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Metabolically Stable *tert*-Butyl Replacement

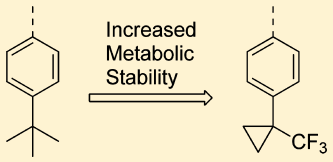
David Barnes-Seeman,*[,†] Monish Jain,[‡] Leslie Bell,[‡] Suzie Ferreira,[‡] Scott Cohen,[†] Xiao-Hui Chen,[‡] Jakal Amin,[‡] Brad Snodgrass,[‡] and Panos Hatsis[‡]

[†]Department of Global Discovery Chemistry, Novartis Institutes for Biomedical Research, 100 Technology Square, Cambridge, Massachusetts 02139, United States

[‡]Department of Metabolism and Pharmacokinetics, Novartis Institutes for Biomedical Research, 250 Massachusetts Avenue, Cambridge, Massachusetts 02139, United States

Supporting Information

ABSTRACT: Susceptibility to metabolism is a common issue with the *tert*-butyl group on compounds of medicinal interest. We demonstrate an approach of removing all the fully sp^3 C−Hs from a *tert*-butyl group: replacing some C−Hs with C−Fs and increasing the s-character of the remaining C−Hs. This approach gave a trifluoromethylcyclopropyl group, which increased metabolic stability. Trifluoromethylcyclopropyl-containing analogues had consistently higher metabolic stability in vitro and in vivo compared to their *tert*-butyl-containing counterparts.



KEYWORDS: *Metabolic stability, metabolism, clearance, tertiary butyl, tert-butyl, t-butyl*

Compounds with high metabolic lability or hepatic clearance often have low oral bioavailability and short half-lives. Consequently, optimization of the metabolic stability of lead molecules is of critical importance in the drug discovery process.[1] In vitro studies such as metabolic stability assays in liver microsomes,[2] biotransformation studies to pinpoint vulnerable spots in the molecule, and in vivo pharmacokinetic studies in preclinical species are some of the tools employed by project teams to arrive at potent compounds with favorable clearance values. Cytochrome P450s (CYPs) are the principal enzymes considered responsible for hepatic metabolism.[3,4]

Susceptibility to metabolism is a common issue with the *tert*-butyl group on compounds of medicinal interest. During the course of a lead optimization effort, we encountered a pocket in a receptor with a strong preference for a *tert*-butyl group. However, compounds bearing this *tert*-butyl group had high clearance in rats and microsomal incubations. The general principals and specific functional group used during the course of overcoming this challenge may be employed in other chemical series and are detailed herein.

We hypothesized that the rapid abstraction of H from the sp^3 hybridized methyl carbons of the *tert*-butyl resulted in rapid oxidative metabolism. Compound **1** served as the prototype *tert*-butyl-containing compound. An in vitro biotransformation study in rat and human liver microsomes (RLM, HLM) showed that the major route of metabolism of **1** is oxidation of the *tert*-butyl to the corresponding alcohol product **2**, confirming the initial hypothesis. Further in vitro studies in human liver microsomes suggested that oxidative metabolism could be attributed to multiple Cytochrome P450 isoform involvement, including CYPs 3A4/5, 2D6, 2C9, and 1A2 (data not shown).

Polar substitution has often been used to increase metabolic stability. As expected, analogues bearing a hydroxyl, **2**, cyano, **3**, or acid, **4**, had increased microsomal stability. However, an oxetane, another group that has recently been reported to improve metabolic stability,[5−8] offered no improvement (**5** vs **1**).

Clearly, in some instances, the introduction of a polar group is not tolerated by the receptor. For such a case, another solution was needed. We hypothesized that removing all fully sp^3 C−Hs from the *tert*-butyl would lower the rate of H-abstraction by metabolizing enzymes. On the basis of this principle, we designed a replacement for *tert*-butyl that has a similar shape and size and retains hydrophobicity but lacks fully sp^3 C−Hs. One of the methyl groups was replaced by CF_3. The other two methyl groups were joined to form a cyclopropyl ring, whose C−Hs have a higher homolytic bond dissociation energy owing to the higher s-character of the C−H bonding orbitals than a typical aliphatic C−H. As shown by comparing compounds **1**, **6**, and **7**, the CF_3 group alone was not adequate nor was just installing the cyclopropyl group (**1** vs **8**). However, when both features were combined in the trifluoromethyl-cyclopropyl[9−11] group or Cp-CF_3, the resulting compound **9** had high metabolic stability. The LogP of compounds **1** and **9** are 4.2 and 4, respectively. Compound **10** bears the Cp-CF_3 and also incorporates a pyridyl-N, which can be useful as a solubilizing functionality.

We sought to probe the consistency of increase in metabolic stability of the Cp-CF_3 group compared to *tert*-butyl. To do so, we tested the metabolic stability of six additional matched pairs of biaryls and amides, where the portion of the compound distal to the *tert*-butyl or Cp-CF_3 contained functionality that was generally relatively metabolically stable. The results are summarized in Table 2 and demonstrate consistent increases in metabolic stability by replacing *tert*-butyl with Cp-CF_3.

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Table 1. In Vitro Clearance of a Series of *tert*-Butyl Replacements

1-5 Ar =	RLM t½ min	HLM t½ min	6-10 Ar =	RLM t½ min	HLM t½ min
1	30	51	**6**	7	9
2	125	202	**7**	25	66
3	70	122	**8**	11	35
4	135	274	**9**	> 400	150
5	37	38	**10**	129	110

Table 2. In Vitro Clearance of Matched Pairs of Compounds Containing Either *tert*-Butyl or Trifluoromethylcyclopropyl (Cp-CF_3)

11-18 / 19-22 Ar =	R *t*-Bu	RLM t½ min	HLM t½ min	R Cp-CF_3	RLM t½ min	HLM t½ min
	11	25	41	**12**	197	>405
	13	24	51	**14**	98	88
	15	12	85	**16**	47	>405
	17	12	29	**18**	40	>405
	19	24	27	**20**	>405	64
	21	8	25	**22**	75	69

Compounds **1**, **5**, **6**, **9**, **11**, **12**, **19**, and **20** were checked for CYP 3A4/5 inhibition with and without a 30 min incubation with NADPH prior to substrate addition as a screen for potential time-dependent inhibition, in order to mitigate against a chance for artifacts in metabolic stability differences due to CYP inhibition. All compounds tested had negligible reversible CYP inhibition (IC_{50} >50 μM) with no evidence of time-dependent inhibition.

We measured clearance in rats for representative compounds in order to determine if the in vitro clearance comparisons were consistent with in vivo results. The results are summarized in Table 3. Despite the addition of a polar atom, oxetane-containing **5** had similar in vivo clearance to *tert*-butyl-containing **1**, consistent with the lack of in vitro improvement. Cp-CF_3-containing **9** and **12** had substantially lower in vivo clearance than their *tert*-butyl-containing counterparts **1** and **11**, respectively.

Table 3. In Vitro and in Vivo Clearance Comparisons

compd	RLM Cl (h) (mL/min/kg)	rat Cl (mL/min/kg)	rat $t_{1/2}$ (h)	rat Vss (L/kg)
5	30	28	0.4	1.3
1 (*t*-Bu)	33	20	2.3	2.5
9 (Cp-CF_3)	<5	4.6	9.1	3.4
11 (*t*-Bu)	36	352	0.2	5.4
12 (Cp-CF_3)	10	25	3.4	6.2

Finasteride (**23**) is known to be metabolized in humans predominantly on the *tert*-butyl (ω) but also at the 6-position (Figure 1).[12,13] Replacing the *tert*-butyl with Cp-CF_3 increased the $t_{1/2}$ in HLM from 63 min (**23**) to 114 min (**24**). This moderate increase is consistent with a caveat of the approach: replacing *tert*-butyl with Cp-CF_3 is not expected to substantially reduce metabolism at distal soft-spots.[14] In this case, at least the 6-position can still be metabolically labile.[12,13]

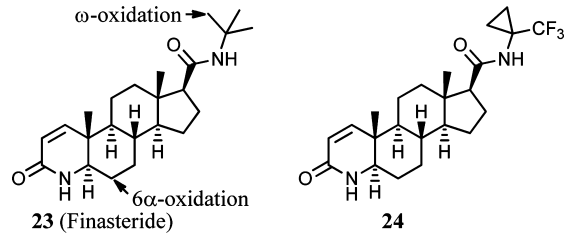


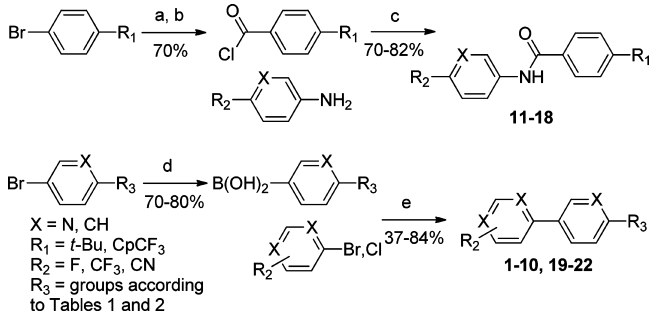
Figure 1. Finasteride and the Cp-CF_3 analogue thereof.

In conclusion, we have demonstrated an approach of removing all the fully sp^3 C–Hs from a *tert*-butyl group: replacing some H with F and increasing the s-character of the remaining C–Hs. This approach gave a trifluoromethylcyclopropyl group that consistently increased metabolic stability in vitro and in vivo compared to *tert*-butyl.

EXPERIMENTAL PROCEDURES

The compounds **1**–**22** were prepared according to Scheme 1.

Scheme 1. General Synthesis of Compounds in Table 1 and 2[a]



[a]Reagents and conditions: (a) *n*-BuLi, THF, −78 to 0 °C, CO_2; (b) $(COCl)_2$, CH_2Cl_2; (c) iPr_2NEt, CH_3CN; (d) *n*-BuLi, THF, −78 to 0 °C, $B(OMe)_3$; (e) SPhos, $Pd(OAc)_2$, K_3PO_4, THF.

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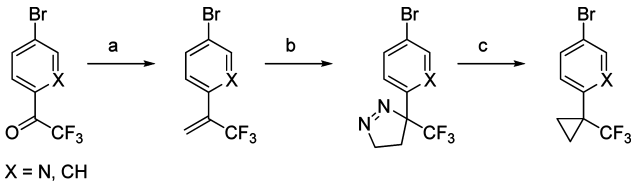
Arylboronic acids and pinacol esters used to make **1**, **3**, **4**, **6**, **19**, and **21** are commercially available. Arylboronic acids to make **5**[15] and **8**[16] are derived from the corresponding arylbromides, which are prepared according to the corresponding references. Compound **4** was converted to the acid chloride with $(COCl)_2$, then reduced to **2** with $NaBH_4$.

Intermediates to make compounds containing Cp-CF_3 groups were prepared according to Scheme 2.

Scheme 2. Synthesis of Cp-CF_3-Containing Aryl Bromides[a]



[a]Reagents and conditions: (a) X = CH, MsCl, 18-crown-6, KF, DMF, 100 °C;[17] X = N: Tebbe reagent;[18] (b) CH_2N_2;[19] (c) Xylenes reflux[19].

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and spectral characterization data. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*(D.B.-S.) Phone: 617-871-7166. E-mail: david.barnes-seeman@novartis.com.

Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

$CpCF_3$, trifluoromethylcyclopropyl; Cl, clearance; Cyps, cytochrome P450s; HLM, human liver microsomes; RLM, rat liver microsomes; SPhos, dicyclohexyl(2′,6′-dimethoxy-[1,1′-biphenyl]-2-yl)phosphine; $t_{1/2}$, half-life; Vss, volume of distribution

■ REFERENCES

(1) Lau, Y. Y.; Krishna, G.; Yumibe, N. P.; Grotz, D. E.; Sapidou, E.; Norton, L.; Chu, I.; Chen, C.; Soares, A. D.; Lin, C. C. The use of in vitro metabolic stability for rapid selection of compounds in early discovery based on their expected hepatic extraction ratios. *Pharm. Res.* **2002**, *19* (11), 1606−1610.

(2) Obach, R. S. Prediction of human clearance of twenty-nine drugs from hepatic microsomal intrinsic clearance data: an examination of in vitro half-life approach and nonspecific binding to microsomes. *Drug Metab. Dispos.* **1999**, *27* (11), 1350−1359.

(3) Guengerich, F. P. Cytochrome P450 and Chemical Toxicology. *Chem. Res. Toxicol.* **2008**, *21* (1), 70−83.

(4) Lu, A. Y.; Wang, R. W.; Lin, J. H. Cytochrome P450 in vitro reaction phenotyping: A re-evaluation of approaches used for P450 isoform identification. *Drug Metab. Dispos.* **2003**, *31* (4), 345−350.

(5) Wuitschik, G.; Carreira, E. M.; Wagner, B.; Fischer, H.; Parrilla, I.; Schuler, F.; Rogers-Evans, M.; Muller, K. Oxetanes in drug discovery: structural and synthetic insights. *J. Med. Chem.* **2010**, *53* (8), 3227−3246.

(6) Wuitschik, G.; Rogers-Evans, M.; Buckl, A.; Bernasconi, M.; Marki, M.; Godel, T.; Fischer, H.; Wagner, G.; Parrilla, I.; Schuler, F.; Schneider, J.; Alker, A.; Schweizer, W. B.; Muller, K.; Carreira, E. M. Spirocyclic oxetanes: synthesis and properties. *Angew. Chem., Int. Ed.* **2008**, *47* (24), 4512−4515.

(7) Wuitschik, G.; Carreira, E. M.; Rogers-Evans, M.; Muller, K. Oxetan-3-one: chemistry and synthesis. *Process Chem. Pharm. Ind.* **2008**, 217−229.

(8) Wuitschik, G.; Rogers-Evans, M.; Muller, K.; Fischer, H.; Wagner, B.; Schuler, F.; Polonnchuk, L.; Carreira, E. M. Oxetanes as promising modules in drug discovery. *Angew. Chem., Int. Ed.* **2006**, *45* (46), 7736−7739.

(9) In vitro potency comparisons have been published for compounds containing either *tert*-butyl or Cp-CF_3 (refs 9−11). To the best of our knowledge, there are no reports of using the Cp-CF_3 group to increase metabolic stability compared to *tert*-butyl: Paone, D. V.; Nguyen, D. N.; Shaw, A. W.; Burgey, C. S.; Potteiger, C. M.; Deng, J. Z.; Mosser, S. D.; Salvatore, C. A.; Yu, S.; Roller, S.; Kane, S. A.; Selnick, H. G.; Vacca, J. P.; Williams, T. M. Orally bioavailable imidazoazepanes as calcitonin gene-related peptide (CGRP) receptor antagonists: Discovery of MK-2918. *Bioorg. Med. Chem. Lett.* **2011**, *21* (9), 2683−2686.

(10) Lee, S. H.; Seo, H. J.; Lee, S.-H.; Jung, M. E.; Park, J.-H.; Park, H.-J.; Yoo, J.; Yun, H.; Na, J.; Kang, S. Y.; Song, K.-S.; Kim, M.-a.; Chang, C.-H.; Kim, J.; Lee, J. Biarylpyrazolyl oxadiazole as potent, selective, orally bioavailable cannabinoid-1 receptor antagonists for the treatment of obesity. *J. Med. Chem.* **2008**, *51* (22), 7216−7233.

(11) Phillips, D. J.; Davenport, R. J.; Demaude, T. A.; Galleway, F. P.; Jones, M. W.; Knerr, L.; Perry, B. G.; Ratcliffe, A. J. Imidazopyridines as VLA-4 integrin antagonists . *Bioorg. Med. Chem. Lett.* **2008**, *18* (14), 4146−4149.

(12) Lundahl, A.; Lennernäs, H.; Knutson, L.; Bondesson, U.; Hedeland, M. Identification of finasteride metabolites in human bile and urine by high-performance liquid chromatography/tandem mass spectrometry. *Drug Metab. Dispos.* **2009**, *37* (10), 2008.

(13) Hulin-Curtis, S. L.; Petit, D.; Figg, W. D.; Hsing, A. W.; Reichardt, J. K. V. Finasteride metabolism and pharmacogenetics: new approaches to personalized prevention of prostate cancer. *Future Oncol.* **2010**, *6* (12), 1897.

(14) For an example, where a site other than *tert*-butyl was the primary site of metabolism, replacing the *tert*-butyl with Cp-CF_3 did not improve clearance: Guo, C.; McAlpine, I.; Zhang, J.; Knighton, D. D.; Kephart, S.; Johnson, M. C.; Li, H.; Bouzida, D.; Yang, A.; Dong, L.; Marakovits, J.; Tikhe, J.; Richardson, P.; Guo, L. C.; Kania, R.; Edwards, M. P.; Kraynov, E.; Christensen, J.; Piraino, J.; Lee, J.; Dagostino, E.; Del-Carmen, C.; Deng, Y.-L.; Smeal, T.; Murray, B. W. Discovery of pyrroloaminopyrazoles as novel PAK inhibitors. *J. Med. Chem.* **2012**, *55* (10), 4728−4739.

(15) Brodney, M. A. Pyridyl piperazines and Their Preparation and Pharmaceutical Compositions, for the Treatment of CNS Disorders or Conditions That Can Be Treated by Serotonin-Mediated Neurotransmission. PCT Int. Appl., 2006000912, 2006.

(16) Gandon, V.; Bertus, P.; Szymoniak, J. A straightforward synthesis of cyclopropanes from aldehydes and ketones. *Eur. J. Org. Chem.* **2000**, *22*, 3713−3719.

(17) Nader, B. S.; Cordova, J. A.; Reese, K. E.; Powell, C. L. A Novel Fluoride Ion Mediated Olefination of Electron-Deficient Aryl Ketones by Alkanesulfonyl Halides. *J. Org. Chem.* **1994**, *59* (10), 2898−2901.

(18) Phillion, D. P.; Cleary, D. G. Disodium salt of 2-[(dihydroxyphosphinyl)difluoromethyl]propenoic acid: an isopolar and isosteric analog of phosphoenolpyruvate. *J. Org. Chem.* **1992**, *57* (9), 2763−2764.

(19) Banks, B. J. Preparation of 1-aryl-4-cyclopropylpyrazoles as medical and agrochemical parasiticides and pesticides. EP 0933363, 1999. Safety note: diazomethane is an explosion hazard.