Bioorganic & Medicinal Chemistry Letters 21 (2011) 947–951

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Influence of pK_a on the biotransformation of indene H_1-antihistamines by CYP2D6

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ARTICLE INFO

Article history:
Received 29 August 2010
Revised 8 December 2010
Accepted 13 December 2010
Available online 16 December 2010

Keywords:
H_1 receptor
H_1-antihistamine
Histamine
Insomnia
Indene
CYP2D6
Biotransformation
pK_a

ABSTRACT

Structure–activity relationship studies were conducted to reduce CYP2D6-mediated metabolism in a series of indene H_1-antihistamines. Reductions in pK_a via incorporation of a β-fluoro substituent or a heteroaryl moiety were shown to reduce contributions to metabolism through this pathway. Several compounds, including **8l**, **8o**, and **12f** were identified with promising primary in vitro profiles and reduced biotransformation via CYP2D6.

Novel and selective H_1-antihistamines with appropriate exposure are of potential interest as an alternative to current medications for the treatment of insomnia. Such agents enhance sleep during the latter third of the night and improve sleep efficiency.[1] Unlike current $GABA_A$ hypnotics,[2] these antihistamines are likely to have low abuse potential. Recently, derivatives of H_1-antihistamine *R*-dimethindene (**1**),[3] namely **2a** and **2b**, were identified as highly selective compounds for the H_1 receptor with sedating properties (Fig. 1).[4]

Analysis of the metabolism of compound **2a** in human liver microsomes (HLM) showed a similar metabolite profile to *R*-dimethindene (**1**),[5] the major metabolite being the 6-hydroxyindene (**3**) (Fig. 2). Semi-quantitative analysis of the enzymatic pathways associated with this metabolism indicated that the enzyme responsible for the vast proportion of metabolism (>90% in human liver microsomes, HLM) was CYP2D6.[6] Analysis of compound **2b** indicated a similar metabolite profile to **2a**. The characterization of these compounds as predominant CYP2D6 substrates presented two issues. In addition to the liability of drug–drug interactions, heterogeneity of CYP2D6 activity within the general population raised the concern of extreme variability in pharmacokinetics for candidate compounds.[7] This was of particular concern for a sleep agent as extended duration of action would be expected in CYP2D6 poor metabolizers resulting in undesirable next day residual effects. We previously described an approach in which modifications to the 6-position of the indene core was utilized to identify a backup candidate, **4**, with significantly reduced biotransformation through the CYP2D6 pathway.[6]

In this Letter, we describe an alternative approach to reduce biotransformation via CYP2D6 in the indene series. Previous reports demonstrated the critical importance of Asp 301 in the CYP2D6 enzyme for substrate transformation,[8] suggesting that binding of the basic amine within compounds such as **2a** and **2b** is important for metabolism. Indeed modulation of pK_a in some basic compounds has been shown to affect metabolism by this enzyme.[9] While indenes **2a** and **2b** are basic (measured pK_a 9.1 for

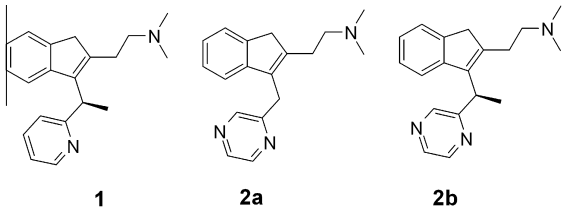

Figure 1. *R*-Dimethindene (**1**), and analogs **2a** and **2b**.

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doi:10.1016/j.bmcl.2010.12.053

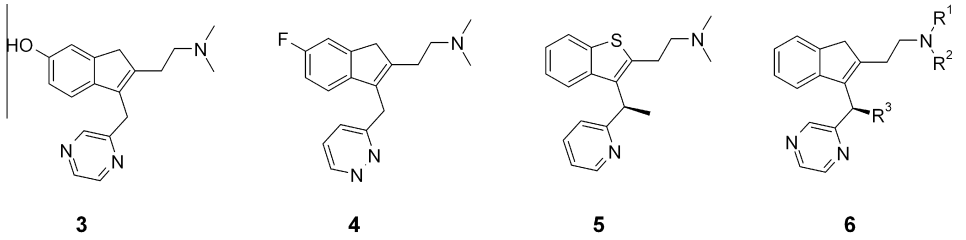


Figure 2. Major metabolite (**3**) of **2a**, backup compound (**4**), clinical compound (**5**), and series described in this Letter (**6**).

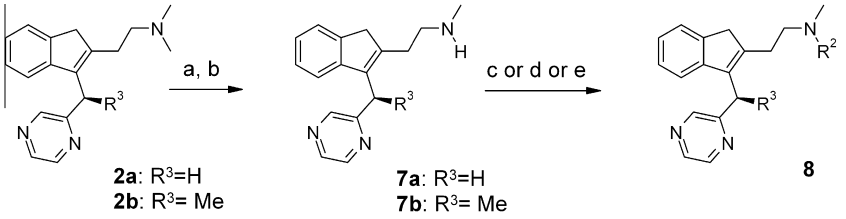
both, calcd pK_a 9.6)[6] and primary substrates for CYP2D6, benzothiophene **5** is less basic (measured pK_a 8.6, calcd pK_a 9.2)[10] and is metabolized by a variety of CYP450 enzymes.[11] We hypothesized that we could reduce susceptibility to metabolism via the CYP2D6 pathway in the indene class by reduction of pK_a through modification of the NR_1R_2 in the general substructure **6**.

Assessment of the biotransformation was measured through a screening strategy previously described using quinidine, a known specific CYP2D6 inhibitor.[6,12] Contributions from CYP2D6 biotransformation to overall compound metabolism vary significantly between normal and poor metabolizer populations. We estimated that a contribution from CYP2D6 greater than 70% could lead to exposure variabilities $\geqslant$3–10-fold. Therefore, to limit PK variability to $\leqslant$2–3-fold, we estimated that CYP2D6 contributions to compound metabolism should not exceed 60%.

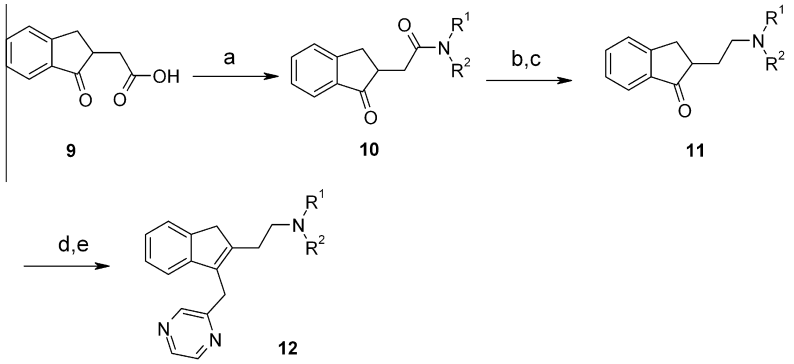
Our objective was to make small modifications to the amine in general structure **6** that maintained H_1 affinity and selectivity, yet reduced pK_a of the basic function. Compounds of interest would then be evaluated for their ability to reduce biotransformation via CYP2D6. To achieve similar selectivity to that described earlier,[4,12] candidate compounds were required to demonstrate high H_1 binding affinity ($K_i \leqslant 10$ nM) with at least 100-fold binding selectivity versus the representative muscarinic M_1 receptor. Initially, selectivity in the range of 100–1000-fold was considered acceptable for CYP enzyme inhibition. It is well known that several antihistamines are potent inhibitors of the hERG channel,[13] implicated in prolongation of cardiac QTc and leading to cardiac arrhythmias. A preliminary assessment of hERG affinity would also be conducted.

Two synthetic schemes were employed to generate indenes (**6**). Indenes **2a**, **2b**[4] were demethylated to **7** under standard conditions and N-alkylated by reductive amination or alkylation to yield **8** (Scheme 1). When the NR^1R^2 fragment in **6** was a cyclic amine, a different route was employed. Indanone **9** was coupled to an amine to give intermediate **10**, as previously reported.[12] A simultaneous reduction of the amide and ketone functionalities with $LiALH_4$, followed by a subsequent oxidation, yielded indanone **11**. Addition of methyl pyrazine to **11**, followed by dehydration afforded indene **12**. (Scheme 2)

Compounds were tested in a histamine H_1 receptor binding assay.[4] To confirm initial selectivity, compounds were subsequently tested for inhibition of cytochrome P450 enzymes CYP2D6 and CYP3A4.[4] Preliminary selectivity over the hERG channel was evaluated using the high-throughput dofetilide binding assay.[14] Representative compounds were also assessed for M_1 affinity.[4] Estimates of biotransformation through CYP2D6 were made through comparison of the predicted intrinsic clearance in HLM with and without the specific CYP2D6 inhibitor quinidine.[12] The

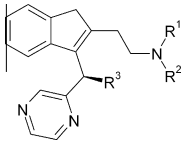


Scheme 1. Reagents and conditions: (a) ACE-Cl, DIPEA, 1,2-DCE, 1 h, 45 °C; (b) MeOH, rt; (c) $NaBH(OEt)_3$, HOAc, $R^2(=O)H$; (d) BH_3·Pyr, MeOH, $R^2(=O)H$; (e) R^2X, K_2CO_3, DMF, 70 °C.



Scheme 2. Reagents and conditions: (a) HNR^1R^2, EDCI, HOBt, DIPEA, DCM; (b) $LiAlH_4$, THF, 0 °C then reflux 3 h; (c) Ph_2CO, ^tBuOK, benzene, 120 °C, 3 h; (d) methyl pyrazine, LDA, THF, 0 °C; (e) 20% HCl, reflux.

Table 1
Exploration of various NR^1R^2 groups with and without electron-withdrawing moieties



Compd	NR^1R^2	R^3	H_1[a,b,c] K_i (nM)	CYP2D6[d] IC_{50} (nM)	CYP3A4[d] IC_{50} (nM)	Calcd pK_a[10]	Pred. Int. Cl.[e] (mL/min/kg) [0 μM quinidine]	Pred. Int. Cl.[e] (mL/min/kg) [3 μM quinidine]	% CYP2D6 quinidine
2a	$-NMe_2$	H	4.0 ± 0.3	6679	>10,000	9.6	13.6	1.5	91
8a	–NMeEt	H	7.2 ± 1.6	4346	>10,000	9.7	NT[f]	NT	NT
8b	–NMePr	H	41 ± 4	973	4038	9.7	NT	NT	NT
8c	$-NMeCH_2CH_2F$	H	24 ± 2	3093	>10,000	8.1	22.6	20.2	10
2b	$-NMe_2$	Me	1.3 ± 0.2	>10,000	>10,000	9.6	4.3	1.5	66
8d	$-NMeCH_2CH_2F$	Me	13 ± 1	2605	>10,000	8.1	122.3	103.2	16
8e	$-NMeCH_2CN$	Me	32 ± 2	>10,000	>10,000	5.1	NT	NT	NT
8f	$-NMeCH_2CH_2CN$	Me	109 ± 11	>10,000	>10,000	8.0	NT	NT	NT
8g	$-NMeCH_2CH_2CF_3$	Me	91 ± 20	7224	>5000	8.3	NT	NT	NT
12a		H	4.3 ± 1.8	8845	>10,000	10.2	15.5	1.5	90
12b		H	2.8 ± 0.2	3385	>10,000	10.2	38.6	1.5	96
12c		H	49 ± 4	6466	>10,000	7.4	63.5	47.8	25
12d		H	4605 ± 4	>10,000	3871	4.5	150.1	137.2	9
12e		H	249 ± 4	7533	1405	5.6	192.3	157.8	18
12f		H	10.5 ± 1.0	4293	>10,000	7.9	35.3	16.3	54

[a] SEM of K_i values derived from dose–response curves generated from triplicate or more data points.
[b] No significant M_1 inhibition (K_i >10 μM) for representative compounds tested: **2a**, **8a**, **2b**, **8d**, **12f**.
[c] hERG dofetilide binding between 0% and 24% at 3 μM for all compounds tested.
[d] IC_{50} values derived from dose–response curves generated from duplicate data points.
[e] Average of two replicates for the entire curve from 0 to 60 min.
[f] NT = not tested.

reduction in predicted intrinsic clearance in the presence of quinidine was used to estimate the contribution of CYP2D6 as a percentage of the total predicted intrinsic clearance in the absence of the enzyme inhibitor. Data are shown in Tables 1 and 2.

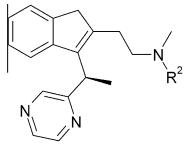
Prior to introduction of electron-withdrawing moieties on the amine portion, the effects of extension of the methyl group to ethyl (**8a**) and propyl (**8b**) in **2a** were explored to establish a baseline SAR (Table 1). Although an ethyl group was still tolerated, the larger propyl group demonstrated that increased hydrophobicity led to both decreased H_1 binding affinity and decreased selectivity versus the CYP enzymes. Since a fluoro atom is only slightly bigger than hydrogen and considered a hydrogen mimic, a fluoroethyl moiety was introduced (**8c**) in a first attempt to reduce pK_a (calcd pK_a is 1.6 log units lower than **2a** and **8a**) and maintain the H_1 affinity observed for **8a**. Although the H_1 binding affinity (K_i 24 nM) did not meet our program criteria (H_1 $K_i \leqslant 10$ nM), biotransformation of this compound by CYP2D6 was evaluated to assess our hypothesis. Gratifyingly, the predicted intrinsic clearance in the absence and presence of quinidine were very similar indicating low amounts of biotransformation through CYP2D6 pathway in contrast to that observed for the original lead **2a**.

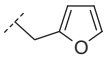
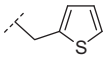
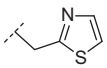
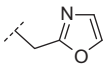
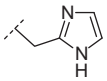
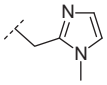
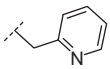
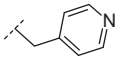
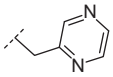
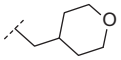
Encouraged by these initial results, we subsequently introduced the fluoroethyl and other electron-withdrawing moieties with reduced pK_a values (at least 1.5 log units) into the chiral analog **2b**. Since the *R*-enantiomer (**2b**) has improved binding affinity for H_1 and improved selectivity over off-targets compared to the achiral indene **2a**,[4] we expected improved primary profiles. Of the variants explored (**8d**–**g**) only the fluoroethyl compound (**8d**) demonstrated H_1 affinity approaching our criteria. This compound exhibited a similar significant reduction in biotransformation through CYP2D6 as observed for **8c**. Unexpectedly, chiral **8d** was more rapidly metabolized than achiral **8c** both in the presence and absence of quinidine. Increased clearance had not been observed for the chiral lead **2b** when compared to the achiral **2a**.

Ring constraints at the NR_1R_2 moiety have been shown to improve affinity in H_1-antihistamines.[15] In this chemical class, compounds **12a** and **12b**, containing an azetidine and pyrrolidine, respectively, displayed comparable/improved H_1 binding affinity to **2a**. The calcd pK_a of these analogs was increased compared to **2a** and it was expected that they displayed similar high levels of biotransformation via CYP2D6 as **2a**. With the objective to improve H_1 affinity over our leads **8c** and **8d** and maintain the low CYP2D6 biotransformation and low clearance, fluoro substituted pyrrolidines and azetidines were incorporated into the achiral indene.

Monosubstituted fluoro-cycloamines (**12c**, **12f**) demonstrated significantly higher affinity for H_1 than the difluoro substituted analogs (**12d**, **12e**). The pyrrolidine analogs demonstrated higher affinity than their azetidine counterparts, a trend also observed for the unsubstituted variants **12a** and **12b**. Overall, the H_1 affinity for all these fluoro substituted variants was lower than **2a**. Interestingly, the difluoro analogs were more potent CYP3A4 inhibitors, but less potent CYP2D6 inhibitors than their monofluoro counterparts. Upon evaluation of the fluoro substituted cyclic amines in the CYP2D6 biotransformation assay, analogs **12c**–**f** demonstrated significant reductions in CYP2D6 contributions to metabolism, suggesting a correlation between pK_a and CYP2D6 biotransformation.

Table 2
Effect of R^2 substituent on H_1 binding, CYP inhibition, and calcd pK_a of –NMeR^2



Compd	R^2	H_1[a,b,c] K_i (nM)	CYP2D6[d] IC_{50} (nM)	CYP3A4[d] IC_{50} (nM)	Cal. pK_a[10] –NMeR^2
8h		8.1 ± 1.1	4363	2751	8.2
8i		6.7 ± 1.0	1427	1826	8.1
8j		48 ± 6	>10,000	2820	6.9
8k		162 ± 70	>10,000	4840	6.6
8l		8.8 ± 0.5	5852	4416	7.4
8m		28 ± 4	5684	1535	8.3
8n		13.9 ± 0.4	5707	4783	7.6
8o		4.9 ± 0.2	7957	1281	7.6
8p		24.7 ± 0.3	>10,000	>10,000	7.6
8q		15.2 ± 0.9	9373	>10,000	9.2

[a] SEM for K_i values derived from dose–response curves generated from triplicate or more data points.
[b] No significant M_1 inhibition (K_i >10 μM) for representative compounds tested: **8l**, **8o**.
[c] hERG dofetilide binding between 0% and 37% at 3 μM for all compounds tested.
[d] IC_{50} values derived from dose–response curves generated from duplicate data points.

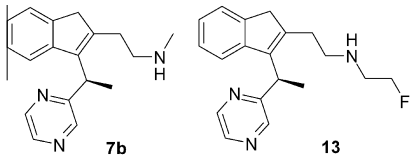


Figure 3. Metabolites of compound **8d**.

Both difluoro substituted analogs **12d** and **12e**, having calcd pK_a values that were at least 2 log units less than their mono fluoro analogs, lost significant affinity for the H_1 receptor. Although contribution of CYP2D6 to their metabolism was low, their overall intrinsic clearance was significantly higher than for the monofluoro variants.

Of the compounds evaluated, monofluoropyrrolidine **12f** showed the best overall profile, with acceptable H_1 binding, weak CYP2D6 inhibition and an estimated level of CYP2D6 biotransformation of 53% that was less than our 60% cut-off criteria. Compound **12f** was dosed in a rat cassette PK study[14] to assess the level of brain-penetration (iv, 1 mg/kg). At 4 h, the concentration in the brain was 57 ng/g (B/P = 3.9), which was comparable to triprolidine (72 ng/g), a known sedating antihistamine. With this compound, we demonstrated that lowering of the pK_a of the basic amine had a profound impact on metabolism via CYP2D6, but did not negatively affect the capability to penetrate the blood–brain-barrier. Unfortunately, neither enantiomer of **12f** demonstrated an improvement in H_1 binding affinity (data not shown).

As an alternative to extended alkyl groups with simple electron-withdrawing features, several heterocycles were explored with the same objective of reducing the pK_a of the basic amine while maintaining high H_1 binding affinity (Table 2). Several modifications were identified with high H_1 affinity, such as the furyl (**8h**), thiophene (**8i**), unsubstituted imidazole (**8l**), and pyridines (**8n** and **8o**). In all examples listed, the calcd pK_a for the tertiary amine was significantly reduced compared to that in **2a** or **2b**. Compound profiling for off-target activity indicated minimal M_1 inhibition[4] and no significant affinity for the hERG channel in the dofetilide binding assay.[14] CYP2D6 inhibition was most significant for **8h** and **8i**, possibly due to the increased hydrophobicity. In contrast to indenes described previously,[4,6,12] several compounds in this subset displayed significant CYP3A4 inhibition. Some structural features of the R^2 side chain (Table 2) have been noted to contribute to increased CYP3A4 inhibition (e.g., imidazole **8l** and 4-pyridine **8o**).[16]

The compounds with the best overall profile, **8l** and **8o**, were subsequently tested for CYP2D6 biotransformation. Both compounds showed minimal changes in estimated intrinsic clearance when quinidine was present (**8l**: 411.7 ml/min/kg at 0 μM quinidine; 403.4 ml/min/kg at 3 μM quinidine and **8o**: 275.5 ml/min/kg at 0 μM quinidine; 224.9 ml/min/kg at 3 μM quinidine), indicating that CYP2D6 metabolism was not a major factor (2% for **8l** and 18%

for **8o**) in the biotransformation of these analogs in contrast to **2a** or **2b**. Unfortunately, both **8l** and **8o** demonstrated poor stability in human liver microsomes compared to **2b** (Cl. int. 4.3 ml/min/kg), a similar result to that observed for the fluoroethyl analog **8d**.

Metabolite identification studies were conducted on **8d**. In contrast to the metabolism profile of **2b**, minimal hydroxylation of the indene 6-position was detected. Two major metabolites were identified, namely the dealkylated indene **7b** (46% of total metabolites) and the demethylated indene **13** (33%) (Fig. 3). This suggested that modification of the basic center promoted a shift away from the CYP2D6-mediated pathway of hydroxylation towards dealkylation pathways that are presumably mediated by other CYP450 enzymes. From the metabolite data for **8d**, it would appear that electron-withdrawing features of the type employed create an activated methylene that is highly susceptible to metabolic oxidation and can contribute to an increase in the rate of substrate metabolism. Overall, while a reduction in pK_a of the basic center could be used to modulate biotransformation through the CYP2D6 pathway, alternative substitution patterns of the amine distinct from the imidazole and pyridine features identified in compounds **8l** and **8o** are likely required to disrupt the other pathways responsible for the extensive metabolism observed in these compounds. Given the considerably higher clearance observed for the tertiary trialkylamines **8l** and **8o**, the more stable analog **12f** containing the substituted pyrrolidine is likely the better starting point for further analog synthesis and will be the focus of future optimization efforts.

In summary, near exclusive metabolism by CYP2D6 of early lead indenes **2a** and **2b** was identified as an issue for further compound development. An approach was described to reduce CYP2D6 biotransformation through a lowering of the pK_a of the NR^1R^2 moiety in series **6** that was achieved by the introduction of electron-withdrawing groups. A clear correlation was discovered between reduction of pK_a and reduced biotransformation through CYP2D6. Compounds **8l**, **8o** and **12f** were identified with sufficient H_1 binding affinity and selectivity that demonstrated reduced contributions to their metabolism through the CYP2D6 pathway. It was further demonstrated that a reduction in pK_a did not negatively impact the capability of one of these compounds to penetrate the blood–brain-barrier. However, these leads demonstrated relatively poor stability in human liver microsomes, making the compounds unsuitable for further development. In our next communication we will examine an expansion of the approach described here to identify H_1-antihistamines with improved stability and selectivity over the CYP enzymes, and a more suitable overall profile for further development as sleep aids.

Acknowledgments

The authors thank John Harman and Chris DeVore for analytical support and Dr. John Saunders, Dr. Paul Conlon and Dr. Haig Bozigian for program support.

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