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FLUOROBENZAMIDRAZONE THROMBIN INHIBITORS: INFLUENCE OF FLUORINE ON ENHANCING ORAL ABSORPTION

Koo Lee,* Won-Hyuk Jung, Sang Yeul Hwang, and Sung-Hack Lee

Biotech Research Institute, LG Chemical Ltd/Research Park, P.O. Box 61 Yu Sung Science Town, Taejon 305-380, Korea

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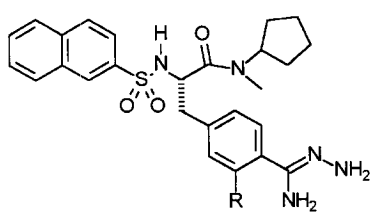
Abstract: LB30057 (**1**) is a selective and efficacious oral thrombin inhibitor. Fluorine-substitution on the phenylene ring of the benzamidrazone portion in both compound **1** and its derivatives gave, in many cases, enhanced oral absorption in rats while maintaining the intrinsic potency and selectivity. Compound **2** demonstrated a 3-fold increase in absorption.

Of the numerous thrombin inhibitors developed,[1] orally bioavailable drugs are most desirable for the treatment of chronic thrombotic disorders such as deep vein thrombosis.[2] Only a few of small molecules in this area have been reported to have promising oral efficacy in animal models of thrombosis.[3] One such compound is LB30057 (**1**) a sulfonamide that was recently identified in our laboratories as an efficacious oral thrombin inhibitor and is now in Phase I clinical trials.[4] While compound **1** demonstrated excellent oral bioavailability in dogs, this agent suffered insufficient oral absorption in rats. Subsequent SAR studies focused on optimizing the naphthyl portion of compound **1** led to a number of compounds (e.g. **7**, **10**) with superior potency but with still limited absorption in rats.[5] Another class of analogs bearing benzylamine P1 in place of the benzamidrazone moiety was also limited by poor absorption behavior in dogs despite excellent bioavailability and long duration of action in rats.[6]

The attractiveness and utility of fluorine in biologically active molecules are well understood: its strong hydrophobic and electronic effect often provided drug candidates with improved pharmacokinetic properties without affecting the intrinsic activity due to the similar steric demands of fluorine and hydrogen.[7] We have thus chosen to adopt this fluorine strategy as an approach to enhance the oral bioavailability of **1**. Given several reports that oral absorption can be enhanced by reducing the basicity of P1 functionality in thrombin inhibitors,[8] we decided to incorporate the electron-withdrawing fluorine substituent onto the phenylene ring of the benzamidrazone P1 as in compound **2**. In this report, we describe the synthesis, SAR, and absorption behavior of some fluorine analogs of the benzamidrazone thrombin inhibitors.



1: R = H ($C_{max,\ rat}$ = 1.6 μM at 30 mpk)
2: R = F ($C_{max,\ rat}$ = 4.1 μM at 30 mpk)

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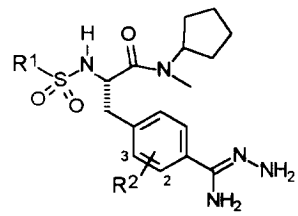
Table 1. Enzyme inhibition constants and pharmacokinetic parameters for compounds **1-11** in rats (n = 3 - 4).



Compd	R^1	R^2	Thrombin[a] Ki (nM)	Trypsin[b] Ki (μM)	Blood concentration[c]	
					C_{max} (μM)	AUC (μM·min)
1	2-naphthyl	H	0.4	33	1.61	140
2	2-naphthyl	2-F	5.5	25	4.13	400
3	2-naphthyl	3-F	2.0	11	1.63	110
4	4-propylphenyl	H	0.2	3.5	1.17	134
5	4-propylphenyl	2-F	1.8	22	2.78	250
6	4-propylphenyl	3-F	0.7	4.0	1.35	161
7	5,6,7,8-tetrahydro -2-naphthyl	H	0.1	4.6	0.36	49
8	5,6,7,8-tetrahydro -2-naphthyl	2-F	0.9	25	1.34	98
9	5,6,7,8-tetrahydro -2-naphthyl	3-F	0.5	3.0	1.03	82
10	6-OMe-2-naphthyl	H	0.06	9.2	1.13	157
11	6-OMe-2-naphthyl	3-F	0.2	2.0	2.41	197

[a] human thrombin. [b] bovine trypsin. [c] after oral dosing at 30 mg/kg as a 20% HPCD solution in water.

As shown in Table 1, the *in vitro* activity of these compounds was expressed as Ki and selectivity for thrombin was evaluated against the prototype serine protease trypsin. The oral absorption behavior of compounds in this study was judged by the peak blood concentration (C_{max}) and area under the curve (AUC) obtained after oral administration in rats. The initially explored fluorine compound **2** gave highly increased absorption but suffered significant loss in potency as compared to the parent compound **1**. The 3-fluoro isomer **3** also was slightly less potent, which indicates that di or multifluoro-substitution on the phenylene ring would have a detrimental effect on potency. However, the lost potency in compounds **2** and **3** could be restored by employing some readily available naphthyl replacements that showed superior thrombin affinity in the previous SAR.[5,6,9,10] As anticipated, most of the derivatives displayed acceptable range of Ki values for thrombin inhibition, with the 6-methoxy-2-naphthyl derivative **11** being the most inhibitory.

Unlike compound **2**, its regioisomer **3** exhibited only comparable absorption to that of compound **1**. This trend also was observed with the 4-propylphenyl derivatives **4-6**. Fortunately, this trend was not seen with the other 3-fluoro isomers **9** and **11** which demonstrated improved absorption in terms of C_{max}. Encouraged by these results in rats, we promptly took these fluorine compounds to dogs. It was however unfortunate that the

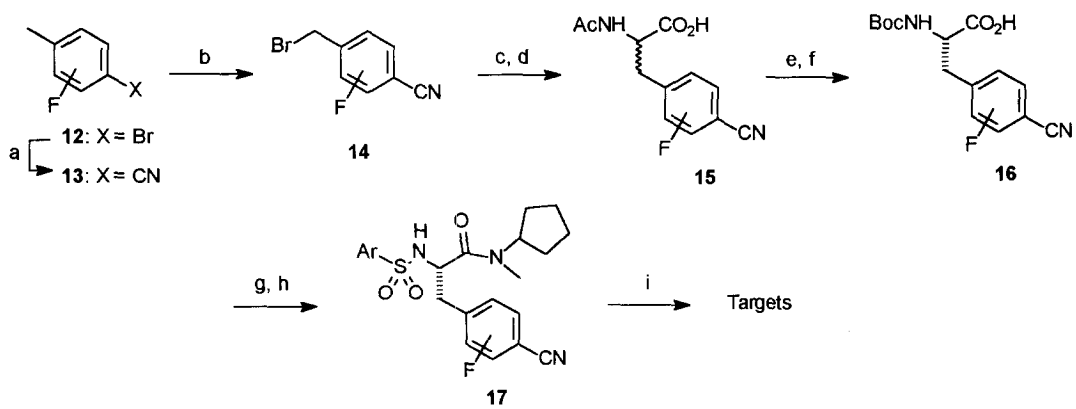
fluorine effect was found to be negligible in the dog pharmacokinetic evaluation: none of the targets showed a better performance of C_{max}/AUC values than the unsubstituted parent.

The *in vivo* antithrombotic activity of compounds was determined by the inhibition extent of the thrombus formation in the rat thromboplastin-induced model of venous thrombosis.[11] At bolus iv doses of both 1 and 5 mg/kg, compound **8** was essentially as effective as compound **1**, whereas other sub-nanomolar analogs were somewhat inferior despite even higher *in vitro* potency.

In summary, we have investigated a series of fluorine analogs of the LB30057 class as a means of enhancing oral absorption. With their intrinsic potency and selectivity retained, a number of compounds in this study demonstrated enhanced absorption in rats as evidenced by the increased blood concentrations upon oral dosing. Although none of these compounds has been chosen for further development due to insufficient overall profiles, the findings in this study have the potential for impact on designing other drug candidates with improved bioavailability. Further structure-activity/absorption relationship study of this class is also currently underway: the details will be reported in due course.[12]

Synthesis

The fluorine compounds in Table 1 were prepared employing essentially the same strategy as described in the synthesis of compound **1** (Scheme 1).[4b,9] The requisite cyanofluorobenzyl bromides **14** were prepared from the bromofluorotoluenes **12** in two steps involving CuCN replacement and subsequent NBS bromination of the methyl group. Condensation with diethyl acetamidomalonate, followed by in situ saponification and decarboxylation provided amino acids **15**. Each racemate was enzymatically resolved, and subsequent protection of the free amino group gave the Boc-protected acids **16**. After elaboration of the C- and N-terminals by successive coupling with *N,N*-cyclopentylmethylamine and arylsulfonyl chlorides, the intermediates **17** were subjected to a modified Pinner reaction to yield the desired amidrazones. Other derivatives listed in Table 1 were similarly prepared from the corresponding arylsulfonyl chlorides. All of the targets were obtained as pure HCl salts after preparative HPLC and ion-exchange chromatography.



Scheme 1

(a) CuCN, DMF, reflux, 76-80%; (b) NBS, benzoylperoxide, CCl_4, reflux, 57-78%; (c) $EtO_2CCH(NHAc)CO_2Et$, KI (cat.), NaOEt, dioxane, reflux; (d) NaOH, H_2O, reflux, 61-67% 2 steps; (e) acylase, H_2O, pH 6.5, 36 °C; (f) Boc_2O, NaOH, H_2O/dioxane; (g) i. *N,N*-cyclopentylmethylamine-HCl, *N*-methylmorpholine, EDC, HOBT, 39-42% from **15**; ii. AcCl, MeOH, 0°C (h) arylsulfonyl chlorides, *N*-methylmorpholine, DMF, 79-98%; (i) i. H_2S, py, Et_3N; ii. MeI, CH_3CN, reflux; iii. NH_2NH_2, MeOH, 63-83%.

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12. Some substituents investigated other than fluorine were disappointing, including chlorine, bromine, and amino groups. These substituents resulted in at least a 30-fold decrease in thrombin inhibitory potency as compared to the unsubstituted parent.