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Naphthamidine urokinase plasminogen activator inhibitors with improved pharmacokinetic properties

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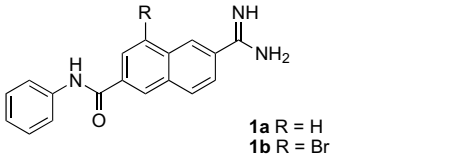
Abstract—A series of non-amide-linked 6-substituted-2-naphthamidine urokinase plasminogen activator (uPA) inhibitors are described. These compounds possess excellent binding activities and selectivities with significantly improved pharmacokinetic profiles versus previously described amide-linked inhibitors.

Urokinase plasminogen activator (uPA) is a trypsin class serine protease that is involved in various biological processes. uPA has been shown to mediate cancer invasion and metastasis by the regulation of extracellular proteolysis through plasminogen activation.[1] Additionally, recent studies have shown broader involvement of uPA in processes involving tumour invasion.[2] The binding of uPA to its receptor induces cellular events such as tumourigenesis, cell proliferation, migration, adhesion and angiogenesis. High expression levels of uPA, uPAR (uPA receptor) and PAIs (plasminogen activator inhibitors) typically correlate with poor prognosis in cancer patients.[2c,3] The involvement of uPA in many cancer related cellular pathways makes it an attractive target for cancer therapy.[2b,4]

We have previously described 6-substituted-phenylamido-2-naphthamidines **1a**, and 8-substituted-2-naphthamidines as inhibitors of uPA.[5] This series was further elaborated to 6,8-disubstituted-2-naphthamidines **1b–d**.[6] Substituents in the 8-position of such analogues occupied the S1β binding pocket of the uPA active site.[7] The resulting compounds showed increased binding affinities against uPA and good selectivities against other proteases. However, all these compounds were poorly bioavailable. The amide linker was identified as a source of a potential metabolic liability,[8] contributed to high crystallinity and poor solubility, which were contributors to poor intestinal absorption. This communication describes compounds designed to probe this hypothesis.



1a R = H
1b R = Br
1c R = 3-Furyl
1d R = 2-Pyrimidylamino

With the goal of developing an oral agent, an effort was made to determine, which functional groups might be adversely affecting bioavailability. The amide-linked naphthamidines showed unsuitable pharmacokinetic properties despite the excellent binding activities and selectivities. Since amidines are generally considered to possess poor bioavailability, we attempted to replace the amidine functional group with less basic groups such

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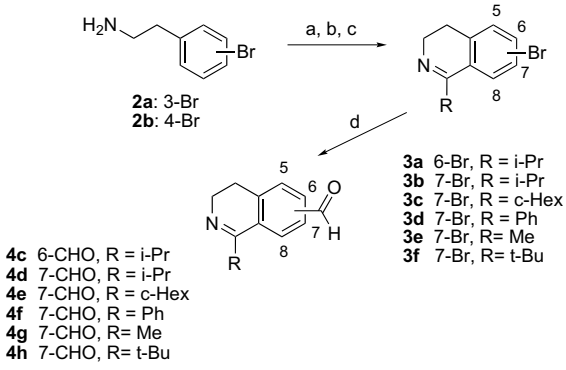
as amines. However, the resulting compounds were much less active. Then, we focused our attention on amide linker replacements. For amide bond isosteres,[9] the most appealing linker replacements were alkenes, alkanes and cyclopropanes whose synthesis has been described below.

Dihydroisoquinoline (DHIQ) carboxaldehydes were synthesized in three steps from bromophenethylamines **2a**,**b** and acyl chlorides (Scheme 1). Conversion of the resultant amides to bromo-DHIQs **3a**–**f** was accomplished via an oxalyl chloride/iron chloride mediated cyclization followed by HCl hydrolysis of the cyclic intermediates.[10] Bromides **3a**–**f** were *trans*-metalated with *n*-butyl lithium and subsequently quenched with DMF providing aldehydes **4c**–**h**.



Scheme 1. Reagents and conditions: (a) RC(=O)Cl, Et_3N; (b) $(COCl)_2$, $FeCl_3$, chloroform; (c) HCl; (d) BuLi, DMF, THF, −78 °C.

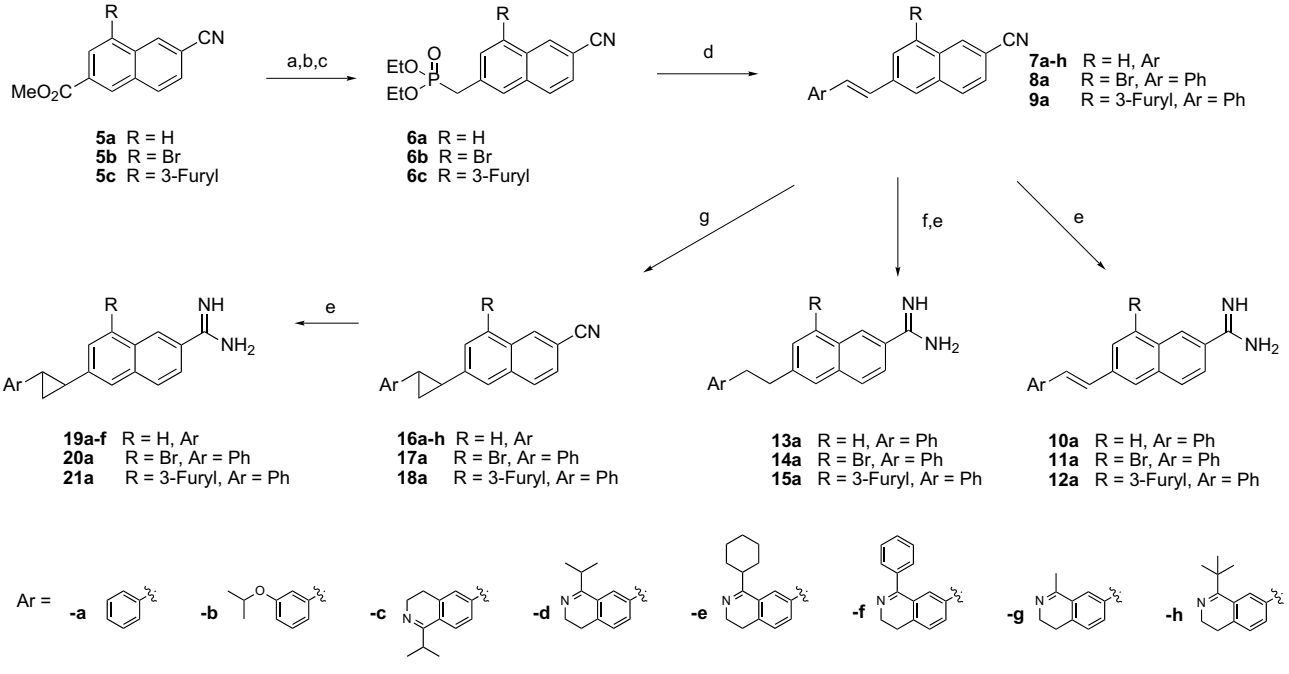
The preparations of the amide replacement naphthamidines are shown in Schemes 2 and 3. Esters of cyanonaphthalenes **5a**–**c** were converted into Horner–Emmons phosphonates **6a**–**c** in three steps. The ester is first reduced to the corresponding alcohol utilizing a calcium chloride assisted sodium borohydride reduction in ethanol. Bromination was accomplished with NBS and triphenylphosphine followed by Arbuzov reaction to yield phosphonates **6a**–**c**. (*E*)-Alkenes **7a**–**h**, **8a** and **9a** were prepared via Horner–Emmons–Wadsworth couplings of phosphonates **6a**–**c** with the appropriate aldehydes **4a**–**h**.

The cyclopropyl linked amidines **19a**–**f**, **20a** and **21a** were synthesized as racemic mixtures in two steps by treating (*E*)-arylalkenes **7a**–**f**, **8a** and **9a** with diazomethane under palladium acetate catalysis. We were able to resolve a mixture of **16d** by using chiral HPLC. The following amidination of nitriles **16a**–**f**, **17a** and **18a** was executed with excess LiHMDS (5 equiv) and subsequent quench with HCl.

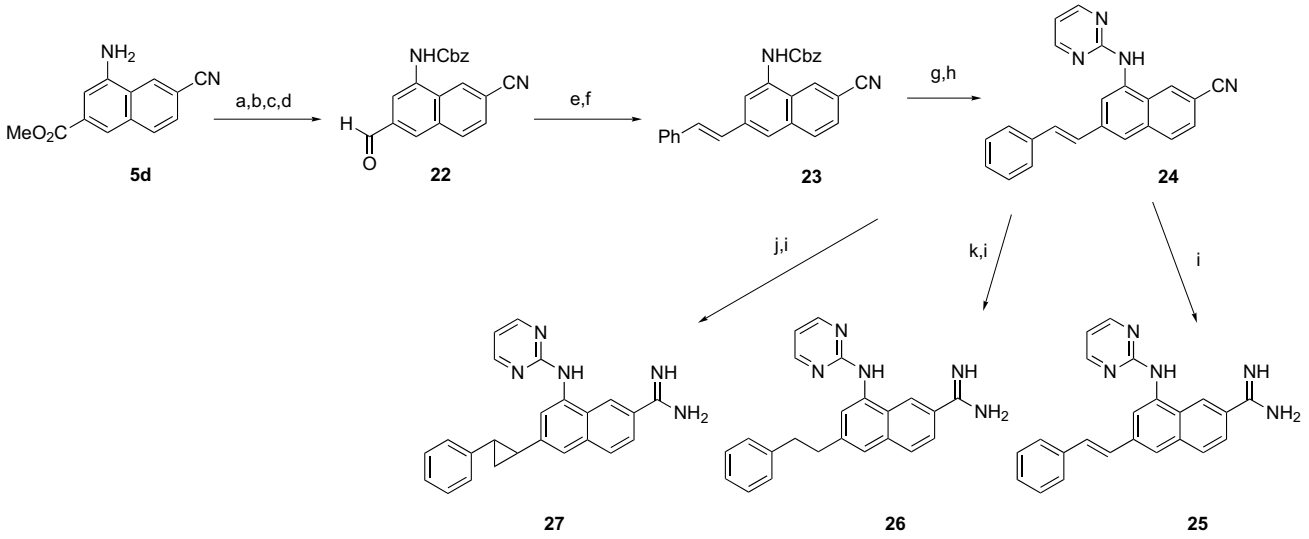
The alkyl linked compounds **13a**, **14a** and **15a** were prepared by Pd/C catalyzed reduction with H_2 followed by standard amidination as described above.

The arylalkene nitriles **7a**, **8a** and **9a** were similarly transformed to the corresponding amidines with excess LiHMDS (5 equiv) followed by quench with HCl.

8-Pyrimidyl naphthamidines **25**, **26**, **27** were prepared by a modified route (Scheme 3) starting with the protection of 8-aminonaphthalene **5d**[6] with Cbz–Cl. The methyl ester was converted to aldehyde **22** in three steps beginning with an ester saponification with LiOH, followed



Scheme 2. Reagents and conditions: (a) $NaBH_4$, $CaCl_2$, EtOH; (b) PPh_3, NBS, dichloromethane; (c) $P(OEt)_3$, 130 °C; (d) LiHMDS, ArCHO **4a**–**h**, THF; (e) LiHMDS, THF, HCl; (f) Pd/C, H_2, MeOH; (g) CH_2N_2, $Pd(OAc)_2$, THF, ether.

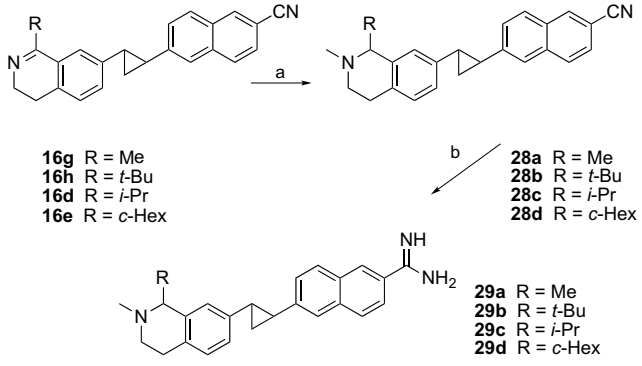


Scheme 3. Reagents and conditions: (a) Cbz–Cl, *i*-PrNEt$_2$, THF; (b) LiOH, dioxane, H_2O; (c) $(COCl)_2$, THF; (d) LiAl(O^{t}Bu)$_3$H, THF, −78 °C; (e) Ph_3PCH_3Br, NaHMDS, THF; (f) PhN_2BF_4, $Pd_2(dba)_3$, DMF; (g) $Ba(OH)_2$, DME, 70 °C; (h) 2-Br-pyrimidine, $Pd_2(dba)_3$, NaO^{t}Bu, toluene; (i) $AlMe_3$, NH_4Cl, toluene; (j) CH_2N_2, $Pd(OAc)_2$, THF, ether; (k) Pd/C, H_2, MeOH.

by formation of the acid chloride with oxalyl chloride, and reduction to aldehyde **22** with LiAl(O^{t}Bu)$_3$H at −78 °C. The formation of alkene **23** was achieved via a two-step process, a Wittig olefination to provide the vinyl intermediate followed by Pd-catalyzed coupling with phenyldiazonium species. The Cbz group was cleaved with $Ba(OH)_2$ in DME at 70 °C. The pyrimidyl group was introduced under Buchwald amination conditions with 2-bromopyrimidine and 8-aminonaphthalene to yield alkene **24**.

(*E*)-Arylalkene **24** was converted to the corresponding arylcyclopropane and arylalkane in the same fashion as described above (Scheme 2). All nitriles in the pyrimidyl series were transformed to amidines **25**, **26** and **27** by treatment with $AlMe_3$ and NH_4Cl in toluene.

Finally, racemic tetrahydroisoquinolines (THIQ) **29a–d** were obtained via a one pot imine reduction/reductive amination of the corresponding DHIQ (**16d**,**e**,**g**,**h**) with formaldehyde and followed by standard amidine formation with LiHMDS (Scheme 4).



Scheme 4. Reagents and conditions: (a) $NaBH_3CN$, CH_2O, MeOH; (b) LiHMDS, THF, HCl.

From previous studies, it was known that the amide linker is quite rigid and the NH participates in a water mediated hydrogen bond to Ser214.[5] By implementing carbon–carbon replacements, we would lose this interaction and possibly some activity, however, this might be acceptably offset by an increase in the oral bioavailability of the compounds. The alkene linker might be expected to be most structurally similar to an amide linker since it has sp^2-hybridized atoms, thus the orientation and vectors of aromatic substituents would be in close proximity. In fact, we found from the X-ray

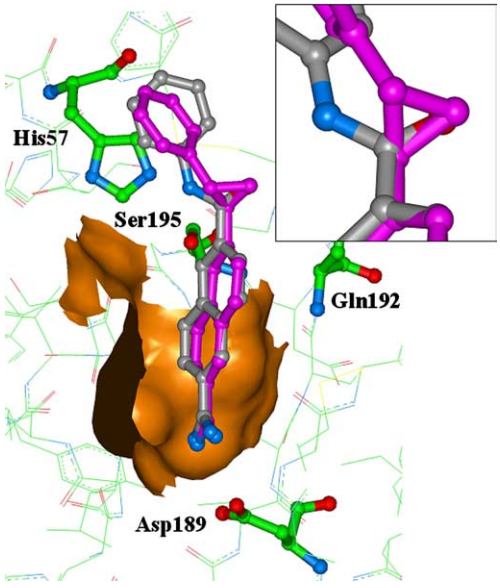
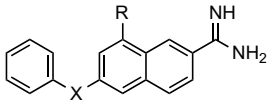
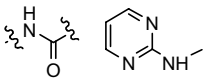
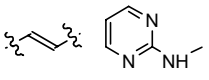
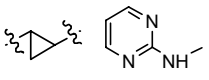
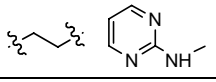


Figure 1. Crystal structure of compound **19a** (pink, cyclopropyl link, PDB entry 1U6Q) bound to urokinase is shown overlaid with compound **1a** (grey, amide link, PDB entry 1OWE). His57, Asp189, Gln192 and Ser195 are shown in thick bonds. The surface of the S1 pocket is shown. The inset shows a close-up view of the cyclopropyl/amide overlay.

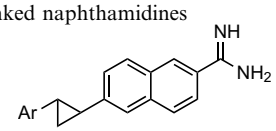
Table 1. Phenyl substituted naphthamidines with variable linker

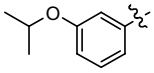
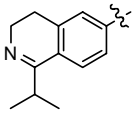
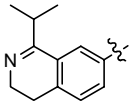
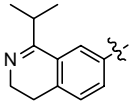
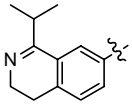
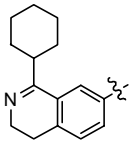
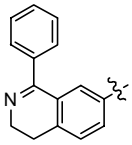
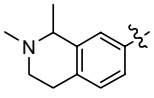
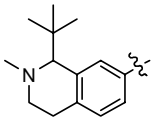
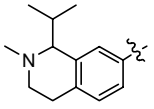
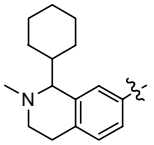


Compd	X	R	K_i[a] (μM)						$t_{1/2}$[b] (h)	Cl[b] (L/h kg)	Vd[b] (L/kg)	AUC[b] (μM h)	F[d] (%)
			uPA	tPA	Thrombin	Plasmin	Kallikrein	Trypsin (porcine)					
1a		H	0.631 ± 0.086	31.8 ± 17.8(9)	5.64 ± 2.16(9)	1.95 ± 0.58(9)	2.50 ± 1.14(9)	0.325 ± 0.175(8)	2.1[e]	2.8	8.5	8.2	3
10a		H	1.20	34.564	5.269	1.216	0.991	0.346	—[c]		—[c]	—[c]	—[c]
19a		H	1.31	69.11	1.89	3.523	2.482	0.720	7.4[e]	2.16	23.0	11.6	30
13a		H	3.183	—[c]	—[c]	—[c]	—[c]	—[c]	1.0	6.75	9.8	1.14	60
1b		Br	0.281	5.59	2.655	1.015	0.992	0.064	1.05	12.3	18.7	0.6	0
11a		Br	0.820	13.38	0.92	1.35	0.77	0.480	—[c]	—[c]	—[c]	—[c]	—[c]
20a		Br	0.622	30.783	0.503	0.239	0.379	0.334	4.71	2.1	14.3	2.72	17
14a		Br	1.37	—[c]	—[c]	—[c]	—[c]	—[c]	3.3	3.8	18	1.75	>32
1c		3-Furyl	0.091 ± 0.011	0.176	0.132	0.321	0.265	0.045	3	5.0	21.7	5	0
12a		3-Furyl	0.168	4.465	0.237	0.134	0.083	0.096	0.62	1.3	1.2[c]	5.8[c]	0
21a		3-Furyl	0.198	—[c]	—[c]	—[c]	—[c]	—[c]	4.2	2.4	14.5	2.0	10
15a		3-Furyl	0.731	—[c]	—[c]	—[c]	—[c]	—[c]	3.31	1.53	17.6	1.83	18
1d			0.020	0.810	0.170	0.090	0.100	0.040	0.6	42.9	37.1	0.14	0
25			0.010	>50	0.395	0.277	0.042	0.084	—[c]	—[c]	—[c]	—[c]	—[c]
27			0.005	5.049	0.048	0.243	0.054	0.054	0.33	21.1	10.0	0.23	0
26			0.047 ± 0.001	5.786	0.191	0.570	0.156	0.167	2[e]	4.7	13.6	4.2	0

[a] ±Standard deviation (number of determinations if more than two). All determinations are triplicate values.
[b] Half life, clearance, volume distribution and area under the curve (AUC) in Sprague Dawley rat, IV dosing 3 mg/kg, vehicle 10% DMSO, 20% EtOH, 27.5% PEG400, 42.5% saline.
[c] Not tested.
[d] Oral bioavailability in rat, dosing 10 mg/kg.
[e] IV dosing 10 mg/kg.

Table 2. SAR and oral bioavailability for cyclopropyl linked naphthamidines



Compd	Ar	K_i[a] (μM)						$t_{1/2}$[b] (h)	Cl[b] (L/h kg)	Vd[b] (L/kg)	AUC[b] (μM h)	F[d] (%)
		uPA	tPA	Thrombin	Plasmin	Kallikrein	Trypsin (porcine)					
19b		0.263	21.924	1.099	2.454	1.402	0.141	5.91	2.26	19.3	3.48	23
19c		0.610 ± 0.045	—[c]	—[c]	—[c]	—[c]	—[c]	0.33	16.4[d]	7.9[d]	0.27[d]	0[d]
19d		0.139 ± 0.006	4.74	2.139	1.046	0.525	0.053	1.33	9.67	18.6	0.51	26
19d (*S*,*S*)		0.065 ± 0.005	10.43	1.79	1.48	0.64	0.03	—[c]	—[c]	—[c]	—[c]	—[c]
19d (*R*,*R*)		0.38 ± 0.01	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
19e		0.050	4.017	1.136	0.594	0.206	0.428	0.27	11.49	4.5	0.57	22
19f		0.142	7.038	2.124	0.955	0.336	0.165	—[c]	—[c]	—[c]	—[c]	—[c]
29a		0.293 ± 0.021	—[c]	—[c]	—[c]	—[c]	—[c]	0.28	7.28	2.9	0.69	0
29b		0.548	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]	—[c]
29c		0.047	6.23	1.796	1.302	0.576	0.090	0.55	12.95	10.3	0.37	55
29d		0.028 ± 0.001	18.943	0.996	0.811	0.295	0.031	1.53	8.49	18.7	0.53	27

[a] ±Standard deviation (number of determinations if more than two). All determinations are triplicate values.
[b] Half life, clearance, volume distribution and area under the curve (AUC) in Sprague Dawley rat, IV dosing 3 mg/kg, vehicle 10% DMSO, 20% EtOH, 27.5% PEG400, 42.5% saline.
[c] Not tested.
[d] Oral bioavailability in rat, dosing 10 mg/kg.

data of compounds **1a** and **19a**[11] bound to urokinase that aryl substituents on the cyclopropane linker overlay and are oriented closely with substituents on the amide linker (Fig. 1).

The PK profiles of the compounds with modified linkers demonstrated significant improvement. In comparison to the corresponding amides (**1a**–**c**), the cyclopropanes (**19a**, **20a**, **21a**) and the alkanes (**14a**, **15a**) had significantly lower clearance rates and longer half lives. The oral bioavailability of alkanes **13a**, **14a** and cyclopropane **19a** were particularly good (30–60%) (Table 1).

Oral bioavailability decreased with introduction of the substituent at the 8-position of naphthamidine. 2-Pyrimidylamino substituted compounds (**1d**, **25**, **26** and **27**) entirely lacked oral bioavailability. The alkene linked amidine **12a** was not also orally bioavailable with a shorter half life compared to cyclopropyl **18a** or alkyl **15a** linked amidines.

Based on Pk data and binding activities we chose the cyclopropane as the most suitable amide bond replacement. We also eliminated the substituent at the 8-position of naphthamidine moiety, since majority of the 8-substituted compounds with both polar and non-polar groups lacked oral bioavailability. Our attention was focused on substituted phenyl analogues (Table 2). Previous work indicated that this series was likely to provide compounds with good activities. 3-Isopropoxyphenyl analogue (**19b**) is fivefold more active against uPA compared to unsubstituted phenyl analogue. The compound **19b** possesses a good half life, lower clearance and is 23% orally bioavailable.

A significant increase in potency was observed by introduction of the DHIQ moiety (**19c**–**f**).

The 7-linked DHIQs were more active compared to 6-linked analogues, so further SAR continued in this series. These efforts yielded 1-*c*-hexyl-substituted analogue (**19f**) that possessed 50 nM potency against uPA and 22% oral bioavailability. The potency of cyclopropyl analogues can be possibly increased by resolving a racemic mixture or preparing a single enantiomer as was shown in example **19d**. Compound **19d** (*S*,*S*) enantiomer is six times more potent against uPA than **19d** (*R*,*R*).

The THIQ and DHIQ analogues were significantly more potent than phenyl substituted cyclopropyl naphthamidines (**19a**,**b**) however they suffered shorter half lives and higher clearances.

The better substituents at the 1-position of the THIQ proved to be a secondary branched alkyl group. Methyl (**29a**) or *t*-butyl (**29b**) were less active. The isopropyl (**29c**) and the *c*-hexyl (**29d**) analogues were highly bioavailable ($F = 55\%$ and 27%) and possessed excellent potencies (47 and 28 nM).

In summary, we have developed a new group of uPA inhibitors by replacing the amide linker in the aryl substituted naphthamidines with a cyclopropyl moiety. These novel compounds possess improved pharmacokinetic profiles with good oral bioavailabilities and extended half lives in rats.

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