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Identification of a novel selective H_1-antihistamine with optimized pharmacokinetic properties for clinical evaluation in the treatment of insomnia

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ABSTRACT

Analogs of the known H_1-antihistamine *R*-dimethindene with suitable selectivity for key GPCRs, P450 enzymes and hERG channel were assessed for metabolism profile and in vivo properties. Several analogs were determined to exhibit diverse metabolism. One of these compounds, **10a**, showed equivalent efficacy in a rat EEG/EMG model to a previously identified clinical candidate and a potentially superior pharmacokinetic profile as determined from a human microdose study.

Insomnia is one of the most common CNS disorders, affecting one third of the general population, with a prevalence range of 10–48% in industrialized nations.[1] The condition has a significant economic impact on managed care[2] and the workplace.[3] Accordingly, treatment options are becoming increasingly desirable for which pharmacologic agents have become a convenient option of choice. While agents that act on the benzodiazepine binding site of the gamma-aminobutyric acid ($GABA_A$) receptor have been approved and provide positive effects for both sleep onset and maintenance, concerns over side effects associated with daytime sedation, cognitive impairment, motor effects, and complex behaviors[4–6] have prompted investigations of other sedative hypnotic mechanisms.[7,8]

Novel selective H_1-antihistamines with appropriate exposure are of potential interest as an alternative to current medications for the treatment of insomnia, particularly for improvements in sleep during the latter third of the night and overall sleep efficiency.[9] These agents would also be expected to have a low potential for abuse and likely would be non-scheduled unlike the current $GABA_A$ hypnotics.[10] Recently, derivatives of the selective and sedating H_1-antihistamine *R*-dimethindene **(1)**,[11] namely **2**, **3a** and **3b**, were identified as highly selective compounds for the H_1 receptor with sedating properties (Fig. 1).[12]

Metabolism of **1** has been reported to likely predominate via first pass mechanisms.[13] Based on this premise it was hypothesized that pharmacokinetic properties of analogs of **1** could be reasonably estimated from projections of clearance, first studied in vitro followed by in vivo confirmation in animals. From the pharmacokinetic analysis of the analogs in Figure 1, compound **2** was determined to have a desirable profile and advanced into clinical trials.[12] Despite suitable selectivity and sedative properties of compounds **3a** and **3b**, a subsequent assessment of their metabolism in human liver microsomes (HLM) identified an issue with their suitability as lead compounds. Analysis of the metabolism of compound **3a** in human liver microsomes (HLM) showed a similar metabolite profile to **1**; the major metabolite being the 6-hydroxyindene, **4** (Fig. 2).[14] Smaller amounts of the des-methyl metabolite, **5**, and N-oxide, **6**, were also observed. However, in an analysis of the enzymatic pathways associated with this metabolism, using either inhibitory antibodies specific to CYP450 enzymes or recombinant CYP450 enzymes, it was determined that the enzyme responsible for the vast proportion of metabolism (>90% in HLM) was CYP2D6. A similar analysis of compound **3b** indicated

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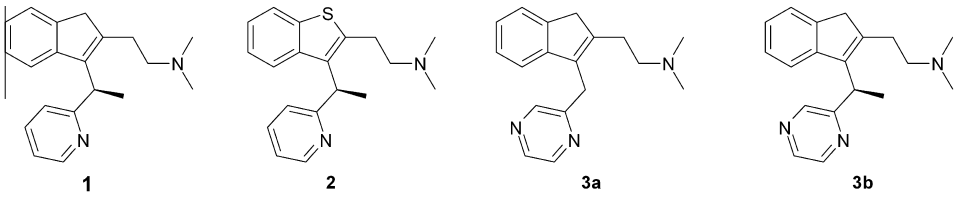


Figure 1. *R*-Dimethindene (**1**) and analogs (**2**, **3a**, **3b**).

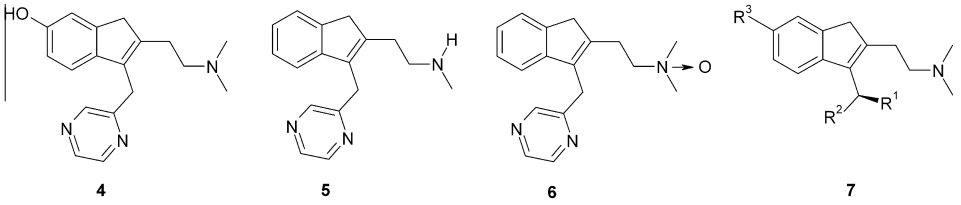
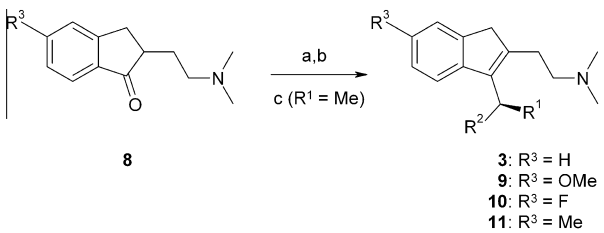


Figure 2. Metabolites of **3a** (**4**–**6**) and indene series (**7**) described in this Letter.

a comparable metabolite profile to **3a** in which CYP2D6 was also determined to be the major contributor to metabolism in the HLM assay. The characterization of these compounds as predominant CYP2D6 substrates presented two issues. Besides the liability of drug interactions, heterogeneity of CYP2D6 activity within the general population raised the concern of extreme variability in pharmacokinetics for candidate compounds.[15] PK variability in our leads was considered an issue because of the potential of varying exposures to adversely impact duration of action.

Assessment of the metabolite profile in the early leads implied that modifications to the 6-position of the indene core would impact metabolic stability and lead to potential changes in the enzymatic pathways contributing to biotransformation of the parent compound. Previous studies have shown that modifications to this position are tolerated within the H_1 pharmacophore.[16] This information suggested a strategy to identify potential backup compounds with diversified biotransformation through the synthesis of analogs of general structure **7**. A follow up study based on this idea led to the identification of a number of potent analogs with suitable receptor, CYP enzyme and hERG channel selectivity.[14] These compounds satisfied the preset criteria of high H_1 binding affinity (K_i <10 nM), greater than 100-fold binding selectivity versus other receptor targets, greater than 1000-fold selectivity for CYP enzyme inhibition and a hERG IC_{50}/H_1 K_i selectivity of 400 or greater.

The study described in this letter had two objectives. The first objective was to profile a set of 8 analogs of **1**, previously identified with suitable selectivity profiles,[14] for risks as predominant CYP2D6 substrates. The second objective was to assess leads of interest arising from the metabolism profiling study for suitable in vitro and in vivo pharmacology required for a sedative hypnotic. This included evaluation of in vitro selectivity versus a broader range of targets, pharmacokinetics, and in vivo efficacy in a rat electroencephalography/electromyography (EEG/EMG) model.



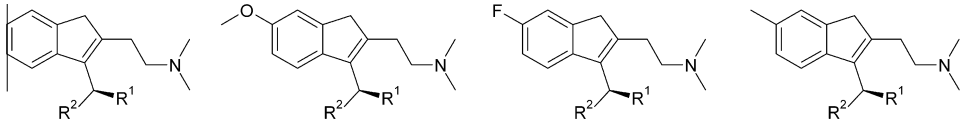
Scheme 1. Reagents and conditions: (a) $R^2CH_2R^1$, LDA, THF, 0 °C; (b) HCl (aq), reflux; (c) Chiral separation by chiral HPLC or SFC.

Synthesis of these analogs is outlined in Scheme 1. Indene analogs were synthesized in analogous fashion to that previously reported[12,14,16] through base mediated coupling of a suitable akyl heteroaromatic to the indanone (**8**) followed by acidic work up and chiral separation (if R^1 = Me) to yield the desired analogs **3**, **9**–**11**.

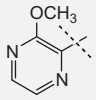
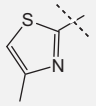
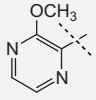
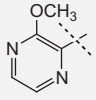
Compounds with CYP2D6 contributions estimated to be less than 60% were hypothesized to have reduced liabilities for drug interactions and PK variability. A screening strategy to determine estimates of biotransformation through CYP2D6 was realized using several methods. In a primary screen a comparison of the predicted intrinsic clearance in HLM with and without the specific CYP2D6 inhibitor quinidine was used to calculate an estimate of biotransformation of the candidate compound through the CYP2D6 pathway. While acceptable as an initial screen, stable compounds with low predicted intrinsic clearance (less than 10 mL/min/kg) were expected to provide false negatives, due to uncertainties in the low clearance values generated, such that inhibition of CYP2D6-related metabolism would be difficult to measure in the microsomal assay. Consequently, follow up studies using specific P450 antibodies and recombinant CYP enzymes were performed to confirm the quinidine results. In the first of these approaches, the formation of the major metabolites was monitored in the presence of antibodies to specific CYP enzymes. Data from this study was used to provide a semi-quantitative assessment of the total contribution of CYP2D6 to biotransformation. The reduction in the amount of each metabolite formed in the presence of CYP2D6 antibody was estimated as a percentage of total metabolism and in comparison to the contributions from other CYP antibodies. In a second and complimentary method, formation of the major metabolite was assessed using individual recombinant CYP enzymes to confirm the estimate from the antibody studies.[17] Although the current literature suggests primary metabolism of compounds of this type to be through phase I mediated mechanisms, metabolism of key compounds was also evaluated in hepatocytes to determine any additional phase II effects on biotransformation.[18]

A primary assessment from the CYP2D6 screen of the *R*-dimethindene analogs is shown in Table 1 in comparison to lead compounds **3a** and **3b**. Of the analogs tested, quinidine inhibition studies immediately excluded several analogs (**3c**, **9b**, **9c**) with too high a contribution from CYP2D6 for further consideration. In these cases, changes in intrinsic clearance in the presence or absence of quinidine were clearly significant. Of the more stable

Table 1
Metabolism profile of *R*-dimethindene analogs



3 **9** **10** **11**

Compd	R^1	R^2	Pred. Int. Cl.[a] (mL/min/kg) [0 μM Quinidine]	Pred. Int. Cl.[a] (mL/min/kg) [3 μM Quinidine]	% CYP2D6[a] Quinidine
3a	H		13.6	1.5	91
3b	CH_3		4.3	1.5	66
3c	CH_3		19.4	4.2	79
3d	CH_3		9.4	4.2	48
9a	CH_3		5.8	4.9	15
9b	CH_3		19.0	6.1	68
9c	CH_3		12.2	2.9	76
10a	H		1.6	1.5	9
10b	CH_3		5.1	5.3	0
11a	CH_3		7.2	4.9	32

[a] Average of two replicates for the entire curve from 0 to 60 min.

compounds, **3d** passed the initial screen. However, follow up studies using CYP450 antibodies or recombinant enzymes clearly indicated that the majority of biotransformation occurred via the CYP2D6 pathway (estimates from these studies indicated metabolism by CYP2D6 as greater than 80%). Interestingly, the 6-methoxy analog **9a** exhibited low metabolism through the CYP2D6 pathway. However in this case, antibody studies indicated that much of the metabolism predominated through the CYP3A4 pathway. Since a predominant CYP3A4 substrate would present similar development issues to a predominant CYP2D6 substrate, this compound was not analyzed further. Of the remaining compounds, **10b** and **11a** showed significantly reduced contributions to metabolism from CYP2D6, further confirmed in follow up studies with antibodies and recombinant enzymes. Compound **10a** demonstrated minimal CYP2D6 contribution in the microsome studies using quinidine. However, the follow up studies with antibodies indicated that the CYP2D6 component (64%) was near the previously set metabolism criteria (60%). Further analysis in hepatocytes for the compounds in Table 1 indicated that both compounds **3b** and **10a** formed significant amounts of a stable *N*-glucuronide (estimated as 26% and 47%, respectively of total metabolites),[18] a phenomenon previously reported for diphenhydramine.[19] This result rekindled interest in these compounds as this was likely to significantly diversify metabolism away from CYP2D6. The other compounds tested showed no second phase metabolism of parent compound.

Generally, indene analogs lacking a 6-substituent (**3**) were significant substrates of CYP2D6. From antibody studies, metabolism of these compounds was characterized by oxidation products at the 6-position.[20] These metabolites appeared to be formed via the 2D6 pathway as observed for the original substrate **3a**. Addition of the chiral methyl in **3b** provided only a modest reduction in biotransformation through this pathway compared to compound **3a.** The identification of an *N*-glucuronide from metabolism in hepatocytes for this compound, however, diversified metabolism sufficiently for this compound to meet our criteria. Of the 6-substituted indene analogs, all the examples in the 6-methoxy series displayed significant biotransformation (estimated >60%) through either CYP2D6 or CYP3A4. In contrast, both 6-fluoro and 6-methyl chiral indenes **10b** and **11a** showed minimal

biotransformation via the CYP2D6 pathway. While the achiral compound **10a** also provided a reduction in CYP2D6 biotransformation compared to the original substrate **3a**, the N-glucuronidation observed in hepatocytes was necessary to fully diversify the metabolism profile for this compound. These data taken together suggest that 6-fluoro or 6-methyl substitutions in combination with the chiral methyl were the most appropriate modifications for modulation of metabolism away from the CYP2D6 pathway.

With the identification of a series of analogs with clearly diversified metabolic profiles, a more detailed comparison of the in vitro profiles of compounds **3b**, **10a–b** and **11a** was made to our clinical candidate (**2**, Table 2).[12] All compounds had high H_1 binding affinity and were highly selective for CYP2D6 inhibition, monoamine receptors associated with other sedation mechanisms [H_3; 5-HT_{2A}; M_1], and receptors that could potentiate side effects [M_1, M_3] associated with known H_1-antihistamines. Broader assessment of the selectivity profile in a Cerep panel indicated minimal binding affinity for other targets with the exception of compounds **10b** and **11a**. Both of these analogs showed significant binding affinity for H_2 receptor with little selectivity for H_1 [**10b**: H_2 K_i = 4.5 nM; **11a**: H_2 94% inhibition at 1 μM]. Typically, known H_1-antihistamines, including dimethindene, display poor binding to the H_2 receptor (selectivity for H_1 versus H_2 is well over 100-fold).[21] The 3-methoxy substituent of the pyrazine was implicated in the increased binding towards the H_2 receptor since this activity was not observed in the unsubstituted analogs. The impact of functional antagonism of H_2 receptors in the central compartment is unclear although safety concerns with certain patient populations have been raised for known H_2 antagonists.[22]

The other factor expected to impact selection of candidate compounds was pharmacokinetic profile. Single dose studies of **1** have indicated sedative effects up to 5 h in humans,[23] the reported half-life of racemic dimethindene being approximately 5 h.[24] More recently doxepin, a potent non-selective H_1-antihistamine with reported elimination half-life of 18 h,[25] has shown sleep maintenance effects up to 8 h at low doses with no next day residual effects.[9] From this data it was hypothesized that analogs of **1** with similar or incrementally longer half-life than **1** would represent suitable candidates for further evaluation of dose, exposure and sedative action.[12] In this study, candidate selection was based on projections of in vitro clearance that was subsequently confirmed through in vivo studies. Comparison of the compounds in Table 2 indicated that analogs **3b** and **10a** were likely to represent incrementally longer exposure compounds, while compounds **10b** and **11a** appeared more similar to the benzothiophene lead (**2**) previously identified.[12]

Overall from this study, **3b** and **10a** retained desirable selectivity profiles and sufficient stability based on clearance measurements to represent potential alternatives to the lead benzothiophene. In vivo properties of **10a** were subsequently assessed and compared to the other leads **2** and **3b** (Table 3). EEG studies for **10a** exhibited a similar and significant increase in NREM sleep in rats without effects on REM sleep (not shown) in accord with reported effects of H_1-antihistamines on sleep-wake parameters under these conditions.[26,12] None of the compounds tested showed appreciable effects on latency to any sleep stage. Plasma concentrations from surrogate animals for **2** and **10a** at 1 h were similarly low despite the 10-fold variance in the minimally effective doses employed. This was likely due to lower clearance and enhanced stability of **10a** in rats,[27] in contrast to that previously observed for **2** and the other analogs examined.[12] Surrogate PK studies of **10a** showed maximum brain levels of 11 ng/g at 1 h following a dose of 3 mg/kg, indicating that this analog was also brain penetrating. Allometric assessment of **10a** projected a human clearance of 5.7 mL/min/kg[28] that was lower than previously estimated for compounds **2** and **3b** or reported for racemic dimethindene.[24] Human microdose data indicated a measured half-life for **10a** of 9.5 h with median clearance of 2.3 mL/min/kg.[28]

Evaluation of the human pharmacokinetic data indicated suitable half-lives for all 3 compounds within the desirable range of 5–18 h.[12] However, for the small sample size assessed, **2** and **3b** showed significant variability as reflected in the ranges of mean half-life and AUC (Table 3) following oral dosing. Variability of this magnitude was also reported for racemic dimethindene[24] and originally attributed to variations in either absorption as a function of pK_a or first pass metabolism. In this study the less basic benzothiophene **2** (measured pK_a 8.5[29]) showed similar variability to the more basic indene **3b** (measured pK_a 9.1). In contrast, variability in all corresponding parameters for **10a** was significantly less despite similar basic character to **3b** (measured pK_a 9.1) suggesting that variation in absorption on the basis of pK_a is unlikely. Interestingly, compounds **2** and **3b** trended to reduced variability in clearance upon IV dosing. Whether these data support a specific effect of first pass metabolism on PK variability or some other factor is unclear. Compound **10a** was confirmed as a highly stable compound which, combined with high measured bioavailability (62%), would be expected to provide a candidate with reduced variation in PK properties. This observed trend was consistent with the high variability in the human PK data previously described for a high clearance benzothiophene analog that was also considered as a backup.[30] Overall, the reduced variability observed in **10a** was more desirable from a pharmacokinetic perspective in order to generate a more reproducible sedation profile devoid of adverse events associated with next day sedation and warrants further evaluation.

In summary, metabolic profiles of a series of 6-substituted indenes were assessed in order to identify a number of novel, selective H_1-antihistamines lacking a CYP2D6 substrate liability previously noted. Substituents, including the fluoro, methyl, and methoxy group, were incorporated into the 6-position of the indene core with varying results on biotransformation through the CYP2D6 pathway. The 6-fluoro or 6-methyl modifications afforded the highest probability of identifying compounds with diversified metabolism. Due to formation of stable *N*-glucuronides, some compounds passed our criteria for diversified biotransformation. An additional key driver in compound selection was focused on pharmacokinetic properties. 6-Fluoro analog **10a** was efficacious in a rat EEG/EMG model and exhibited suitable pharmacokinetics with a trend to reduced variability. This compound was adjudged to have a profile superior to two previously identified lead com-

Table 2
Profile of lead indene analogs with diversified metabolism in comparison to clinical compound (**2**)

Compd	H_1 K_i [a] (nM)	Monoamine receptor selectivity [H_3;5-HT_{2A}; M_1; M_3]	CYP2D6[b] IC_{50} (μM)	Selectivity hERG IC_{50}/H_1 K_i	Pred Sys. Cl. (mL/min/kg)
2	4.0 ± 0.5	>1000	28	336	9.7
3b	1.5 ± 0.2	>1000	50	933	4.7
10a	1.7 ± 0.2	>400	22	529	2.8
10b	0.7 ± 0.1	>1000	6.4	2571	9.6
11a	2.7 ± 0.3	>1000	11	1407	10.8

[a] SEM for K_i values derived from dose–response curves generated from triplicate or more data points.
[b] CYP3A4 IC_{50} >10 μM for all compounds.

Table 3
In vivo profile of *R*-dimethindene analogs in comparison to clinical compound (**2**)

Compd	Dose (mg/kg)	% NREM in 4 h[a,b]	Latency to 1 min NREM[a,b]	Plasma concentration (1 h) (ng/mL)	Pred. human Sys. Cl.[c] (mL/min/kg)	Obs. human Sys. Cl.[d,e] (mL/min/kg)	Human[d] $t_{1/2}$ (h)	Human[d] AUC_{0-t} (h ng/mL)
2	30	142 ± 6	124 ± 12	4.0 ± 0.1	9.0	5.2 (3.8–6.4)	6.8 (3.6–10)	2.8 (1.9–4.2)
3b	60	145 ± 5	109 ± 7	86 ± 45	9.7	3.3 (2.5–4.0)	12 (7.4–17)	3.1 (2.3–5.2)
10a	3	136 ± 10	106 ± 12	5.2 ± 1.4	5.7	2.3 (2.0–3.3)	9.5 (5.7–9.8)	6.5 (5.1–7.5)

[a] Calculated as a percentage of vehicle.
[b] Average data for zolpidem control (30 mg/kg) calculated as a percentage of vehicle: % NREM in 4 h: 205 ± 7; latency to 1 min NREM: 17 ± 2.
[c] Estimated from allometry.
[d] Human microdose of 0.1 mg/kg.
[e] Median values of clearance quoted. Calculated on an average human weight of 70 kg.

pounds and as such may be a suitable candidate for further assessment as a sleep agent.

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References and notes

1. Ohayon, M. M.; Lemoine, P. *L'Encephale* **2004**, *30*, 135.
2. Fullerton, D. S. P. *Am. J. Manag. Care* **2006**, *12*, S246.
3. Zammit, G. K.; Weiner, J.; Damato, N.; Sillup, G. P.; McMillan, C. A. *Sleep* **1999**, *22*, S379.
4. Glass, J.; Lanctot, K. L.; Herrmann, N.; Sproule, B. A.; Busto, U. E. *Br. Med. J.* **2005**, *331*, 1169.
5. Vermeeren, A. *CNS Drugs* **2004**, *18*, 297.
6. Tsai, M.-J.; Tsai, Y.-H.; Huang, Y.-B. *Clin. Toxicol.* **2007**, *45*, 179.
7. Navab, P.; Guilleminault, C. *Expert Opin. Pharmacother.* **2006**, *7*, 1731.
8. Roecker, A. J.; Coleman, P. J. *Curr. Top. Med. Chem.* **2008**, *8*, 977.
9. Roth, T.; Rogowski, R.; Hull, S.; Schwartz, H.; Koshorek, G. *Sleep* **2007**, *30*, 1555.
10. Hajak, G.; Muller, W. E.; Wittchen, H. U.; Pittrow, D.; Kirch, W. *Addiction* **2003**, *98*, 1371.
11. Nicholson, A. N.; Pascoe, P. A.; Turner, C.; Ganellin, C. R.; Greengrass, P. M.; Casy, A. F.; Mercer, A. D. *Br. J. Pharmacol.* **1991**, *104*, 270.
12. Moree, W. J.; Li, B.-F.; Jovic, F.; Coon, T.; Yu, J.; Gross, R. S.; Tucci, F. C.; Marinkovic, D.; Malany, S.; Bradbury, M. J.; Hernandez, L. M.; O'Brien, L.; Wen, J.; Wang, H.; Hoare, S. R. J.; Petroski, R. E.; Sacaan, A.; Madan, A.; Crowe, P. D.; Beaton, G. *J. Med. Chem.* **2009**, *52*, 5307.
13. Prein, D.; Blaschke, G. *J. Chromatogr., B: Biomed. Sci. Appl.* **1997**, *688*, 309.
14. Li, B.-F.; Moree, W. J.; Yu, J.; Coon, T.; Zamani-Kord, S.; Malany, S.; Jalali, K.; Wen, J.; Wang, H.; Yang, Y.; Hoare, S. R. J.; Petroski, R. E.; Madan, A.; Crowe, P. D.; Beaton, G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2629.
15. (a) Zineh, I.; Beitelshees, A. L.; Gaedigk, A.; Walker, J. R.; Pauly, D. F.; Eberst, K.; Leeder, J. S.; Phillips, M. S.; Gelfand, C. A.; Johnson, J. A. *Clin. Pharm. Ther.* **2004**, *76*, 536; (b) Hamelin, B. A.; Bouayard, A.; Méthot, J.; Jobin, J.; Desgagnés, P.; Poirier, P.; Allaire, J.; Dumesnil, J.; Turgeon, J. *Clin. Pharmacol. Ther.* **2000**, *67*, 466.
16. Böhme, T. M.; Keim, C.; Kreutzmann, K.; Linder, M.; Dingermann, T.; Dannhardt, G.; Mutschler, E.; Lambrecht, G. *J. Med. Chem.* **2003**, *46*, 856.
17. A detailed description of the methods and calculations to estimate CYP2D6 contributions to metabolism is provided in the supplementary data of Ref. 14.
18. Test compound (20–50 μM) was incubated with pooled hepatocytes (~1×10^6 cells/mL) in Waymouth's medium at 37 °C in a shaking water bath for 0, 1 and 2 h. All concentrations and cell contents were relative to the final incubation volume of 500 μL. The incubation was terminated by mixing 500 μL of ice-cold acetonitrile for LC/MS analysis using methods described in supplementary data of Ref. 14.
19. Luo, H.; Hawes, E. M.; McKay, G.; Korchinski, E. D.; Midha, K. K. *Xenobiotica* **1991**, *21*, 1281.
20. In the case of the thiazole analog, **3d**, a second oxidation product was noted on the 4-methylthiazole moiety. Antibody inhibition studies indicated this transformation occurred predominantly through the CYP2D6 pathway.
21. Sharif, N. A.; Xu, S. X.; Miller, S. T.; Gamache, D. A.; Yanni, J. M. *J. Pharmacol. Exp. Ther.* **1996**, *278*, 1252.
22. Berlin, R. G. *Drug Dev. Res.* **1989**, *17*, 97.
23. Cicurel, L.; Péchadre, J. C.; Grandjean, E.; Duchêne-Marullaz, P. *Agents Actions* **1992**, C440.
24. Arnera, V.; Wermeille, M.; Wellman, M.; Llull, M.; Althaus, M. A.; Balant, L. P. *Arzneim.-Forsch./Drug Res.* **1990**, *40*, 1346. Clearance calculations estimated as 6.9 mL/min/kg based on an average human weight of 70 kg.
25. Virtanen, R.; Scheinin, M.; Iisalo, E. *Acta Pharmacol. Toxicol.* **1980**, *47*, 371.
26. Saitou, K.; Kaneko, Y.; Suginoto, Y.; Chen, Z.; Kamei, C. *Biol. Pharm. Bull.* **1999**, *22*, 1079.
27. Clearance for **10a** was 58 mL/min/kg while the clearance for all other compounds studied exceeded blood flow (>68 mL/min/kg). Rat bioavailability was estimated as 25% in rat PK studies compared to <4% for all other compounds examined.
28. Madan, A.; O'Brien, Z.; Wen, J.; O'Brien, C.; Farber, R. H.; Beaton, G.; Crowe, P. D.; Oosterhuis, B.; Garner, R. C.; Lappin, G.; Bozigian, H. P. *Br. J. Clin. Pharmacol.* **2009**, *67*, 288.
29. Standard methods were used to measure pK_a values with Sirius GLPKa instrumentation from pION Inc.
30. Moree, W. J.; Jovic, F.; Coon, T.; Yu, J.; Li, B.-F.; Tucci, F. C.; Marinkovic, D.; Gross, R. S.; Malany, S.; Bradbury, M. J.; Hernandez, L. M.; O'Brien, Z.; Wen, J.; Wang, H.; Hoare, S. R. J.; Petroski, R. E.; Sacaan, A.; Madan, A.; Crowe, P. D.; Beaton, G. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 2316.