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Selectivity profiling of novel indene H_1-antihistamines for the treatment of insomnia

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ABSTRACT

A series of indene analogs of the H_1-antihistamine (−)-*R*-dimethindene was evaluated for selectivity in the search for potentially improved sedative-hypnotics. Variation of the 6-substitutent in the indene core in combination with a pendant electron rich heterocycle led to the identification of several potent H_1-antihistamines with desirable selectivity over CYP enzymes, the M_1 muscarinic receptor and the hERG channel. These compounds were candidates for further ADME profiling and in vivo evaluation.

First generation H_1-antihistamines are sedating and are used in over-the-counter sleep aids. These agents are functional antagonists of muscarinic receptors,[1] a property thought to cause undesirable side effects such as dry mouth, blurred vision, constipation, tachycardia, urinary retention, and memory deficits.[2,3] Next-day impairment has also been observed with these compounds, presumed to result from protracted CNS exposure.[4,5] Thus, selective H_1-antihistamines with appropriate duration of CNS exposure may provide an alternative to current medications for the treatment of insomnia.

Previously, we described the use of the selective (−)-*R*-dimethindene[6] as a starting point to generate novel selective and brain penetrating H_1-antihistamines with an improved pharmacokinetic profile suitable for use as night-time sleep aids. [7] In addition to the benzothiophene **1** (Fig. 1) that was selected for clinical evaluation, indenes **2** and **3** were discovered with suitable receptor selectivity and demonstrated effects on NREM sleep in rats. However, metabolism studies in human liver microsomes (HLM) of **2** and **3** demonstrated that the major portion of metabolism occurred at the 6-position of the indene core via the CYP2D6 pathway (Supplementary data). The characterization of these compounds as predominant CYP2D6 substrates presented two issues. Besides a liability for drug interactions, heterogeneity of CYP2D6 activity within the general population raised the concern of extreme variability in pharmacokinetics for candidate compounds.[8]

PK variability in lead compounds was considered a significant issue because of the potential for varying exposures to adversely impact duration of action.

In this Letter we describe initial SAR around indene **4.** The objective of this work was to identify analogs of **2** and **3** that retained suitable selectivity profiles to justify further metabolism and pharmacokinetic profiling. It has been reported that substitutions in the 6-position of the indene core are accommodated within the H_1 pharmacophore.[9] Our previous studies also indicated the requirement for a heteroaryl moiety in the substructure **4** to confer selectivity versus CYP2D6 inhibition.[7] We therefore elected to evaluate the effects of substituents in the 6-position of the indene core in conjunction with substitutions of the heteroaryl moiety with and without the presence of a chiral center (R^2 = Me or H). Substitutions at the 6-position would be anticipated to improve metabolic stability and potentially modulate the biotransformation pathways encountered as an issue for the CYP2D6 substrates **2** and **3**. From our previous work modification of the heteroaryl moiety and chiral center would be expected to further modify the overall PK profile of candidate compounds.[7].

To achieve similar selectivity to that described earlier,[7] candidate compounds were required to demonstrate high H_1 binding affinity (K_i <10 nM) with at least 100-fold binding selectivity

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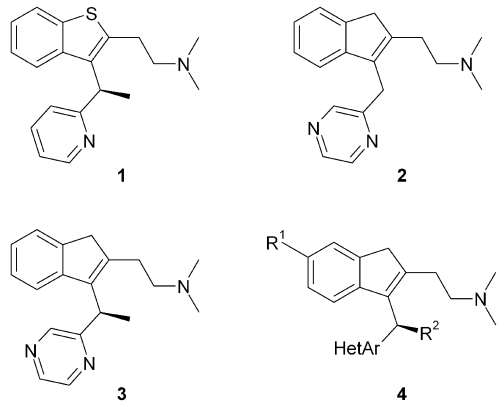
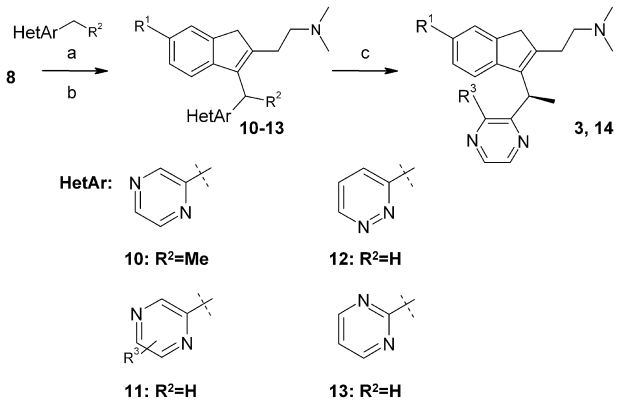


Figure 1. Benzothiophene candidate (**1**), indene leads (**2**) and (**3**) from (−)-*R*-dimethindene, and series (**4**) described in this Letter.



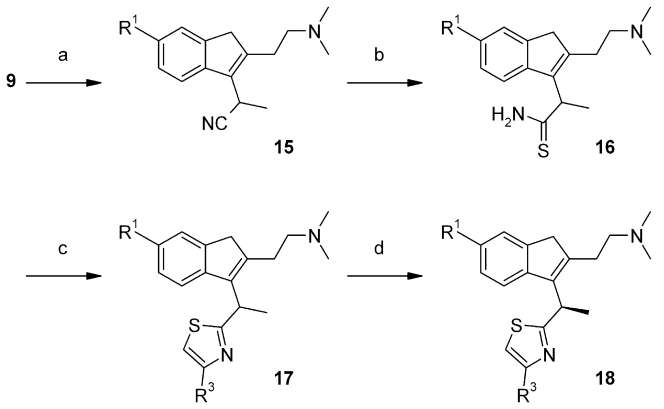
Scheme 2. Reagents and conditions: (a) LDA, THF, 0 °C; (b) 20% HCl, reflux; (c) Chiral separation by chiral HPLC or SFC.

versus the representative muscarinic M_1 receptor. Selectivity of the order of 1000-fold was deemed acceptable for CYP enzyme inhibition. It is well known that several antihistamines are potent inhibitors of the hERG channel[10] implicated in prolongation of cardiac QTc and leading to cardiac arrhythmias. Although benzothiophene **1** had shown weak hERG channel inhibition (hERG IC_{50}/H_1 K_i selectivity of 336[7]), in vivo characterization indicated an absence of cardiovascular risks and safety margins significantly higher than previous guidelines for the assessment of hERG inhibitors.[10a] Based on these data we reasoned that hERG IC_{50}/H_1 K_i selectivity of 400 or greater would be acceptable for candidate compounds.

Compounds were evaluated for H_1 binding affinity, selectivity versus M_1 and inhibition of CYP2D6 and CYP3A4.[7] We also screened compounds in this series against hERG, initially using a high-throughput dofetilide binding assay, followed by hERG channel current (patch clamp) analysis for the more promising candidates.[7]

Various synthetic schemes were employed to generate indenes (**4**) (Schemes 1–4). Indanone **5** was converted to **6** in either a single step by alkylation with *N*,*N*-dimethyl-2-chloroacetamide or by a four step process using a condensation with glycolic acid followed by a reduction with Zn in acetic acid, acyl chloride formation and an amide coupling. Simultaneous reduction of the amide and ketone functionality in **6** with $LiAlH_4$ to alcohol **7** was followed by oxidation with benzophenone to indanone **8** or acid mediated dehydration to give indene **9**.

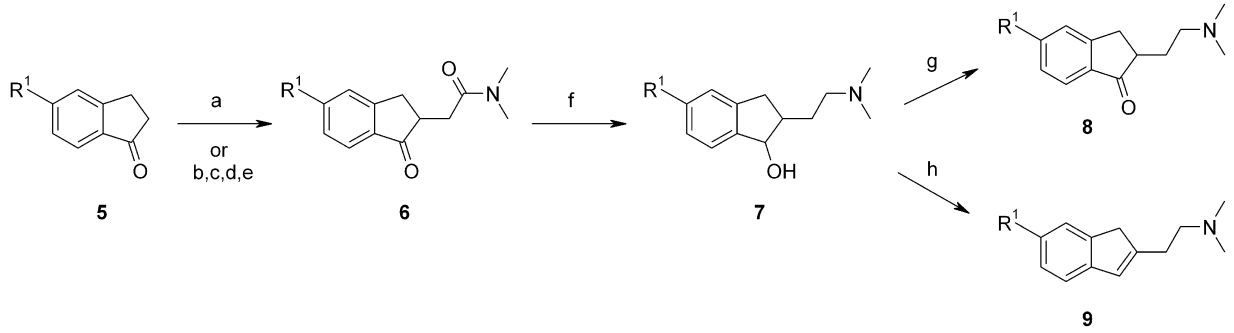
The lithium salts from various alkyl heteroaryls, generated by treatment of alkyl heteroarenes with LDA, were added to indanone **8**, followed by an acid mediated dehydration to afford indenes **10**–**13**. When R^2 was a methyl group, the enantiomers were separated by chiral HPLC or SFC. By analogy to (−)-*R*-dimethindine (**1**),[6] the (−) enantiomers (**3**, **14**) were used for biological profiling.



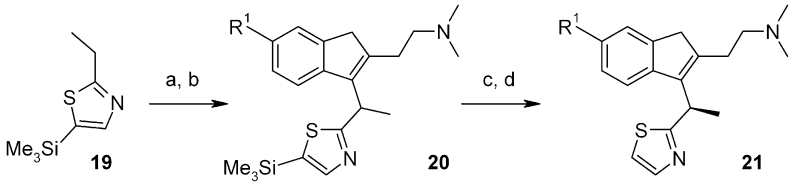
Scheme 3. Reagents and conditions: (a) (i) *n*-BuLi, THF, −78 °C; (ii) BrCH(Me)CN, −78 °C to rt; (b) *i*PrNH$_2$, $(NH_4)_2S$, H_2S, DMA, 60 °C or S=P(OEt)$_2$S, HCl(g); (c) R^3 = H: $BrCH_2CH(OEt)_2$, concd H_2SO_4, dioxane/EtOH; R^3 = Me:CH_3COCH_2Cl, EtOH, 80 °C; (d) Chiral separation by chiral HPLC or SFC.

Indenes **4**, containing a thiazole moiety were synthesized using two different routes (Schemes 3 and 4). Alkylation of indene **9** with 2-bromopropionitrile yielded nitrile **15**. Conversion of **15** to the corresponding thioamide **16** was followed by cyclization to thiazole **17**. Enantiomers **18** were separated by chiral HPLC or SFC.

When R^1 was not hydrogen, alkylation of **9** not only generated the desired R^1 6-substituted indenes **15** but also the 5-substituted regioisomers, which were difficult to separate. Therefore another synthetic route (Scheme 4) was employed, using direct alkylation of indanone **8** with the lithium salt of 2-ethyl-5-trimethylsilanyl-

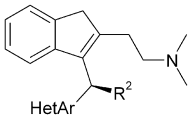


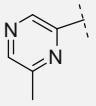
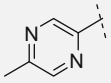
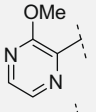
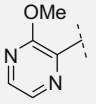
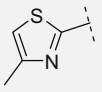
Scheme 1. Reagents and conditions: (a) LDA, *N*,*N*-dimethyl-2-chloroacetamide, THF, −78 °C; (b) Glycolic acid (40% in H_2O), concd H_2SO_4, dioxane, reflux; (c) Zn, HOAc, H_2O, 100 °C; (d) $(COCl)_2$, cat. DMF, DCM; (e) $HNMe_2$, Et_3N, DCM; (f) $LiAlH_4$, THF, 0 °C; (g) Ph_2CO, *t*-BuOK, benzene, reflux; (h) Concd HCl in HOAc, reflux.



Scheme 4. Reagents and conditions: (a) (i) *n*-BuLi, THF, −50 °C; (ii) **8**; (b) 20% HCl, reflux; (c) aq HF in CH_3CN; (d) Chiral separation by chiral HPLC or SFC.

Table 1
SAR of indenes with different heteroaryl groups



Compd	R^2	HetAr	H_1[a] K_i (nM)	M_1[b] K_i (μM)	CYP2D6[c] IC_{50} (μM)	hERG[d,e] IC_{50} (μM)
2	H		4.0 ± 0.3	19	6.8	5.4
11a	H		12 ± 1	>10	6.0	NT[f]
11b	H		63 ± 30	>10	22	NT
11c	H		35 ± 3	>10	3.2	NT
11d	H		2.2 ± 0.5	15	0.9	7.6
12a	H		7.7 ± 1.5	17	>10	NT
13a	H		15 ± 3	>10	>10	NT
3	Me		1.3 ± 0.1	20	51	1.4
14a	Me		1.0 ± 0.1	3.7	17	7.6
18a	Me		0.8 ± 0.1	2.4	3.8	3.7
18b	Me		3.1 ± 0.4	3.6	>10	1.9

[a] K_i values derived from dose–response curves generated from triplicate or more data points.
[b] K_i values derived from dose–response curves generated from duplicate data points.
[c] CYP3A4 IC_{50} >10 μM for all compounds tested.
[d] hERG patch clamp assay.
[e] In hERG dofetilide binding assay all compounds had K_i >3 μM.
[f] NT = not tested.

thiazole (**19**)[11] followed by acid treatment to generate indene **20**. Aqueous HF mediated removal of the TMS group and subsequent chiral HPLC or SFC gave the desired (−) enantiomers **21**.

Effects of changes to the heteroarene and chiral center in the absence of 6-substitution are shown in Table 1. Achiral indene **2**[7] containing an unsubstituted 1,4-pyrazine, displayed potent H_1 binding and was >1000-fold selective over M_1, the CYP enzymes tested, and the hERG channel. Incorporation of a methyl substituent at various positions in the pyrazine ring (**11a–c**) decreased H_1 affinity. In contrast, a 3-methoxy substituent (**11d**) demonstrated similar H_1 affinity compared to **2** with a slightly improved selectivity profile versus M_1 and hERG. However CYP2D6 inhibition

was increased to unacceptable levels. Alternative heterocyclic isosteres such as the pyridazine (**12a**) and pyrimidine (**13a**) had less binding affinity for H_1, although their selectivity profile was acceptable. To increase H_1 affinity and selectivity, a chiral center was incorporated into the compounds with the most promising profiles. Indeed, **3** and **14** demonstrated higher H_1 binding affinity than the achiral analogs **2** and **11d,** improved selectivity versus CYP2D6 inhibition, and comparable selectivity versus M1 and hERG. Incorporation of the pyridine isostere, thiazole (**18a**), provided a compound with sub-nanomolar H_1 affinity and >2000 selectivity over the anti-targets screened. Addition of a methyl group to the thiazole ring (**18b**) decreased H_1 affinity, as was observed for the 1,4-pyrazine series, although an acceptable selectivity profile was maintained.

In initial studies, chlorine, fluorine, methoxy, and methyl substituents in the 6-position of the indene core were investigated to explore the effects on both H_1 affinity and selectivity, especially with respect to the hERG channel. Comparisons were made with the previously described achiral indenes shown to give the best affinity and selectivity profile. (Table 2).

Methyl and methoxy groups (**11g**, **11h**) led to a decrease in H_1 affinity while the selectivity profile for M_1 and CYP2D6 inhibition was maintained. Methyl substitution (**11g**) led to a significant increase in hERG potency. Electron-withdrawing chlorine and fluorine substituents (**11i**, **11j**) increased H_1 binding, but also significantly increased hERG inhibition. This effect was more profound for chlorine than fluorine. Increases for hERG potency were attributed to relative increases in hydrophobicity (substituent contribution to *c*log *P*: chlorine > methyl > fluorine > methoxy, hydrogen[12]). A reduction in electron density to the indene also appeared to contribute to increased hERG activity. Despite these observations, some compounds from the 6-fluoro series (**11j** and **12c**) passed our in vitro criterion for hERG selectivity (hERG IC_{50}/H_1 K_i >400). Notably, the methoxy substituent in the pyrazine moiety of 6-fluoro and 6-chloro substituted indenes (**11k** and **11l**) caused a significant increase in CYP2D6 inhibition compared to **11i** and **11j.** This phenomenon was also observed in the unsubstituted indene core (**11d** compared to **2**).

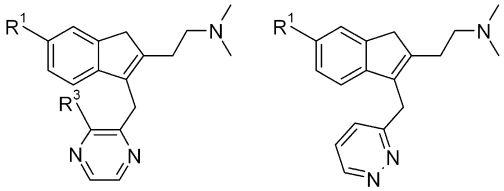
We previously demonstrated that chiral analogs were generally more selective with a marginal increase in H_1 affinity. Based on these observations we decided to incorporate the chiral center to improve H_1 affinity in the less active 6-substituted indene core (methoxy, methyl) and improve selectivity in the more active 6-fluoro substituted indene core. The 6-chloro substituted indene core was abandoned due to irreconcilable high hERG inhibition (Table 3).

Introduction of a chiral center was a successful strategy for improving H_1 affinity and selectivity of 6-methyl and 6-methoxy substituted indenes (**14b** and **14c**), compared to the achiral analogs (**11g** and **11h**). 6-Methoxy substituted indenes indicated a trend to improved hERG selectivity compared to the corresponding 6-methyl indenes (comparisons of **14b**–**14c**, **14d**–**14e** and **21b**–**21c**) although **14d** retained good selectivity for the channel. A similar trend was observed for the achiral pair **11g** and **11h**. In the case of analogs **14c**–**f** and **21c**, hERG selectivity was greater than 1000-fold versus H_1. In general, the 3-methoxypyrazine moiety led to comparable H_1 affinity and improved hERG selectivity compared to the corresponding pyrazine analogs (comparisons of **14b**–**14d**, **14c**–**14e**, **14a**–**3**). CYP2D6 inhibition in the methoxypyrazine analogs was increased although the selectivity for these compounds was still acceptable [CYP2D6 IC_{50}/H_1 K_i >1000]. Introduction of a chiral center appeared to be a general approach for maintaining selectivity versus CYP2D6 inhibition in the 6-substituted series with the 3-methoxypyrazine feature (**14d**–**f**). While the 3-methoxypyrazine contributed to significant CYP2D6 inhibition in achiral 6-fluoro indene (**11l**), this liability was removed in the corresponding chiral analog (**14f**).

The 6-fluoro indene in combination with the thiazole group (**21a**) retained H_1 binding affinity but increased hERG inhibition relative to the thiazole analog with the unsubstituted indene core (**18a**). A similar effect of the 6-fluoro substituent had been observed for the pyrazine pair **2** and **11j**. In contrast, 6-methoxy indene **21c** provided an excellent overall screening profile with >10,000-fold selectivity over the hERG channel.

These observations demonstrated that a desirable profile with potent H_1 binding and selectivity over hERG could be achieved

Table 2
Effect of R^1 substituent in various indenes without a chiral center on H_1 binding affinity and selectivity

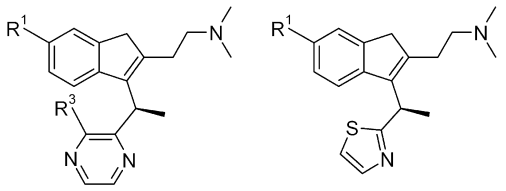


11 **12**

Compd	R^1	R^3	H_1[a,b] K_i (nM)	CYP2D6[c,d] IC_{50} (μM)	hERG[c,e] K_i (μM)	hERG[f] IC_{50} (μM)
2	H	H	4.0 ± 0.3	6.8	>3.0	5.4
11g	Me	H	11.9 ± 0.5	6.6	>3.0	0.52
11h	OMe	H	8.9 ± 0.6	5.3	>3.0	2.1
11i	Cl	H	1.4 ± 0.3	36	1.4	0.11
11j	F	H	1.4 ± 0.3	15	0.95	0.59
11k	Cl	OMe	1.5 ± 0.4	0.69	1.3	0.23
11l	F	OMe	0.8 ± 0.2	0.32	>3.0	NT[g]
12b	Cl	H	2.1 ± 0.3	12	0.38	0.22
12c	F	H	1.7 ± 0.2	12	>3.0	0.89

[a] K_i values derived from dose–response curves generated from triplicate or more data points.
[b] M_1 K_i >10 μM for all compounds except **11k** (K_i = 2.0 μM).
[c] K_i values derived from dose–response curves generated from duplicate data points.
[d] CYP3A4 IC_{50} >10 μM for all compounds tested.
[e] hERG dofetilide binding assay.
[f] hERG patch clamp assay.
[g] NT = not tested.

Table 3
H_1 binding affinity and selectivity of (−)-enantiomers with different R^1 and heteroaryl groups



14 **21**

Compd	R^1	R^3	H_1[a,b] K_i (nM)	CYP2D6[c] IC_{50} (μM)	hERG[d] K_i (μM)	hERG[e] IC_{50} (μM)
14b	Me	H	4.2 ± 1.1	22	5.1	0.36
14c	OMe	H	3.1 ± 0.4	12	>3	3.4
14d	Me	OMe	2.7 ± 0.3	11	>3	3.8
14e	OMe	OMe	1.3 ± 0.1	1.9	>3	5.0
14f	F	OMe	0.7 ± 0.1	6.4	>3	1.8
21a	F	—	0.3 ± 0.1	1.0	1.2	NT[f]
21b	Me	—	2.2 ± 0.2	1.4	>3	0.62
21c	OMe	—	2.5 ± 0.1	5.6	>3	22

[a] K_i values derived from dose–response curves generated from triplicate or more data points.
[b] M_1 K_i >10 μM for all compounds except **21a** (K_i = 3.5 μM).
[c] CYP3A4 IC_{50} >10 μM for all compounds tested.
[d] hERG dofetilide binding assay.
[e] hERG patch clamp assay.
[f] NT = not tested.

through the right balance between electron rich heterocycles, the presence of a chiral center and 6-substitution in the indene core.

In summary, detailed SAR around our lead indene **2** led to the identification of several compounds with potent H_1 affinity and >1000-fold selectivity over M_1, CYP2D6, and hERG. Optimization of the 6-substituent in the indene core was required with each heterocycle to obtain the most beneficial balance between H_1 affinity and selectivity over the hERG channel. A chiral center typically led to a small increase in H_1 affinity and overall selectivity improvement but was not always required to obtain the desired profile. Numerous compounds with an acceptable in vitro profile were identified. Additional metabolism studies and further selectivity profiling, followed by in vivo evaluation of a subset of these compounds led to the identification of our clinical backup compound. These studies will be the subject of our next communication.

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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.2010.02.055.

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11. 2-Ethyl-5-trimethylsilanyl-thiazole (**19**) was prepared by treatment of 2-ethylthiazole with *n*-BuLi in THF at −50 °C for 30 min followed by addition of TMSCl at −50 °C. The reaction was allowed to warm to room temperature prior to workup.
12. Calculated using ACD/Log *P* and pK_a DB, version 9.02, Advanced Chemistry Development, Inc., Toronto, ON, Canada.