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Evaluation of *endo*- and *exo*-aryl-substitutions and central scaffold modifications on diphenyl substituted alkanes as 5-lipoxygenase activating protein inhibitors

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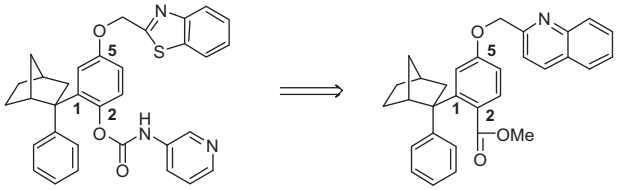
ABSTRACT

A search for a suitable replacement for the central norbornyl scaffold presented in the recently disclosed novel FLAP inhibitors is herein described, as well as the SAR study performed on the *endo* and *exo*-aryl groups.

Inhibition of leukotriene biosynthesis has been reported for the treatment of allergic rhinitis, asthma, and other inflammatory conditions.[1] Inhibitors of the membrane protein 5-Lipoxygenase Acting Protein (FLAP) have been described to block the formation of cellular leukotrienes.[2] Recently, Helgadottir et al. reported that FLAP was genetically linked to the risk of myocardial infarction and stroke in humans.[3] FLAP inhibition therefore may be of therapeutic potential as a novel treatment for atherosclerotic disease.

A previous communication from our colleagues described the identification of 5-benzothiazolyl-methoxy-2-pyridinyl carbamate derivative (**1**, FLAP binding IC_{50} = 2.7 nM, HWBA IC_{50} = 36 nM) and 5-quinolinemethoxy-2-methyl ester **2** as pharmacologically active (in vitro) FLAP inhibitors.[4] The ester functionality in **2** not only presented a potential synthetic handle to access other structure–activity relationships, but also served as an acceptable benchmarking substituent with which to explore SAR in other regions of the molecule. In this communication, we disclose the SARs in the methyl ester series (**2**) for: replacement of the central norbornyl scaffold; investigation of various amide and heterocyclic substituents at the 2-position of the *exo*-aryl ring, and substitutions on the *endo*-aryl ring. All compounds were prepared as racemic mixtures unless indicated otherwise.



1 **2**

We first explored substitution on the *endo*-aryl ring. The synthetic route to these analogues is outlined in Scheme 1 and is similar to that shown in a previous report.[4] The principal modifications to the historic route are incorporation of *tert*-butyl-diphenylsilyl and Boc protecting groups to avoid over-alkylation in step f. Table 1 summarizes our systematic survey of substituents on the *endo*-phenyl ring. In short, *meta*-substitution generally furnished compounds with improved potency in the human whole blood assay (HWBA) versus *ortho*- or *para*-substitution (not all data shown). Among all of the substituents surveyed, the 3-methoxy group afforded analogue (**13f**) with the best activity in human whole blood.

Concurrently, we investigated different central scaffolds. Liver microsome stability experiments with compound **2** and other related analogues revealed the sensitivity of the norbornyl group toward metabolic oxidation. We reasoned that this may be a contribution factor to the generally short half lives ($t_{1/2}$) observed

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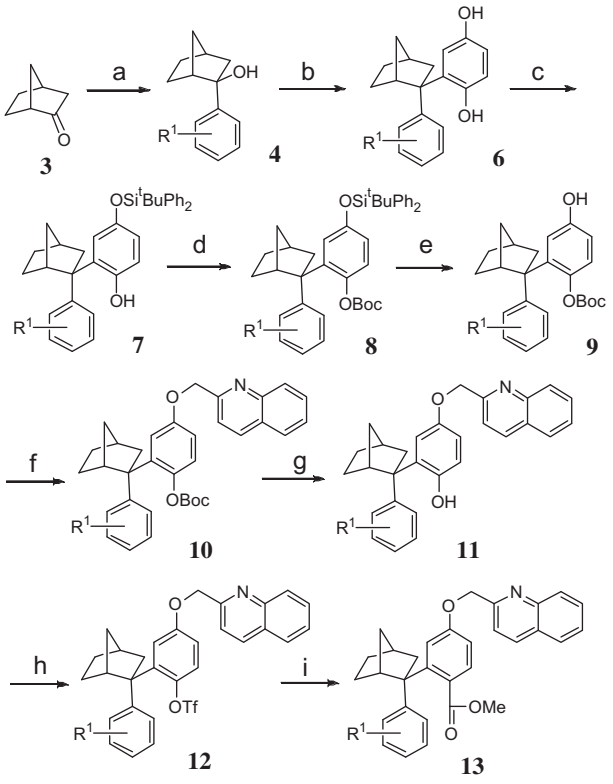
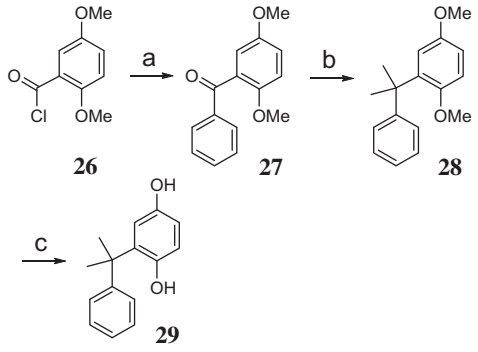


Scheme 1. Synthesis of compounds for investigation of *endo*-aryl SAR. Reagents and conditions: (a) R^1MgX, 0 °C in THF or ether; (b) Hydroquinone (**5**), $AlCl_3$, reflux in toluene; (c) *tert*-butyl-diphenylsilyl chloride, imidazole, DMF, rt; (d) Boc anhydride; (e) TBAF, THF, 0 °C; (f) 2-chloromethylquinoline, NaH, DMF; (g) KOH, MeOH/THF, reflux; (h) triflic anhydride, pyridine, toluene; (i) $Pd(OAc)_2$, dppf, triethylamine, CO, DMF/MeOH.

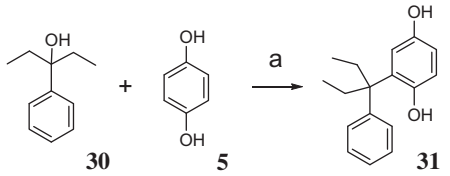
Table 1
Selected analogues from endo-aryl SAR study and their corresponding in vitro assay data

Compounds	R^1	FLAP binding[5] IC_{50} (nM)	HWBA[6] IC_{50} (nM)
2	H	2.1	460
13a	2-F	4.0	1600
13b	3-F	2.3	472
13c	4-F	9.5	1400
13d	3-Me	5.5	940
13e	3-CF_3	7.4	>8600
13f	3-OMe	2.5	322
13g	3,5-di-F	3.4	1315
13h	3,5-di-Me	3.5	2304

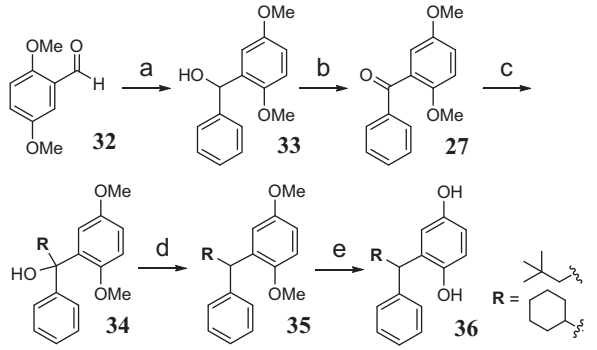
with these compounds when dosed in rats. Therefore, we focused our efforts on designing metabolically robust scaffolds, in the hope of improving $t_{1/2}$. Syntheses of these analogues are described in Schemes 2–8. Scheme 2 describes the synthesis of hydroquinone **29**. Interestingly, while treatment of ketone **27** with dimethyl zinc and titanium trichloride in DCM afforded the dimethyl analogue **28** in good yields, the analogous diethyl compound could not be obtained via this route. Instead, a Friedel–Crafts approach was utilized (Scheme 3). In addition, although the Grignard reactions of ketone **27** (Scheme 4) with both the neopentyl or cyclohexyl derived reagents went smoothly, no product was detected with *tert*-butyl Grignard. This is presumably due to steric congestion, which led to the development of an alternative in which the *tert*-butyl group is introduced at an earlier stage via a Pd-catalyzed transformation (Scheme 5).



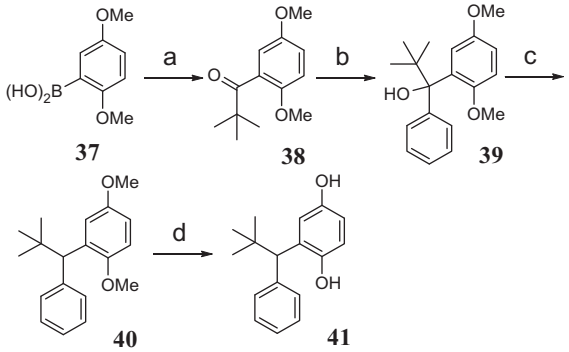
Scheme 2. Partial synthesis of compound **14**. Reagents and conditions: (a) $PhB(OH)_2$, $Pd(PPh_3)_4$, Cs_2CO_3, toluene, 100 °C; (b) dimethyl zinc, $TiCl_3$, DCM, −50 °C; (c) boron tribrimide, DCM, 0 °C to rt.



Scheme 3. Partial synthesis of compound **15**. Reagents and conditions: (a) pTsOH hydrate, toluene, reflux.

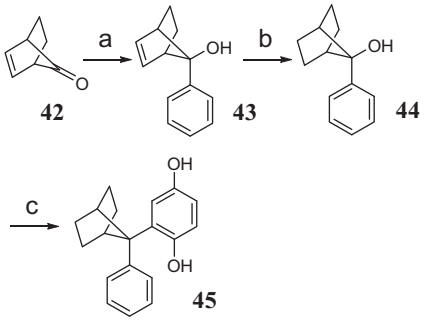


Scheme 4. Partial synthesis of compounds **16** & **18**. Reagents and conditions: (a) PhMgBr, THF, −10 °C; (b) Swern; (c) RMgX, ether, −10 °C; (d) triethyl silane, TFA, DCM, rt; (e) boron tribromide, DCM, 0 °C to rt.

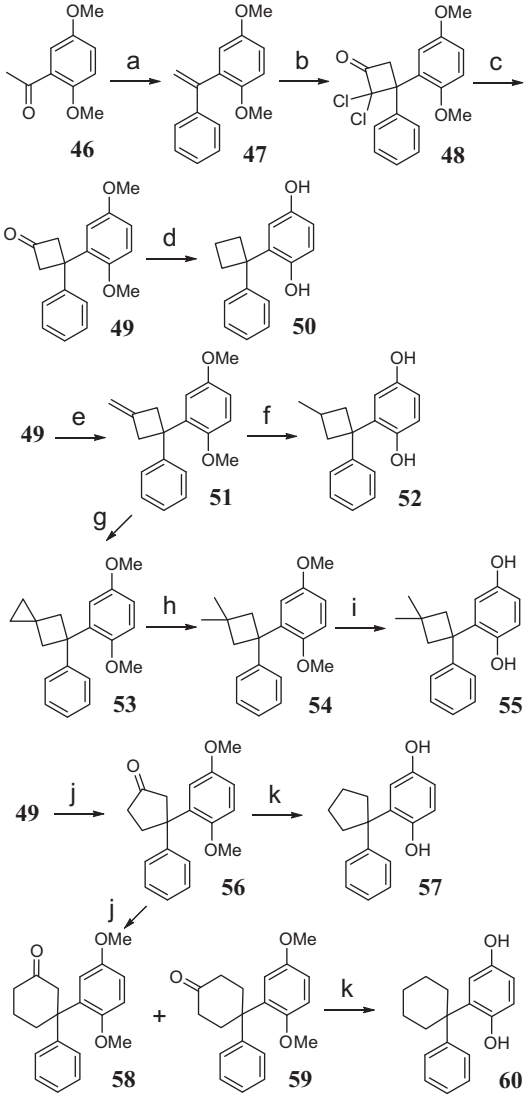


Scheme 5. Partial synthesis of compound **17**. Reagents and conditions: (a) $Pd(PPh_3)_4$, Cs_2CO_3, pivaloyl chloride, toluene, 100 °C; (b) PhMgBr, ether, 0 °C; (c) triethyl silane, TFA, DCM, rt; (d) boron tribromide, DCM, 0 °C to rt.

The achiral norbonyl analogue **19** was prepared in analogous fashion to the approach outlined in Scheme 1 (Scheme 6).



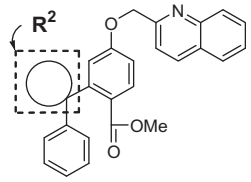
Scheme 6. Partial synthesis of compound **19**. Reagents and conditions: (a) PhMgBr, THF, 0 °C; (b) Pd on carbon, H_2, EtOH; (c) hydroquinone (**5**), pTsOH hydrate, toluene, reflux.



Scheme 7. Partial synthesis of compound **20**,**21**,**22**, **23**, & **25**. Reagents and conditions: (a) PhMgBr, ether, −10 °C; pTsOH hydrate, MeOH, 90 °C; (b) trichloroacetyl chloride, Zn dust, ether, sonication; (c) Zn, AcOH, 70 °C; (d) hydrazine, 160 °C, then diethylene glycol, KOH, 150 °C; boron tribromide, DCM, 0 °C to rt; (e) MeMgBr, ether, 0 °C; pTsOH, benzene, 90 °C; (f) Pd on carbon, H_2, EtOH; boron tribromide, DCM, 0 °C to rt; (g) diethyl zinc, chloroiodomethane, DCE, 0 °C; (h) Pt on carbon (sulfided), H_2, MeOH, AcOH overnight; (i) Boron tribromide, DCM, 0 °C to rt; (j) TMS-diazomethane, trimethyl aluminum, DCM, −78 °C to −25 °C; (k) hydrazine, 160 °C, then diethylene glycol, KOH, 150 °C; boron tribromide, DCM, 0 °C to rt.

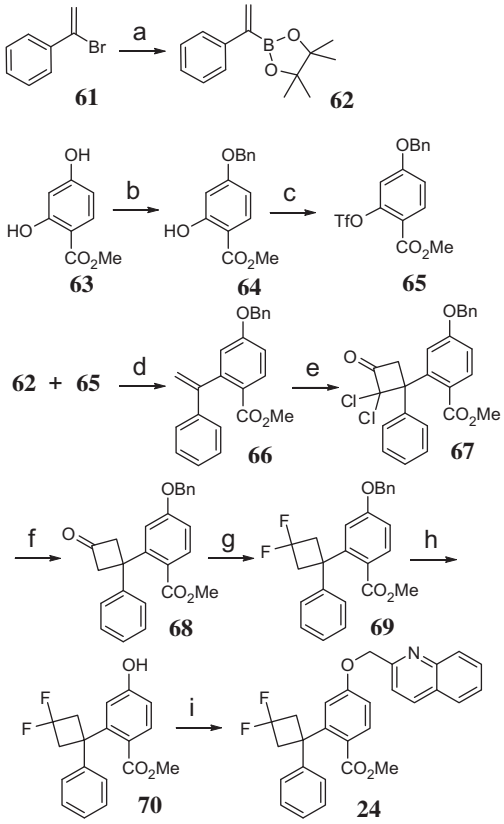
A systematic survey of the optimal ring size in the central scaffold was conducted. Cyclobutyl (**22**), cyclopentyl (**21**), and cyclohexyl (**20**) compounds and their derivatives were synthesized (Scheme 7, Table 2). Another reaction of interest was the ring opening by hydrogenation of the spiro-cyclopropyl group in compound **53** (Scheme 7). No reaction was observed when palladium on carbon was used. When platinum oxide was employed, the desired cyclopropyl ring opening was accomplished with undesired, concomitant saturation of the *endo*-phenyl ring. Fortunately, the

Table 2
Summary of central scaffold replacements and their corresponding in vitro assay data

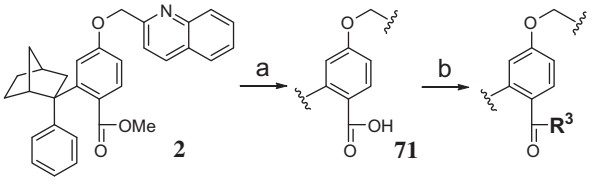


Compounds	R^2	FLAP binding[5] IC_{50} (nM)	HWBA[6] IC_{50} (nM)
2		2.1	460
14[a]		58	>9000
15[b]		96	>9000
16[c]		16	>9000
17[d]		5.5	670
18[c]		3	2000
19[e]		5.6	1300
20[f]		15	1560
21[f]		8.5	3700
22[f]		20	>7000
23[f]		3.2	1100
24[g]		50	>9000
25[f]		5.2	1400

[a] For synthesis to intermediate **29**, see Scheme 2, then follow the analogous transformations outlined in Scheme 1.
[b] For synthesis to intermediate **31**, see Scheme 3, then follow the analogous transformations outlined in Scheme 1.
[c] For synthesis to intermediate **36**, see Scheme 4, then follow the analogous transformations outlined in Scheme 1.
[d] For synthesis to intermediate **41**, see Scheme 5, then follow the analogous transformations outlined in Scheme 1.
[e] For synthesis to intermediate **45**, see Scheme 6, then follow the analogous transformations outlined in Scheme 1.
[f] For synthesis to intermediates **50**, **52**, **55**, **57**, **60**, see Scheme 7, then follow the analogous transformations outlined in Scheme 1.
[g] For synthesis of **24**, see Scheme 8.

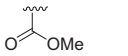
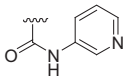
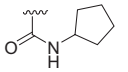
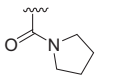


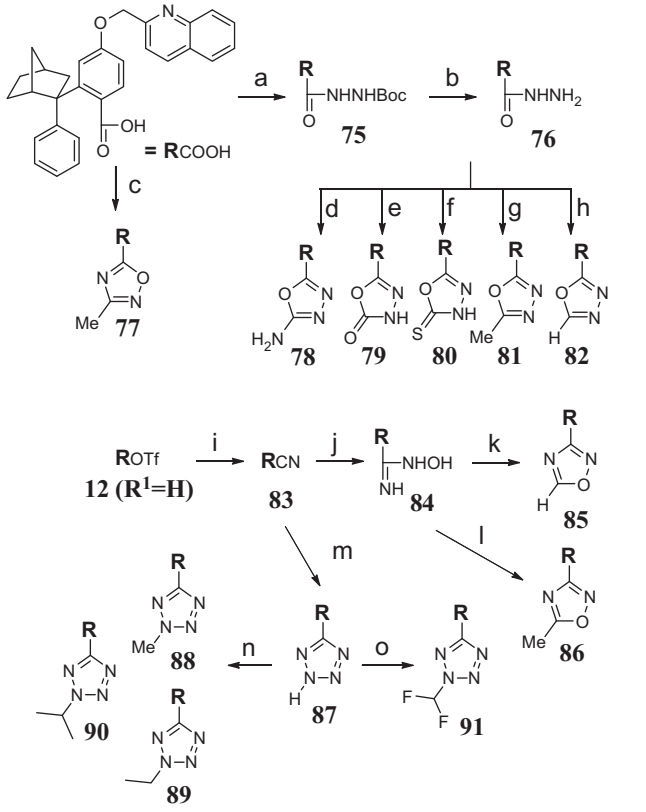
Scheme 8. Synthesis of compound **24**. Reagents and conditions: (a) Bis(pinacolato)diboron, $PdCl_2(PPh_3)_2$, triphenylphosphine, PhOK, toluene, 50 °C; (b) Benzyl alcohol, triphenylphosphine, DIAD, THF, 0 °C; (c) triflic anhydride pyridine, DCM; (d) $PdCl_2(dppf)_2$, K_2CO_3, DMF, 80 °C; (e) trichloroacetyl chloride, Zn-Cu couple, ether/DME, 50 °C; (f) Zn, AcOH, 70 °C; (g) Deoxofluor®, DCM, EtOH; (h) Pd on carbon, H_2, EtOH; (i) 2-chloromethylquinoline, KI, K_2CO_3, DMF.



Scheme 9. Synthesis of compounds **72**–**74**. Reagents and conditions: (a) KOH, THF/propylene glycol, 110 °C; (b) R^3 H, HATU, DIPEA, DMF.

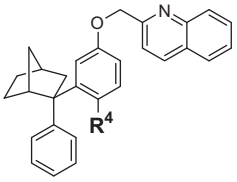
Table 3
Compounds **72**–**74** and their in vitro assay data

Compounds	R^3	FLAP binding[5] IC_{50} (nM)	HWBA[6] IC_{50} (nM)
2		2.1	460
72		5.8	2164
73		48	964
74		7.1	1727



Scheme 10. Synthesis of compounds **77**–**91**. Reagents and conditions: (a) Boc-hydrazide, HATU, DIPEA, DMF; (b) HCl/dioxane, DCM; (c) (1) acetamide oxime, DIC, HOBT, DMF/DCM. (2) CH_3CO_2Na, EtOH, H_2O, 85 °C;[7] (d) $NaHCO_3$, BrCN, dioxane/H_2O, 25 °C; (e) Phosgene, DCM, -78 °C; (f) thiophosgene, THF, −78 °C; (g) (1) Acetyl chloride, TEA, DCM. (2) $SOCl_2$, 25 °C; (h) $CH(OEt)_3$, cat. TsOH; (i) $Zn(CN)_2$, $Pd_2(dba)_3$, dppf, NMP, 140 °C; (j) NH_2OH hydrate, EtOH, 120 °C; (k) $CH(OEt)_3$, cat. TsOH, 125 °C; (l) Acetyl chloride, pyridine; (m) Me_3SnN_3, toluene, 120 °C; (n) K_2CO_3, R'X, DMF, rt; (o) NaH, $ClCHF_2$, rt.

Table 4
Compounds **77**–**91** and their in vitro assay data



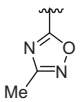
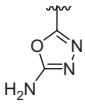
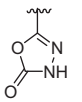
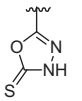
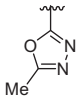
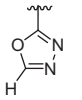
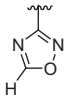
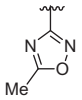
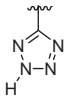
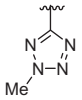
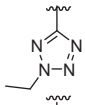
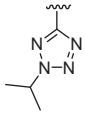
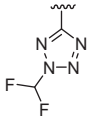
Compounds	R^4	FLAP binding[5] IC_{50} (nM)	HWBA[6] IC_{50} (nM)
77		6.3	1018
78		2.2	258
79		2.5	796
80		1.1	753

Table 4 (*continued*)

Compounds	R^4	FLAP binding[5] IC_{50} (nM)	HWBA[6] IC_{50} (nM)
81		5.0	456
82		2.9	376
85		5.4	721
86		1.6	189
87		2.9	5718
88		2.8	313
89		2.4	177
90		1.7	149
91		2.4	502

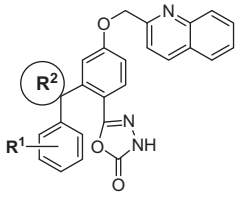
application of platinum on sulfided carbon (100 wt%) resolved the selectivity issue.

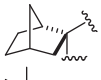
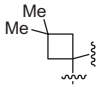
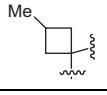
When a similar approach as described in Scheme 7 was adopted to prepare the difluoro analogue **24** from ketone **49**, the difluoro cyclobutyl moiety decomposed during the de-methylation step. Redesign of the synthesis focused on incorporation of the methyl ester functionality early in the synthetic sequence (Scheme 8). Vinyl boronate ester **62** was obtained in moderate but reproducible yields only when freshly prepared potassium phenoxide was used in the coupling step. The palladium catalyzed coupling of boronate ester **62** with triflate **65** went smoothly. The resulting styrene compound **66** was subjected to dichloroketene cyclization followed by reaction with Deoxofluor® to generate the difluorocyclobutane **69**. Subsequent debenzylation and alkylation afforded the desired compound **24**.

Activities of compounds prepared according to the routes described in Schemes 2–8 are summarized in Table 2. While acyclic gem-disubstituted analogues (**14**, **15**) showed substantial loss of FLAP binding and whole blood activity, cyclic variations such as cyclobutyl (**22**), cyclopentyl (**21**) and cyclohexyl (**20**) groups were better tolerated. Among these, the cyclopentyl analogue (**21**) exhibited consistently better activity in the FLAP binding assay. However, if the cyclobutyl ring was appropriately substituted, improvements in potency could be realized (**23**–**25**), which is consistent with a hypothesis that the central scaffold binds within a lipophilic pocket. Finally, mono-alkyl replacements, such as those shown in the *tert*-butyl (**17**) and neopentyl (**18**) analogues demonstrate comparable in vitro activities to the bench-mark norbornyl compound **2**.

Since the SAR of the 5-substituent on the *exo*-aryl ring had been previously optimized,[4] we focused our attention to the modifications at the 2-position of the *exo*-ring. Various amides were prepared initially (Scheme 9). We quickly discovered that although this modification could maintain binding affinity to FLAP, it routinely came at the expense of human whole blood activity (Table 3), possibly due to a large serum shift and/or poor cellular permeability. We subsequently explored the possibility of using heterocycles

Table 5
Compounds 92–96 and their in vitro assay data and pharmacokinetic data[9]



Compounds	R^1	R^2	FLAP binding[5] IC_{50} (nM)	HWBA[6] IC_{50} (nM)	Cl (ml/min/kg)	V_d (L/kg)	iv AUCN (μM. h. kg/mg)	C_{max} (μM)	$t_{1/2}$ (h)	*F* (%)
92[a]	H		0.8	469	7.3	1.0	0.58	0.40	1.0	12
93[a]	3-OMe		1.1	375	7.2	0.7	0.79	0.67	1.2	17
94[a]	H		1.9	534	29	5.3	0.34	0.25	4.3	27
95[a]	H		3.6	834	71	7.5	0.05	0.01	1.7	9
96[a]	H		2.6	695	32	3.1	0.19	0.12	2.0	17

[a] Compounds **92**–**95** are chiral compounds with stereochemistry as shown. Compound **96** is either *cis* or *trans* in configuration.

as replacements. In contrast to the amides prepared, a wide variety of heterocycles were well tolerated at the 2-position of the *exo*-phenyl ring (Scheme 10, Table 4). Regioisomeric oxadiazoles as well as alkylated tetrazoles all exhibited comparable FLAP binding.

The most striking disconnect between binding and HWB was observed with analogue **87**, which was likely due to the poor cellular permeability of this acidic-proton containing compound at physiologoical pH[8] and/or high degree of plasma protein binding. Although alkylated tetrazoles exhibited good HWB potency, they generally suffered from poor PK due to rapid dealkylation in vivo. We then combined various findings from our SAR investigations, specifically the *meta*-methoxy substituent from the *endo*-aryl study and the *tert*-butyl and dimethyl/monomethyl-cyclobutyl groups from the central scaffold. For the substituent at the 2-position of the *exo*-ring, we decided to use the keto-oxadiazole unit (**79**) as our standard template due to the balance of PK and potency. The general synthetic sequence was similar to that outlined in Scheme 10. The in vitro assay results and the pharmacokinetic evaluation of these hybrids are summarized in Table 5. Among the compounds tabulated, the *tert*-butyl analogue **94** was shown to have some slight advantage in its PK profile (improved V_d and bioavailability) over the others while retaining FLAP inhibition activity.

In conclusion, we have shown that an unsubstituted *endo*-phenyl group is optimal, although substitution at the 3-position is consistently tolerated. In addition, the *tert*-butyl group appears to be an effective surrogate for the bicyclic moiety in the central scaffold. Its smaller size provides opportunities for additional substitutions elsewhere in the molecule without exceeding a desirable molecular weight range. Furthermore, we have demonstrated that a wide variety of heterocyles were well tolereated at the 2-position of the *exo*-phenyl ring, laying the groundwork for further studies which will be the subject of future publications.

References and notes

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