupled to **1** and the amide **12** reduced to give the target **13**.

In order to determine the contribution of the tertiary amine of the central piperidine ring to hERG binding and H_1 activity, the cyclohexyl analog **17** was prepared (Scheme 4). Formation of the Wittig reagent **15** from alcohol **14** proceeded in 62% overall yield. Reaction of the ylide of **15** with ethyl 4-oxocyclohexanecarboxylate followed by reduction with H_2 over Pd/C gave cyclohexyl compound **16** in 50% yield over two steps. Reaction with excess phenyl



Scheme 2. Reagents and conditions: (a) [2-(1,3-dioxolan-2-yl)ethyl]triphenylphosphonium bromide, $KN(SiMe_3)_2$, THF, 54%; (b) 5% Pd/C, H_2, MeOH, 91%; (c) PPTS, acetone, 50 °C, 62%; (d) 1, $NaBH(OAc)_3$, CH_2Cl_2, 60%; (e) KOH, MeOH, 80%.

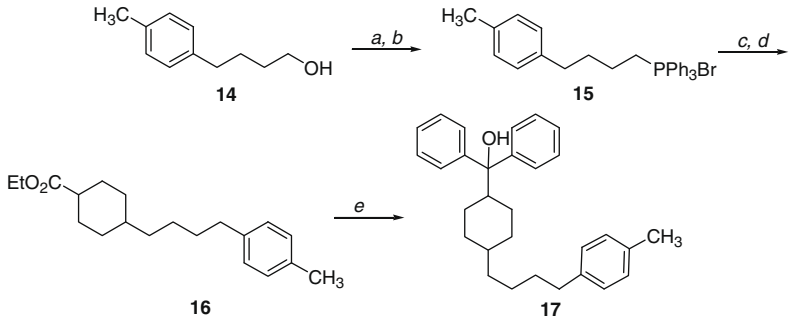


Scheme 3. Reagents: (a) 5% Rh/C, H_2, MeOH, 59%; (b) **1**, HBTA, DEC, CH_2Cl_2, 74%; (c) LAH, THF, 56%.

Grignard gave the tertiary alcohol **17** in 70% yield. The relative stereochemistry about the cyclohexane ring was determined to be trans by NMR spectroscopy.

Table 1 lists the results from the patch clamp assay as a % inhibition at 10 μM and human H_1 binding affinity for the terfenadine analogs prepared in this study. Entry 3, which is the simplified analog of terfenadine, has an identical in vitro profile to the parent compound (entry 1) for both hERG activity and H_1 binding affinity. Interestingly, the simplified acid analog (entry 4) of terfenadine carboxylate is slightly more potent at the hERG channel than would be anticipated. Based on these data, it seems that excising the secondary hydroxyl and *t*-butyl groups of terfenadine may not significantly impact the hERG and H_1 profiles of the analogs compared to the parent, but the effect is less pronounced if a carboxylic acid is present.

Considering the benzhydryl region of terfenadine next (A in Fig. 1), the data in Table 1 imply that both hERG affinity as well as H_1 binding affinity are dependent on the nature of the substituents in this area. Complete removal of the benzhydryl alcohol moiety as in entry 10 causes a decrease in both hERG affinity as well as H_1 affinity, although H_1 affinity seems to be effected to a greater extent. Reintroduction of the alcohol moiety as in entry 11 does not significantly increase affinity for the H_1 receptor, although it does result in a slight improvement in hERG affinity, perhaps due to the polar nature of the hydroxyl group. However, reintroduction of a single phenyl ring in addition to the hydroxyl group as in entry 12 causes an increase in both hERG and H_1 affinity. This is not surprising. Many hERG active compounds such as droperidol and haloperidol contain a piperidine ring substituted with a single phenyl ring at the 4-position. It is also consistent with site directed mutagenesis studies[9] and pharmacophore models of the hERG channel.[10] These studies show that an aromatic group capable of

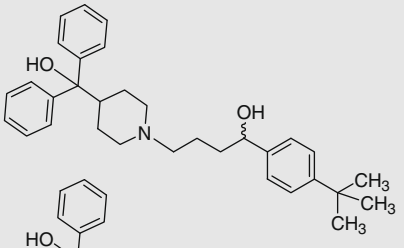
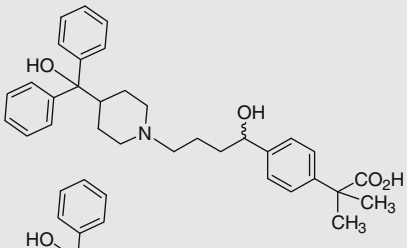
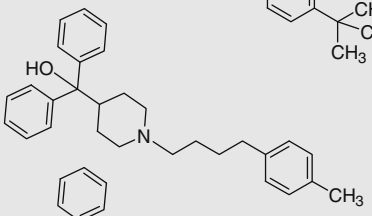
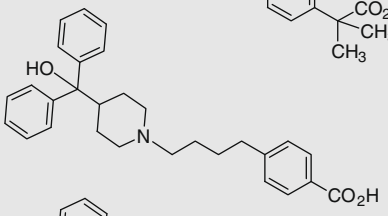
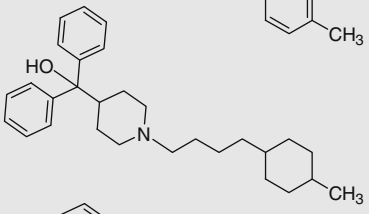
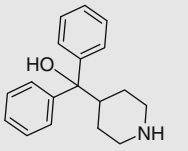
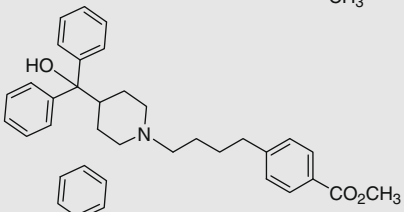
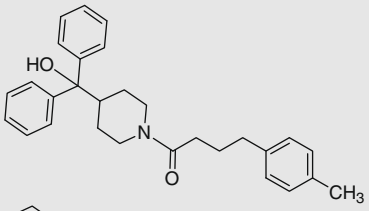
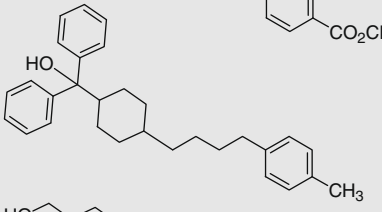
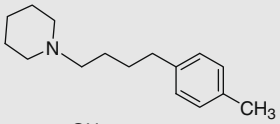
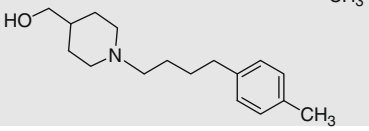
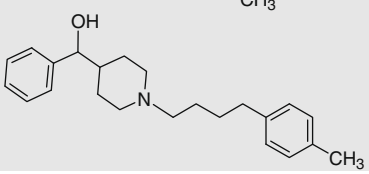


Scheme 4. Reagent: (a) PBr_3, room temperature, 76%; (b) PPh_3, toluene, reflux, 81%; (c) *n*-BuLi, ethyl 4-oxocyclohexanecarboxylate, THF, −78 °C to room temperature, 54%; (d) H_2, Pd/C, MeOH, 92%; (e) PhMgBr, THF, 0 °C to room temperature, 70%.

a π-stacking interaction with Phe656 in the hERG channel contributes significantly to the binding interaction of terfenadine and related compounds. Interestingly, site directed mutagenesis and pharmacophore modeling studies of the H_1 receptor have also demonstrated the importance of three aromatic amino acid residues, Trp167, Phe433 and Phe436, as the putative interaction points of the aromatic rings of H_1 antagonists.[11,12] Therefore, in this region of terfenadine it would appear that hERG affinity and H_1 binding affinity parallel each other due to similar binding interactions in the channel and receptor.

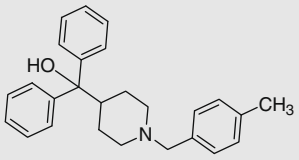
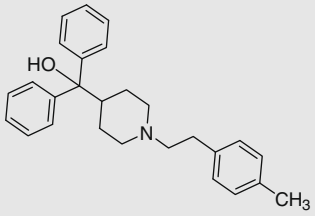
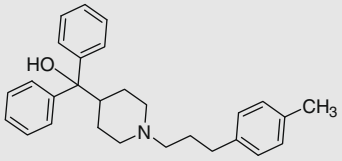
The contribution to hERG activity and H_1 binding affinity of the basic, tertiary amine in region **B** was then evaluated by converting

Table 1
Ionworks patch clamp and H_1 binding data for terfenadine analogs[a]

Entry	Structure	Patch clamp[b] (%)	H_1 Binding K_i	Entry	Structure	Patch clamp[b]	H_1 Binding K_i
1		96	40 nM	2		3	27 nM
3		99	39 nM	4		56	203 nM
5		85	61 nM	6		14	659 nM
7		100	20 nM	8		11	NA
9		35	NA	10		71	3.2 μM
11		54	NA	12		97	121 nM

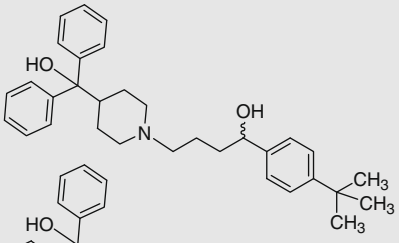
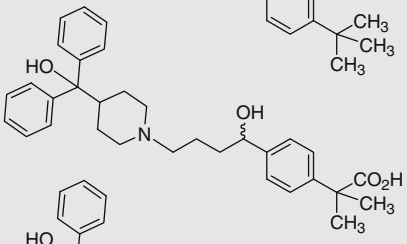
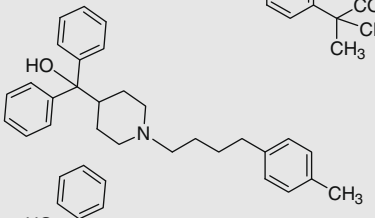
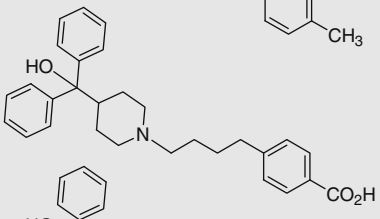
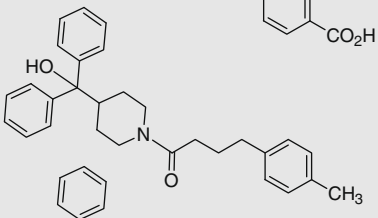
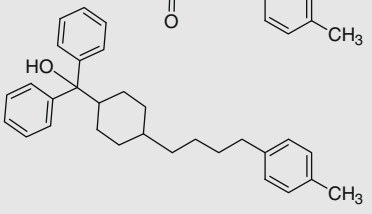
(*continued on next page*)

Table 1 (*continued*)

Entry	Structure	Patch clamp[b] (%)	H_1 Binding K_i	Entry	Structure	Patch clamp[b]	H_1 Binding K_i
13		100	16 nM	14		100	64 nM
15		99	155nM				

NA = not active.
[a] Data are the results of at least two independent determinations.
[b] % Inhibition @ 10 μM.

Table 2
In vitro patch clamp data and in vivo data in the guinea pig

Entry	Structure	Whole cell patch clamp (IC_{50})	QTc MED (mg/kg)	Antihistamine ED_{50} (mg/kg)
1		312 nM	0.03	0.8
2		>100 μM	NA@ 100 mg/kg	0.08
3		396 nM	0.1	0.9
4		11 μM	NA@ 50 mg/kg	0.1
5		>100 μM	N.T.	N.T.
6		>100 μM	N.T.	N.T.

NA = not active.
N.T. = not tested.
MED = minimum effective dose.

the amine either to a non-basic amide group or completely removing the basic center by substitution with carbon (entries 8 and 9). Both analogs showed decreased hERG and H_1 binding affinity. These results were anticipated. It is well known for antihistamines that a basic amine significantly contributes to H_1 binding affinity due to interaction with Asp116 in transmembrane domain III of the histamine H_1 receptor.[13] Furthermore, significant evidence exists that a cationic binding site in the hERG channel, most probably Tyr652,[9] contributes to binding affinity of hERG blockers via a cation–π interaction, although a recent modeling study suggests that Tyr652 may be too far away from the basic center in molecules that block hERG to interact.[9,10,14] These authors propose instead a polar interaction with Ser624. These data do in fact show a decreased affinity for the hERG channel for both analogs. Our data seem to support the former hypothesis since the amide analog, which may be able to maintain a polar interaction, but not a cationic one, is weaker at the hERG channel than the parent.

Turning last to the lipophilic tail of terfenadine (region **C**), our data indicates that a phenyl ring is not required for hERG affinity since the cyclohexyl analog is also a potent hERG blocker (entry 5). This suggests that a hydrophobic interaction may be all that is required for binding in this region of the pore, consistent with some models.[14] Moreover, an aromatic group in this region does not appear to be necessary to H_1 binding affinity, either. The cyclohexyl analog is nearly equipotent as an H_1 ligand compared to its aromatic counterpart. Complete removal of the lipophilic side chain does cause a loss of binding affinity for both the hERG channel and the H_1 receptor (entry 6). This is qualitatively similar to results obtained with sertindole wherein removal of the sertindole side chain decreased but did not abolish hERG affinity.[10b] The effect of the spatial relationship of the terminal aromatic residue to the central piperidine ring on hERG and H_1 binding affinity was also evaluated. hERG affinity remains consistent with that of the parent regardless of the chain length as in examples 13, 14, and 15. However, the best H_1 binding affinity appears to reside in the shorter chain analog.

Interestingly, in contrast to the carboxylic acid, the methyl ester (entry 7) shows surprisingly high hERG affinity, a result that is consistent with sertindole analogs.[10b] This implies that a species that is negatively charged at physiological pH may be necessary to abolish hERG activity. Alternatively, the ester may not be able to adopt the same conformation as the carboxylic acid does to decrease its hERG activity.[10b]

Having identified the structural features of these terfenadine analogs that contribute to hERG and H_1 binding affinity, we next looked to confirm these observations. Towards this, the whole cell patch clamp assay (Ionworks) was used to determine hERG IC_{50} values in vitro and the guinea pig hERG model was used to confirm hERG activity in vivo. Antihistaminic activity was confirmed in vivo in the guinea pig histamine-induced bronchospasm assay. Table 2 presents the results for selected compounds.

These data in Table 2 demonstrate that the in vitro data from Table 1 is predictive of the cell-based and in vivo data, and also help to demonstrate the validity of simplifying the analogs. For example, terfenadine is a potent hERG blocker using the patch clamp assay (IC_{50} = 312 nM) which was further confirmed in vivo in the guinea pig (MED = 0.03 mg/kg). Additionally, as expected, terfenadine was a potent antihistamine (ED_{50} = 0.8 mg/kg). Conversely, terfenadine carboxylate was inactive both in vitro and in vivo against hERG but displayed excellent antihistaminic activity (ED_{50} = 0.08 mg/kg). Moreover, the simplified terfenadine and terfenadine carboxylate analogs (entries 3 and 4) paralleled the activity of their counterparts. Interestingly, the carboxylic acid analogs (Table 2, entries 2 and 4) both appear to be significantly more potent antihistamines in vivo in the guinea pig than their corresponding reduced analogs. The data in Table 2 also confirm the importance of a basic nitrogen to hERG affinity (entries 5 and 6).

Based on a simplified structural model, a structure–activity profile for hERG activity and H_1 binding in a series of terfenadine analogs was elucidated. Structural features that appear to contribute to both hERG activity and H_1 binding include a basic, tertiary amine, a lipophilic tail incorporating either an aromatic moiety or a cyclohexyl ring, and at least one aromatic ring approximately 6 Å from the basic nitrogen center. Overall, with the exception of the carboxylic acid analogs, it appears that in this series of terfenadine analogs, hERG activity generally parallels the antihistamine activity.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.07.047.

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