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Characterization of Novel Selective H_1-Antihistamines for Clinical Evaluation in the Treatment of Insomnia

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Abstract: Analogues of the known H_1-antihistamine *R*-dimethindene were profiled as potential agents for the treatment of insomnia. Several highly selective compounds were efficacious in rodent sleep models. On the basis of overall profile, indene **1d** and benzothiophene **2a** had pharmacokinetic properties suitable for evaluation in night time dosing. Compound **2a** did not show an in vivo cardiovascular effect from weak hERG channel inhibition.

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Current epidemiological data suggest that insomnia is one of the most common central nervous system (CNS[a]) disorders with high prevalence in industrialized nations.[1] Insomnia has a significant economic impact on managed care[2] with detrimental effects on nations' workforces resulting from higher work absenteeism and decreased job performance.[3]

The most common treatments for insomnia are pharmacologic agents that act on the benzodiazepine binding site of the γ-aminobutyric acid ($GABA_A$) receptor and include zolpidem, temazepam, and eszopiclone. These agents provide positive effects in terms of sleep onset and sleep maintenance. However, side effects including daytime sedation, cognitive impairment, and motor effects raise concerns of increased risk of motor vehicle accidents and injuries from falls.[4,5] Moreover, recent safety labeling changes warn of complex behaviors with amnesia following sedative−hypnotic use.[6] These agents have also been associated with the potential for abuse and dependence in at-risk populations and are classified as schedule IV controlled substances in the U.S.[7] Accordingly, there remains significant interest in developing alternative sleep agents that demonstrate sleep onset and maintenance and improved sleep quality throughout the night without next day sedation. Novel hypnotic mechanisms[8] that have been recently evaluated or are still under investigation include the atypical $GABA_A$ receptor agonist gaboxadol, the recently approved melatonin receptor agonist ramelteon, serotonin 5-HT_{2A} receptor modulators, and orexin receptor antagonists.[9]

Histamine exerts a number of well documented peripheral effects.[10] In addition histamine is a neurotransmitter that has acknowledged effects on arousal and wakefulness through central action at H_1 and H_3 receptor subtypes.[11] Indeed, first generation H_1-antihistamines including diphenhydramine, doxylamine, and chlorpheniramine are sedating and have been used for many years in over-the-counter sleep aids. However, these agents also exhibit functional antagonism of muscarinic receptors,[12] a property thought to contribute to undesirable side effects such as dry mouth, blurred vision, constipation, tachycardia, urinary retention, and memory deficits.[13] Next-day impairment after bedtime use of these antihistamines is common and has been attributed to long plasma half-lives and protracted CNS exposure,[14] suggesting that an improved pharmacokinetic profile would be an important determinant in the selection of novel H_1-antihistamines for insomnia. Thus, selective H_1-antihistamines with appropriate duration of CNS exposure may provide an alternative to current medications for the treatment of insomnia, particularly for improved sleep throughout the night and sleep efficiency.[15] These agents would have low potential for abuse and likely would be nonscheduled.

While many first generation H_1-antihistamines are relatively nonselective, some notable exceptions have been reported including a derivative of the substructure **1** (Figure 1), *R*-dimethindene (**1a**). **1a** is the *R* enantiomer of the commercially available racemate dimethindene and is highly selective for the H_1 receptor with sedating properties in daytime studies.[16] Single dose studies have indicated sedative effects lasting up to 5 h,[17] consistent with the reported half-life of racemic dimethindene (~5 h).[18] More recently, doxepin, a potent antihistamine with elimination half-life of 18 h,[19] has shown sleep maintenance effects lasting up to 8 h at low doses with no next day residual effects.[15] While **1a** exhibited less than ideal duration of action for the treatment of insomnia, the compound represented an excellent starting point to identify novel analogues for clinical evaluation that retained receptor selectivity with potentially improved pharmacokinetic profile. Since metabolism of **1a** occurs primarily via "first pass" metabolic processes,[20] pharmacokinetics of new related compounds could be reasonably modeled from projections of clearance. Subtle modifications around the structure of **1a**

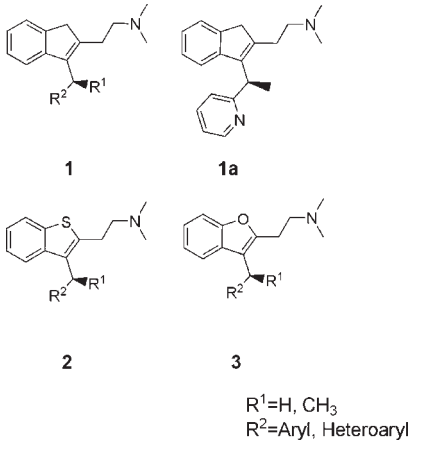

Figure 1. *R*-Dimethindene **1a** and related substructures.

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[a] Abbreviations: CNS, central nervous system; $GABA_A$, γ-aminobutyric acid; QTc, corrected QT interval; EEG, electroencephalography; NREM, nonrapid eye movement; REM, rapid eye movement.

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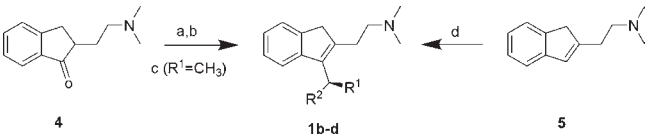
Scheme 1. Synthesis of Indene Analogues[a]



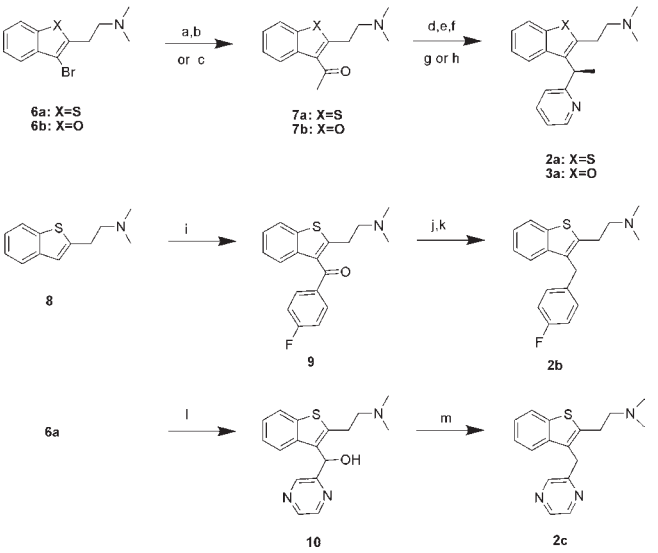
[a] (a) $C_4H_3N_2CH_2R^1$, LDA, THF, 0 °C; (b) HCl (aq) 100 °C; (c) chiral SFC; (d) *n*-BuLi, *p*-$FC_6H_4CH_2Br$, THF, −78 °C.

were anticipated to display differences in pharmacokinetic parameters such as clearance and volume of distribution while maintaining the favorable H_1 activity and selectivity profile. On the basis of **1a**, three substructures were targeted: **1**, benzothiophene (**2**), and benzofuran (**3**). Novelty was generated by selected R^1 (methyl or hydrogen) and R^2 (heteroaryl) substitutions of the indene core or more generalized substitution in the benzothiophene or benzofuran structures. This study had two objectives. The first was to identify potent, brain penetrating H_1-antihistamines with selectivity relative to other key targets. Low muscarinic receptor affinity was required to avoid anticholinergic side effects previously noted. Selectivity relative to $5HT_{2A}$ and H_3 receptors was of interest, since activity at these targets could affect the sleep profile of candidate compounds. hERG potassium channel inhibition was also an important consideration, as a number of antihistamines are potent inhibitors of this channel.[21] Some of these, such as astemizole, are potent hERG channel inhibitors and are thought to cause QT interval or corrected QT interval (QTc) prolongation leading to potentially fatal cardiac arrhythmias. P450 enzyme selectivity and metabolic stability were other important considerations in primary profile assessment. The second objective was to evaluate in vivo properties of the more selective compounds for utility as sleep agents for efficacy in a rat electroencephalography (EEG) model and projected pharmacokinetic profile in humans.

The synthesis of seven analogues is outlined in Schemes 1 and 2. Indene analogues **1b-d** (Scheme 1) were either synthesized in a fashion analogous to that previously reported for **1a**,[22] through base-mediated coupling of a suitable alkyl pyrazine to indanone **4** and acidic work up (analogues **1c, 1d**) or alkylation of the indene **5** (**1b**). Benzothiophene or benzofuran versions of **1a** were synthesized according to one of three approaches outlined in Scheme 2. Direct analogues of **1a** were prepared from acetyl intermediates **7a** or **7b.** Compound **7a** was obtained from a Heck coupling with **6a,** while **7b** was prepared by lithiation of intermediate **6b** followed by quenching with acetic anhydride. Final products **2a** and **3a** were obtained following addition of 2-lithiopyridine to **7a** or **7b** and stepwise dehydration and reduction. Arene substituted benzothiophene **2b** was prepared by Friedel−Crafts acylation of the intermediate **8**. Reduction of the ketone **9** afforded **2b**. Pyrazine **2c** was prepared from lithiation of intermediate 6a and quenching with pyrazine carboxaldehyde. Deoxygenation of the alcohol **10** yielded **2c**. Chiral materials were obtained by fractional crystallization or preparative chiral SFC or HPLC of racemate. The chiral products were characterized by chiral SFC and/or optical rotation. Crystallographic data were obtained for one product to confirm absolute stereochemistry.

Initial profile data of **1a** and its analogues are shown in Table 1. No significant inhibition of CYP3A4 was observed

Scheme 2. Synthesis of Benzothiophene and Benzofuran Analogues[a]



[a] (a) *n*-$BuOCH{=}CH_2$, $Pd(OAc)_2$, P(*o*-Tol)$_3$, Et_3N, CH_3CN, 100 °C; (b) 20% HCl, THF; (c) *n*-BuLi, TMEDA, Ac_2O, toluene, −78 °C; (d) C_5H_4NLi, TMEDA, toluene or DCM, −78 °C; (e) H_2SO_4, TFA; (f) H_2, Pd/C, MeOH; (g) fractional crystallization; (h) chiral HPLC; (i) *p*-FC_6H_4COCl, 1M $AlCl_3$ in $C_6H_5NO_2$, $C_2H_4Cl_2$, 0 °C; (j) $LiAlH_4$, THF, 0 °C; (k) $NaBH_4$, TFA; (l) *n*-BuLi, TMEDA, $C_4H_3N_2CHO$, toluene, −78 °C; (m) HI, HOAc.

in any of the examples. Human microsome stability data displayed sufficient variation in predicted clearance rates, suggesting that it would be possible to identify compounds with varying pharmacokinetic profiles in the analogues prepared. With the exception of **2c**, all of the compounds tested were potent binders to the H_1 receptor with greater than 100-fold selectivity relative to M_1. Examples **1a**−**d** in the indene series and benzothiophene **2a** were greater than 1000-fold selective for this receptor. Data for **1a**−**d** indicated that secondary and tertiary carbon analogues had similar H_1 potency. In the benzothiophene class, arene substitution in **2b** provided a slightly less potent compound. Interestingly, pyrazine analogue **2c** was a markedly less potent H_1-antihistamine, indicating subtle differences between the indene and benzothiophene classes. Introduction of the *p*-fluorophenyl substitution to **1b** and **2b** resulted in significant CYP2D6 inhibition and potent affinity for $5HT_{2A}$. In contrast, **1a**, **1c**, **1d**, **2a**, and **3a** were greater than 1000-fold selective for CYP2D6, highlighting the value of the heterocyclic group for improved drug likeness. Benzofuran **3a** was potent in H_1 binding but an order of magnitude less selective for M_1 compared to **1a** and benzothiophene **2a**. Compound **3a** also displayed significant affinity for M_3 (K_i=76 nM) that was not observed for the other analogues. Indene **1c** was less selective versus $5HT_{2A}$ compared to its chiral analogues **1a** and **1d**. None of the compounds showed significant affinity for H_3 receptor. hERG channel data for **1c**, **1d**, **2a**, and **3a** showed weak inhibition comparable to **1a**. Overall these data indicated suitable receptor selectivity profiles for **1c**, **1d**, and **2a**. These compounds were further assessed for effects on sleep−wake parameters in rats.

Table 2 summarizes sleep parameters for **1a**, **1c**, **1d**, and **2a**. All the compounds tested exhibited a similar and significant increase in nonrapid eye movement (NREM) sleep. None of the compounds tested showed appreciable effects on latency

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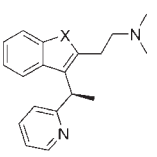
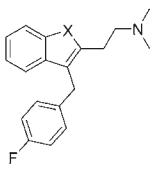
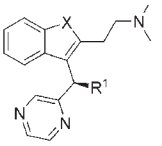
Table 1. In Vitro Profile of *R*-Dimethindene Analogues[a]



1a: X=CH_2
2a: X=S
3a: X=O



1b: X=CH_2
2b: X=S



1c: X=CH_2; R^1=H
1d: X=CH_2: R^1=CH_3
2c: X=S; R^1=H

compound	H_1, $K_i \pm$ SEM[b] (nM)	M_1, $K_i \pm$ SEM[b] (μM)	$5HT_{2A}$, $K_i \pm$ SEM[b] (μM)	CYP2D6, IC_{50} (μM)	hERG,[c] IC_{50} (μM)	human systemic clearance[d] ((mL/min)/kg)
1a	0.4 ± 0.04	2.4 ± 0.2	33% at 10 μM	32.5	0.9	7
1b	0.60 ± 0.03	14% at 10 μM	0.14 ± 0.15	0.3	ND	13
1c	4.0 ± 0.3	19 ± 2	0.60 ± 0.04	6.7	5.5	12
1d	1.3 ± 0.2	20% at 10 μM	32% at 10 μM	50.5	1.4	5
2a	4.0 ± 0.5	5.3 ± 0.3	31% at 10 μM	28.2	1.4	10
2b	11 ± 5	2.0 ± 0.0	0.09 ± 0.04	0.2	ND	16
2c	75 ± 6	ND	ND	ND	ND	ND
3a	0.9 ± 0.2	0.32 ± 0.03	62% at 10 μM	3.3	1.2	10

[a] ND: not determined. [b] Minimum of 3 experiments. [c] For comparison, cisapride and the H_1-antihistamines astemizole and diphenhydramine were tested in these cells (IC_{50} = 36 nM, 10 nM, and 2 μM, respectively). [d] Predicted from human liver microsomes.

Table 2. Efficacy Profile of *R*-Dimethindene Analogues[a]

compound	dose, (mg/kg)	NREM in 4 h[b]	latency to 1 min NREM[b]	plasma concn (1 h), (ng/mL)	rat intrinsic clearance,[c] ((mL/min)/kg)
1a	60	132 ± 7	134 ± 7	ND	2600
1c	60	159 ± 6	79 ± 7	16 ± 13	1500
1d	60	145 ± 5	109 ± 7	86 ± 45	1500
2a	30	142 ± 6	124 ± 12	4.0 ± 0.1	2300
zolpidem	30	220 ± 6	18 ± 2	ND	ND

[a] ND: not determined. [b] Calculated as a percentage of vehicle. [c] Predicted from rat liver microsomes.

to NREM or rapid eye movement (REM) sleep parameters. Only **2a** displayed a significant increase in REM sleep (168% of vehicle). Plasma concentrations from surrogate animals for **1c**, **1d**, and **2a** were low despite the large doses employed. While relatively stable in human microsomes, all the compounds were rapidly metabolized by the rat counterpart (Table 2) with observed clearance values at the limit of assay detection. Surrogate PK studies of **2a** at a dose of 30 mg/kg showed brain levels of 29 ng/g at 1 h and 23 ng/g at 4 h. These data indicated that this analogue was brain penetrating and achieved suitable exposure over the course of the EEG experiment despite relatively low plasma concentrations. Overall, these studies were consistent with reported effects of H_1-antihistamines on sleep−wake parameters in rats.[23]

From the available data, compounds **1c**, **1d**, and **2a** were selective H_1-antihistamines with rodent efficacy comparable to **1a**. It was hypothesized that analogues of **1a** with similar or incrementally longer half-lives would represent suitable candidates for further evaluation of dose, exposure, and sedative action. Human microsome stability data indicated that **1c** displayed higher clearance compared to **1a**. In contrast, **1d** and **2a** exhibited predicted systemic clearance values similar to **1a** [7 (mL/min)/kg]. Allometric scaling of these two compounds projected human clearances of approximately 9 (mL/min)/kg,[24] a value close to that reported for racemic dimethindene [8.0 (mL/min)/kg],[18] indicating that these were candidates of interest. Both compounds were subsequently assessed in a human microdose study where measured half-lives for compounds **2a** and **1d** were 6.8 and 12 h, respectively.[24] Measured clearance values for **2a** and **1d** were low at 5.2 and 3.3 (mL/min)/kg, respectively.

The absence of specific reports concerning QT effects in the use of dimethindene and the weak hERG inhibition signal observed for these compounds prompted further assessment of the risk for QTc prolongation. A cardiovascular safety study of **2a** in conscious monkeys produced a slight decrease in heart rate (16−19%) compared to vehicle control at all doses up to 200 mg/kg, beginning 1 h postdose and returning to near control levels approximately 10 h postdose. Reflective of this decrease, there were slight increases in the electrocardiograph RR and PR intervals, QTc and duration of the QRS complex.[25] When QTc interval was corrected for heart rate, there were no notable differences between the test article treated groups and the control group (range of QTc change is −1 to 5 ms) at exposures at least 100-fold higher than those projected for clinically efficacious doses of **2a**. This margin was significantly higher than previous guidelines for the assessment of hERG inhibitors[21] and underscored the importance of an in vivo assessment of **2a** to qualify the in vitro findings. From the overall data profile **2a** was advanced to clinical trials for further assessment.

In summary, a number of novel, selective H_1-antihistamines were identified and examined for utility in the treatment of insomnia. A key driver in compound selection focused on pharmacokinetic properties which led to the identification of analogues **1d** and **2a** that were efficacious in a rat EEG model with suitable clearance profiles. While they are weak inhibitors of the hERG channel, in vivo assessment of **2a** in monkeys showed no significant effects on QTc, indicating a large safety margin for this parameter. As such, **2a** appears to be a suitable candidate for further assessment as a sleep agent.

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Supporting Information Available: Experimental procedures, analytical and spectral characterization data, EEG data and

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crystallographic information in CIF format. This material is available free of charge via the Internet at http://pubs.acs.org.

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