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Synthesis and evaluation of non-basic inhibitors of urokinase-type plasminogen activator (uPA)

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ABSTRACT

Recent drug discovery programs targeting urokinase plasminogen activator (uPA) have resulted in non-peptidic inhibitors consisting of amidine or guanidine functional groups attached to aromatic or heteroaromatic scaffolds. There is a general problem of poor oral bioavailability of these charged inhibitors. In this paper, we report the synthesis and evaluation of a series of naphthamide and naphthalene sulfonamides as uPA inhibitors containing non-basic groups as substitute for amidine or guanidine groups.

1. Introduction

Urokinase plasminogen activator (uPA),[1] a trypsin-like serine protease,[2] is a key initiator of the extracellular proteolytic cascade driving cellular invasiveness.[3] Proteolytically active uPA is a disulfide-linked two-chain protein generated from proteolytically inactive pro-uPA by the hydrolysis of the Lys158-Ile159 peptide bond.[4] The central function of uPA is to convert plasminogen to plasmin, which digests the components of the extracellular matrix and basement membranes either directly or by activation of proenzymes of matrix metalloproteinases.[3,5] uPA secreted by tumor cells or the adjacent stroma exists as a free enzyme or bound to the cell surface receptor, uPAR.[6] Binding to uPAR significantly increases the rate of cell surface-associated plasminogen activation by uPA and can serve to spatially focus its activity. uPA in particular has been shown to be involved in various malignancies including cancer of breast, ovarian, kidney, prostate, lung, bladder, stomach, cervix and the thyroid gland.[7] Higher levels of uPA have been correlated with poor patient prognosis and uPA is involved in extracellular matrix degradation, tumor cell invasion, angiogenesis and metastasis.[8] The role of the plasminogen activator system in various cancers has been reviewed extensively.[7,9] Also, uPA has been associated with several diseases and disorders including wound healing, macular degeneration, stroke, multiple sclerosis and rheumatoid arthritis.[10]

Most published inhibitors of uPA contain an amidino or guanidino group that forms a salt bridge with the carboxylate of Asp189 in the S1 pocket of the active site.[11] An amidine based, peptide-derived inhibitor (**1**, Fig. 1) reduces the number of experimental lung metastases in a fibrosarcoma model in mice,[12] and two clinical phase I trials with uPA inhibitors **2a** (WX-UK1)[13] and its prodrug **2b** (WX-671 or MESUPRON®) have been successfully completed. Moreover, compound **2b** showed favorable results in randomized phase II trial in patients with locally advanced nonmetastatic pancreatic cancer and phase II trial in patients with HER2-negative metastatic breast cancer (MBC) has been initiated.[14] Wendt et al. reported a series of substituted naphthamidines as inhibitors of uPA (**3a–c** as representative examples) and **3c** was shown to possess high selectivity towards tPA, thrombin and plasmin.[15] Our research group reported potent and highly selective nonpeptidic irreversible diaryl phosphonate inhibitors (**4a** and **4b**) of uPA with a strong anti-metastatic effect in the BN-472 rat mammary carcinoma model (Fig. 1).[16] Zhu and co-workers reported a novel uPA inhibitor **5** (UK122) that is highly potent and specific against uPA in cell-free assays and exhibits potent antimigration and invasion activities.[17] These and other amidine and guanidine containing inhibitors tend to have a very low oral bioavailability.[14–18] More recently, less basic orally bioavailable inhibitors of uPA (**6**, **7b** and **7c**) containing primary amines were reported.[19] The selectivity profile of all these uPA inhibitors is presented in Table 1.

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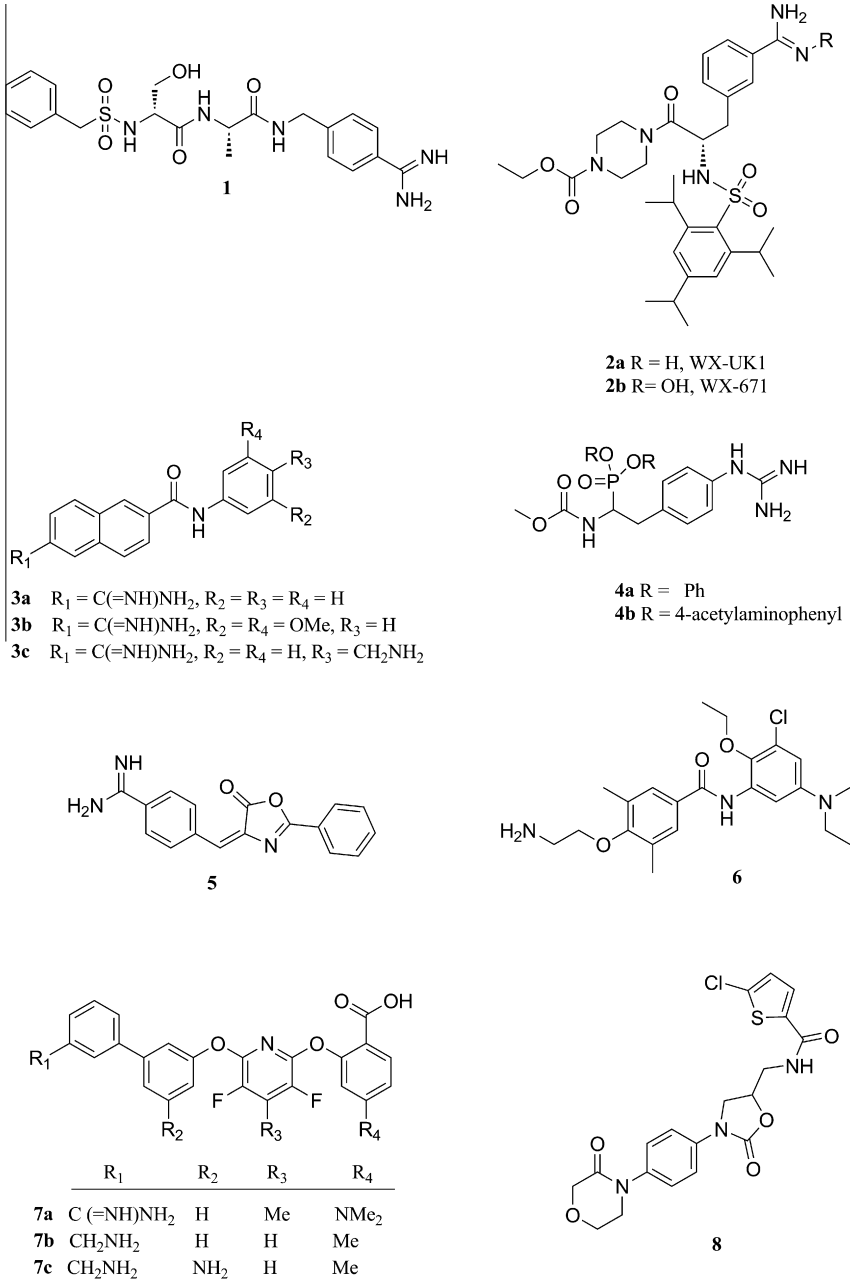


Figure 1. Selection of uPA inhibitors reported in the literature.

For other trypsin-like serine proteases such as FXa non-basic inhibitors have been developed (Fig. 1). A successful example is rivaroxaban (**8**), a novel factor Xa inhibitor that recently entered the market as an oral anticoagulant.[20] However, until now there is no report on similar non-basic uPA inhibitors. This prompted us to develop the new class of non-basic uPA inhibitors reported in this paper.

As a starting point for our design, the naphthamidine inhibitors **3a–c** attracted our attention because of their low molecular weight combined with a good inhibitory activity and selectivity.[15] Similar to the FXa inhibitors, we replaced the amidine moiety by small groups such as –H, –Br, –OMe, –CN, –COOH, –COOMe and $-CONH_2$ (Series A, **9a–k**). Also, we investigated compounds with a biphenyl instead of a phenyl moiety (Series B, **10a–u**), and with a sulfonamide linker (Series C, **11a–p**) (Fig. 2). The reason for selecting the polar sulfonamide group is to increase solubility and lower lipo-

Table 1
Selectivity profile of uPA inhibitors from the literature

Compd	K_i[a]/IC_{50}[b] (μM)[c]			
	uPA	tPA	Thrombin	Plasmin
1[a]	0.0077	7.4	0.11	0.54
2a[a]	0.41	4.9	0.49	0.39
3a[a]	0.631 ± 0.086	31.8 ± 17.8	5.64 ± 2.16	1.95 ± 0.58
3b[a]	0.139 ± 0.011	—	—	—
3c[a]	0.04	58.4	>100	16 ± 0.9
4a[b]	0.0031 ± 0.0005	23 ± 6	17 ± 2.3	13 ± 2
4b[b]	0.0034 ± 0.0004	6.1 ± 0.7	1.13 ± 0.06	2.8 ± 0.5
5[b]	0.2	>100	>100	>100
6[b]	0.072	>3.6	—	>3.6
7a[b]	0.098	3.9	—	2.1
7b[b]	0.24	22	—	5
7c[b]	0.039	>30	—	5

[a] K_i values for compounds **1**,[12] **2a**[28] and **3a–c**.[15]
[b] IC_{50} values for compounds **4a–b**,[16] **5**,[17] **6**[19a] and **7a–c**.[19b]
[c] ±Standard deviation.

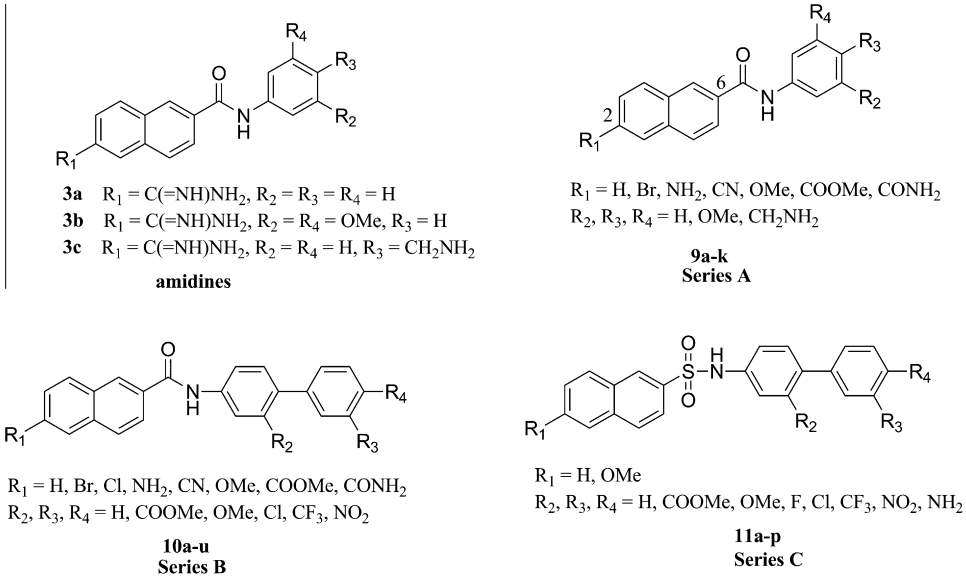
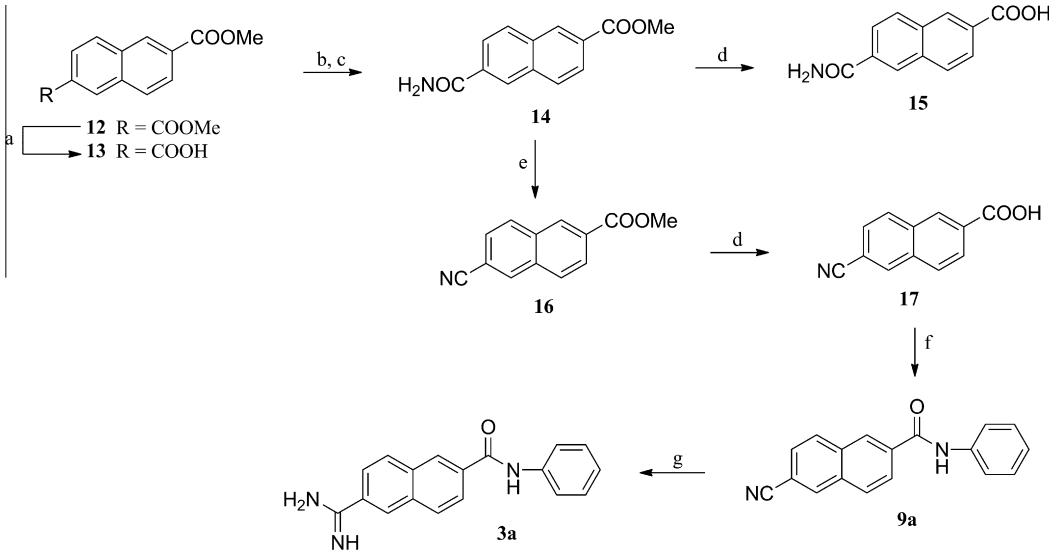


Figure 2. Three different series designed from reference amidine compounds **3a–c**.



Scheme 1. Reagents: (a) KOH, dioxane, Δ; (b) $SOCl_2$, toluene, Δ; (c) 7 N NH_3 in MeOH; (d) LiOH, THF, H_2O; (e) TFAA, pyridine, dioxane; (f) aniline, TBTU, iPr_2NEt, DMF; (g) $LiN(TMS)_2$, THF and then aq HCl.

philicity. The sulfonamide group was also reported as linker in uPA inhibitors such as 1-(7-sulfonamidoisoquinolinyl)guanidines.[18e]

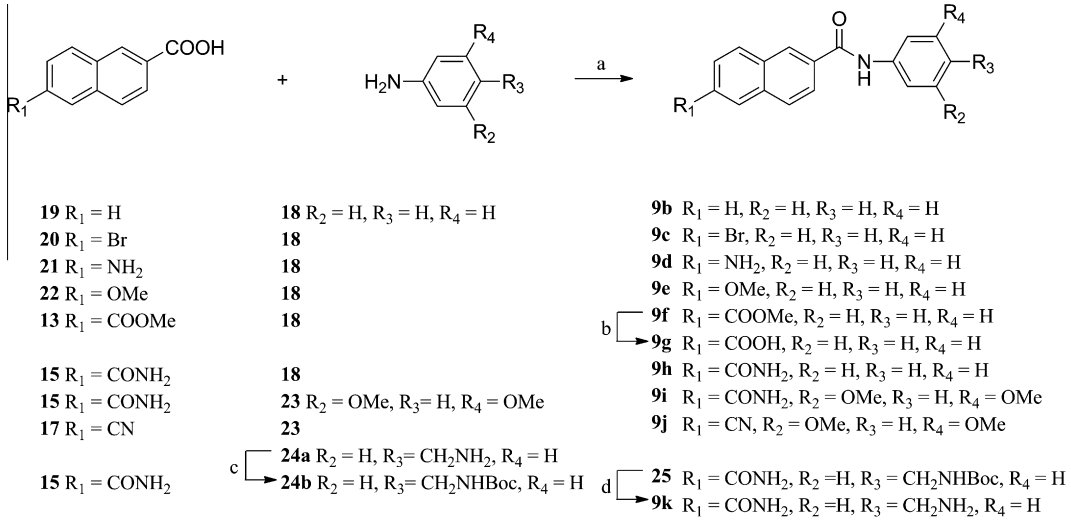
2. Chemistry

The synthesis of compounds belonging to Series A–C is outlined in Schemes 1–7. Monosaponification of diester **12** gave acid **13** which was converted to nitrile **16** via dehydration of amide **14**. Hydrolysis of the remaining ester group in **14** and **16** gave the 6-carbamoyl- and 6-cyano-naphthalene-2-carboxylic acids (**15** and **17**). Coupling of **17** with aniline using TBTU in DMF afforded **9a** which was converted into amidine **3a**[15,21] (Scheme 1).

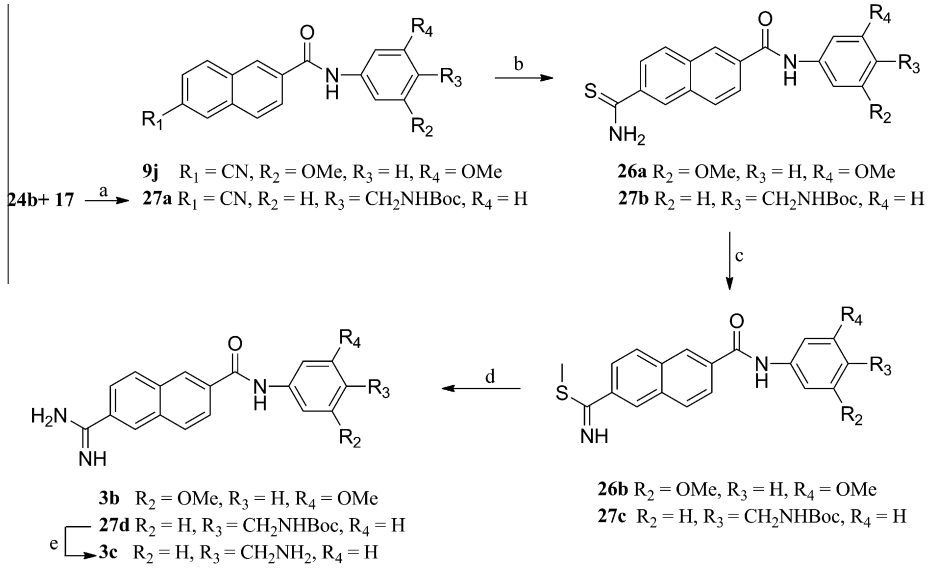
The 6-substituted-*N*-phenyl-2-naphthamides **9b–f** and **9h–j** were obtained by coupling the 6-substituted-naphthalene-2-carboxylic acids with the respective anilines. Compound **9g** was obtained from **9f** by hydrolysis (Scheme 2). The primary amine function of 4-(aminomethyl)aniline **24a** was selectively protected with a Boc-group using di-*tert*-butyl dicarbonate to give **24b**. Coupling of **24b** with acid **15** using TBTU in DMF afforded **25** which on deprotection with TFA yielded **9k** (Scheme 2).

Synthesis of reference amidines **3b** and **3c** with $LiN(TMS)_2$ using a reported procedure failed in our hands.[18d] This was due to incomplete reaction even after using excess $LiN(TMS)_2$ and practical difficulties in separation of the amidines from the complex reaction mixture. Hence we synthesized these amidines by converting nitriles to the thioamide, followed by conversion to the thioimidate and reaction with ammonium acetate (Scheme 3).[22]

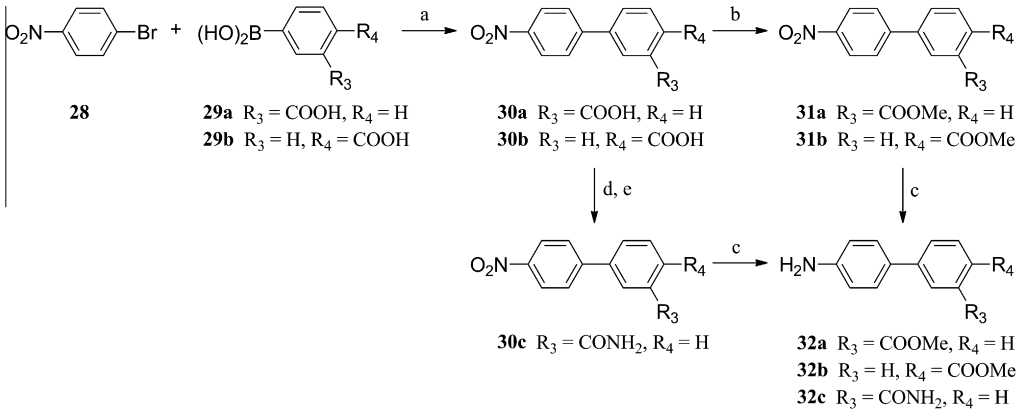
Suzuki coupling of 4-bromonitrobenzene (**28**) with boronic acids **29a** and **29b** afforded nitrobiphenyl acids **30a** and **30b** which were converted into nitrobiphenylmethyl esters **31a** and **31b**. Reduction of nitro compounds using Pd/C in methanol yielded biphenylamines **32a** and **32b**.[23] Compound **32c** was prepared by reduction of **30c** (Scheme 4). Scheme 5 shows the synthesis of biphenyl amines **35a–i**.[24] The compounds **35a–h** were prepared



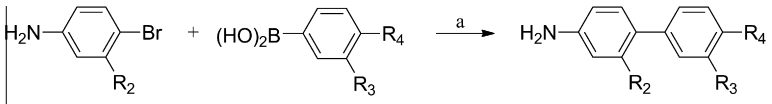
Scheme 2. Reagents: (a) TBTU, *i*Pr$_2$NEt, DMF; (b) LiOH, THF, H$_2$O; (c) (Boc$_2$)O, THF, H$_2$O; (d) TFA, CH$_2$Cl$_2$.



Scheme 3. Reagents: (a) TBTU, *i*Pr$_2$NEt, DMF; (b) diethyl dithiophosphate, *i*Pr$_2$NEt, H$_2$O, 1,2-dimethoxyethane, Δ; (c) MeI, acetone; (d) NH$_4$OAc, MeOH; (e) TFA, CH$_2$Cl$_2$.

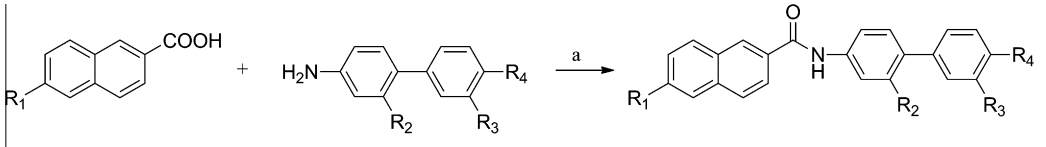


Scheme 4. Reagents: (a) Pd(PPh$_3$)$_4$, K$_2$CO$_3$, toluene, EtOH, H$_2$O, Δ; (b) SOCl$_2$, MeOH, Δ; (c) H$_2$, Pd/C, MeOH; (d) SOCl$_2$, toluene, Δ; (e) 7 N NH$_3$ in MeOH.



33 R_2 = H	**34a** R_3 = H, R_4 = H	**35a** R_2 = H, R_3 = H, R_4 = H
	34b R_3 = OMe, R_4 = H	**35b** R_2 = H, R_3 = OMe, R_4 = H
	34c R_3 = NO_2, R_4 = H	**35c** R_2 = H, R_3 = NO_2, R_4 = H
	34d R_3 = CF_3, R_4 = H	**35d** R_2 = H, R_3 = CF_3, R_4 = H
	34e R_3 = F, R_4 = H	**35e** R_2 = H, R_3 = F, R_4 = H
	34f R_3 = Cl, R_4 = H	**35f** R_2 = H, R_3 = Cl, R_4 = H
	34g R_3 = H, R_4 = OMe	**35g** R_2 = H, R_3 = H, R_4 = OMe
	34h R_3 = OMe, R_4 = OMe	**35h** R_2 = H, R_3 = OMe, R_4 = OMe
36 R_2 = OMe	**34g** R_3 = H, R_4 = OMe	**35i** R_2 = OMe, R_3 = H, R_4 = OMe

Scheme 5. Reagents: (a) $Pd(PPh_3)_4$, Na_2CO_3, toluene, EtOH, H_2O, Δ.



19 R_1 = H	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10a** R_1 = H, R_2 = H, R_3 = COOMe, R_4 = H
19 R_1 = H	**35b** R_2 = H, R_3 = OMe, R_4 = H	**10b** R_1 = H, R_2 = H, R_3 = OMe, R_4 = H
15 R_1 = $CONH_2$	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10c** R_1 = $CONH_2$, R_2 = H, R_3 = COOMe, R_4 = H
15 R_1 = $CONH_2$	**35b** R_2 = H, R_3 = OMe, R_4 = H	**10d** R_1 = $CONH_2$, R_2 = H, R_3 = OMe, R_4 = H
15 R_1 = $CONH_2$	**35d** R_2 = H, R_3 = CF_3, R_4 = H	**10e** R_1 = $CONH_2$, R_2 = H, R_3 = CF_3, R_4 = H
15 R_1 = $CONH_2$	**35f** R_2 = H, R_3 = Cl, R_4 = H	**10f** R_1 = $CONH_2$, R_2 = H, R_3 = Cl, R_4 = H
15 R_1 = $CONH_2$	**35i** R_2 = OMe, R_3 = H, R_4 = OMe	**10g** R_1 = $CONH_2$, R_2 = OMe, R_3 = H, R_4 = OMe
13 R_1 = COOMe	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10h** R_1 = COOMe, R_2 = H, R_3 = COOMe, R_4 = H
17 R_1 = CN	**35b** R_2 = H, R_3 = OMe, R_4 = H	**10i** R_1 = CN, R_2 = H, R_3 = OMe, R_4 = H
17 R_1 = CN	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10j** R_1 = CN, R_2 = H, R_3 = COOMe, R_4 = H
		10k R_1 = CN, R_2 = H, R_3 = COOH, R_4 = H
22 R_1 = OMe	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10l** R_1 = OMe, R_2 = H, R_3 = COOMe, R_4 = H
22 R_1 = OMe	**35b** R_2 = H, R_3 = OMe, R_4 = H	**10m** R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = H
22 R_1 = OMe	**35c** R_2 = H, R_3 = NO_2, R_4 = H	**10n** R_1 = OMe, R_2 = H, R_3 = NO_2, R_4 = H
22 R_1 = OMe	**35d** R_2 = H, R_3 = CF_3, R_4 = H	**10o** R_1 = OMe, R_2 = H, R_3 = CF_3, R_4 = H
22 R_1 = OMe	**35g** R_2 = H, R_3 = H, R_4 = OMe	**10p** R_1 = OMe, R_2 = H, R_3 = H, R_4 = OMe
22 R_1 = OMe	**35h** R_2 = H, R_3 = OMe, R_4 = OMe	**10q** R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = OMe
22 R_1 = OMe	**35i** R_2 = OMe, R_3 = H, R_4 = OMe	**10r** R_1 = OMe, R_2 = OMe, R_3 = H, R_4 = OMe
37 R_1 = Cl	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10s** R_1 = Cl, R_2 = H, R_3 = COOMe, R_4 = H
20 R_1 = Br	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10t** R_1 = Br, R_2 = H, R_3 = COOMe, R_4 = H
21 R_1 = NH_2	**32a** R_2 = H, R_3 = COOMe, R_4 = H	**10u** R_1 = NH_2, R_2 = H, R_3 = COOMe, R_4 = H

Scheme 6. Reagents: (a) TBTU, *i*Pr_2NEt, DMF; (b) LiOH, THF, H_2O.

relying on Suzuki coupling of 4-bromoaniline **33** with substituted boronic acids **34a–h**. Similarly, Suzuki coupling of 3-methoxy-4-bromoaniline (**36**) with 4-methoxyphenylboronic acid (**34g**) afforded **35i**.

The synthesis of the target compounds **10a–u** is shown in Scheme 6. Compound **37** was obtained from **20** by a reported procedure.[25] The compounds **10a–j** and **10l–u** were made by coupling of substituted naphthalene carboxylic acids with substituted biphenyl amines using TBTU. Compound **10k** was obtained from **10j** by hydrolysis. Scheme 7 shows the synthesis of biphenyl sulfonamides **11a–p**. 6-Methoxynapthalene-sulfonylchloride was prepared by a reported procedure.[26] The reaction of sulfonyl chlorides **38** and **39** with substituted biphenylamines in pyridine afforded biphenyl sulfonamides **11a–k** and **11m–p**. Compound **11l** was obtained by reduction of **11k** using Pd/C in methanol.

3. Results and discussion

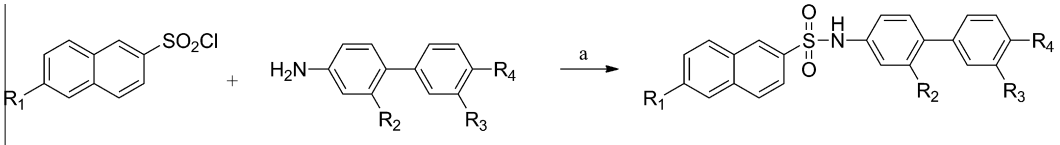
3.1. Biochemical evaluation

The inhibitory potential toward uPA was determined for all compounds. The amidine group of **3a** was replaced by small moieties such as –H, –CN, –Br, $–NH_2$, –OMe, –COOMe, –COOH and $–CONH_2$ (**9a–h**, Table 2). Compounds **9a–g** were found to be inactive but amide **9h** showed uPA inhibition comparable with the reference amidine **3a**. However, replacement of the amidine group of the more potent compounds **3b** and **3c** by an amide group (**9i** and **9k**) did not result in a similar uPA inhibition.

The amide **9h** was chosen as a starting point for further optimization in Series B (**10a–u**, Table 3). Replacement of the phenyl amide of **9h** by a biphenyl amide containing a methyl ester functionality at R_3 increased the activity fourfold (**10c**, IC_{50} = 3.6 μM). The introduction of functional groups $–CONH_2$ and –OMe at R_1 and –COOMe, –OMe, $–NO_2$ and $–CF_3$ at R_3 was more favorable and the best compounds obtained in this series were **10c, 10d**, **10e**, **10l, 10m, 10n** and **10o**. Remarkably, potency of these compounds is comparable with amidines **3b** and **3c**.

Table 4 shows the IC_{50} values of the compounds **11a–p** (Series C). In this series we replaced the amide linker by a sulfonamide linker. Potency of **11e** and **11g** was comparable with their respective amide analogs **10l** and **10o**. The other sulfonamides are less potent.

Selectivity of our compounds was profiled against the highly related enzymes tPA, thrombin and plasmin. Since these enzymes are involved in the blood coagulation cascade and fibrinolysis, an



38 R_1 = H
39 R_1 = OMe

32a R_2 = H, R_3 = COOMe, R_4 = H
32b R_2 = H, R_3 = H, R_4 = COOMe
35b R_2 = H, R_3 = OMe, R_4 = H

35a R_2 = H, R_3 = H, R_4 = H
32a R_2 = H, R_3 = COOMe, R_4 = H
32c R_2 = H, R_3 = OMe, R_4 = H
35d R_2 = H, R_3 = CF_3, R_4 = H
35e R_2 = H, R_3 = F, R_4 = H
35f R_2 = H, R_3 = Cl, R_4 = H
35b R_2 = H, R_3 = OMe, R_4 = H
35c R_2 = H, R_3 = NO_2, R_4 = H

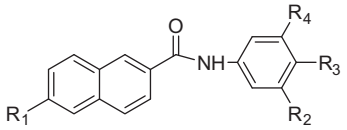
32b R_2 = H, R_3 = H, R_4 = COOMe
35g R_2 = H, R_3 = H, R_4 = OMe
35h R_2 = H, R_3 = OMe, R_4 = OMe
35i R_2 = OMe, R_3 = H, R_4 =OMe

11a R_1 = H, R_2 = H, R_3 = COOMe, R_4 = H
11b R_1 = H, R_2 = H, R_3 = H, R_4 = COOMe
11c R_1 = H, R_2 = H, R_3 = OMe, R_4 = H

11d R_1 = OMe, R_2 = H, R_3 = H, R_4 = H
11e R_1 = OMe, R_2 = H, R_3 = COOMe, R_4 = H
11f R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = H
11g R_1 = OMe, R_2 = H, R_3 = CF_3, R_4 = H
11h R_1 = OMe, R_2 = H, R_3 = F, R_4 = H
11i R_1 = OMe, R_2 = H, R_3 = Cl, R_4 = H
11j R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = H
11k R_1 = OMe, R_2 = H, R_3 = NO_2, R_4 = H
11l R_1 = OMe, R_2 = H, R_3 = NH_2, R_4 = H
11m R_1 = OMe, R_2 = H, R_3 = H, R_4 = COOMe
11n R_1 = OMe, R_2 = H, R_3 = H, R_4 = OMe
11o R_1 = OMe, R_2 = H, R_3 = OMe, R_4 = OMe
11p R_1 = OMe, R_2 = OMe, R_3 = H, R_4 =OMe

Scheme 7. Reagents: (a) Pyridine; (b) H_2, Pd/C, MeOH.

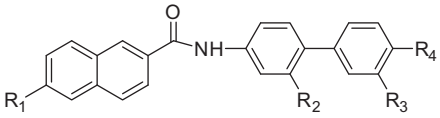
Table 2
uPA inhibitory activity of non-basic compounds **9a–k** and amidines **3a–c**



Compd	R_1	R_2	R_3	R_4	IC_{50}[a] (μM)
9a	CN	H	H	H	>62
9b	H	H	H	H	>62
9c	Br	H	H	H	>62
9d	NH_2	H	H	H	>62
9e	OMe	H	H	H	>62
9f	COOMe	H	H	H	>62
9g	COOH	H	H	H	>62
9h[b]	$CONH_2$	H	H	H	15 ± 1
9i	$CONH_2$	OMe	H	OMe	30 ± 12
9k	$CONH_2$	H	CH_2NH_2	H	45 ± 7
3a[c]	$C(=NH)NH_2$	H	H	H	13 ± 2
3b	$C(=NH)NH_2$	OMe	H	OMe	2.6 ± 0.7
3c	$C(=NH)NH_2$	H	CH_2NH_2	H	1.2 ± 0.2

[a] ±Standard deviation.
[b] Slow binding and non-competitive inhibitor.
[c] Competitive inhibitor (K_i = 1.9 ± 0.2 μM).

Table 3
uPA inhibitory activity of naphthamide biphenyl analogs **10a–u**



Compd	R_1	R_2	R_3	R_4	IC_{50}[a] (μM)
10a	H	H	COOMe	H	16
10b	H	H	OMe	H	9.8 ± 1.2
10c	$CONH_2$	H	COOMe	H	3.6 ± 0.2
10d	$CONH_2$	H	OMe	H	2.9 ± 0.2
10e[b]	$CONH_2$	H	CF_3	H	2.4 ± 0.7
10f	$CONH_2$	H	Cl	H	8.1 ± 1.6
10g	$CONH_2$	OMe	H	OMe	6.4 ± 0.6
10h	COOMe	H	COOMe	H	14 ± 1
10i	CN	H	OMe	H	20 ± 11
10j	CN	H	COOMe	H	9 ± 2
10k	CN	H	COOH	H	>250
10l	OMe	H	COOMe	H	4.0 ± 0.2
10m	OMe	H	OMe	H	4.0 ± 0.2
10n	OMe	H	NO_2	H	2.6 ± 0.1
10o[b]	OMe	H	CF_3	H	2.1 ± 0.6
10p	OMe	H	H	OMe	12 ± 2
10q	OMe	H	OMe	OMe	31
10r	OMe	OMe	H	OMe	>31
10s	Cl	H	COOMe	H	7.0 ± 0.5
10t	Br	H	COOMe	H	11 ± 2
10u	NH_2	H	COOMe	H	31
3b					2.6 ± 0.7
3c					1.2 ± 0.2

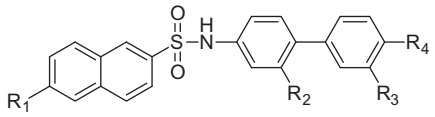
[a] ±Standard deviation.
[b] Slow binding and non-competitive inhibitor.

adequate degree of selectivity is required.[27] The most interesting compounds **10c–e**, **10g**, **10l–o**, **11e** and **11g** were selected for evaluation (Table 5). We found that **11e** showed good uPA selectivity towards both thrombin and plasmin, comparable to the selectivity of **3c**. The selectivity of **11e** towards tPA was only 10-fold, whereas for **3c** this selectivity was more than 50-fold. However, our compound **11e** compares favorably with the clinically relevant compound **2a** which has only 12-fold selectivity of uPA over tPA, and no selectivity towards plasmin and thrombin[28] (K_i μM: uPA = 0.41, tPA = 4.9, plasmin = 0.39 and thrombin = 0.49).

4. Conclusion

This is the first report on uPA inhibitors without a basic moiety. In summary, we designed and synthesized three series of non-basic compounds **9a–k** (Series A), **10a–u** (Series B) and **11a–p** (Series C) and evaluated their uPA inhibition in comparison with reference amidines **3a–c**. Several non-basic compounds show comparable uPA inhibition with reference amidines **3b** and **3c**. The selectivity profile of **11e** is similar with that of the amidine **3c** for thrombin and plasmin, but **11e** is much more selective than the clinically relevant amidine **2a**. The remarkable potency and selectivity of these first non-basic uPA inhibitors promises a good starting point for the future development of compounds with potential to reduce

Table 4
uPA inhibitory activity for naphthalene sulfonamide biphenyl analogs **11a–p**



Compd	R_1	R_2	R_3	R_4	IC_{50}[a] (μM)
11a	H	H	COOMe	H	16
11b	H	H	H	COOMe	9.8 ± 1.2
11c	H	H	OMe	H	31
11d	OMe	H	H	H	31
11e[b]	OMe	H	COOMe	H	2.8 ± 0.1
11f	OMe	H	$CONH_2$	H	>62
11g	OMe	H	CF_3	H	4.1 ± 0.6
11h	OMe	H	F	H	>62
11i	OMe	H	Cl	H	>62
11j	OMe	H	OMe	H	19 ± 4
11k	OMe	H	NO_2	H	6.9 ± 0.3
11l	OMe	H	NH_2	H	62
11m	OMe	H	H	COOMe	16
11n	OMe	H	H	OMe	23 ± 10
11o	OMe	H	OMe	OMe	8
11p	OMe	OMe	H	OMe	8
3b					2.6 ± 0.7
3c					1.2 ± 0.2

[a] ±Standard deviation.
[b] Slow binding and non-competitive inhibitor.

Table 5
uPA selectivity profile of selected compounds and reference compounds **3a–c**

Compd	IC_{50} (μM)			
	uPA	tPA	Thrombin	Plasmin
10c	3.6 ± 0.2	11 ± 1	>125	>125
10d	2.9 ± 0.2	5.7 ± 0.1	>62	12 ± 1
10e	2.4 ± 0.7	3.4 ± 0.2	>62	6.3 ± 0.4
10g	6.4 ± 0.6	9.7 ± 0.5	—	—
10l	4.0 ± 0.2	8.4 ± 0.2	>62	4
10m	4.0 ± 0.2	21 ± 1	>62	20 ± 1
10n	2.6 ± 0.1	8.6 ± 0.6	>62	31
10o	2.1 ± 0.6	5.3 ± 0.3	>62	6.5 ± 0.5
11e	2.8 ± 0.1	26 ± 3	>62	>62
11g	4.1 ± 0.6	13 ± 3	—	—
3a	13 ± 2	125	56 ± 10	22 ± 2
3b	2.6 ± 0.7	>62	>62	13 ± 2
3c	1.2 ± 0.2	>62	>62	>62

[a]±Standard deviation.

invasion and metastasis of malignant cancer types and with improved pharmacokinetic properties.

5. Experimental section

5.1. General

Reagents were obtained from Sigma–Aldrich or Acros. Melting points were determined using Electrothermal melting point apparatus (Thermo Scientific) and are uncorrected. Characterization of all compounds was done with ^{1}H NMR and mass spectrometry. ^{1}H NMR spectra were recorded on a 400 MHz Bruker Avance DRX-400 spectrometer. ES mass spectra were obtained from an Esquire 3000plus iontrap mass spectrometer from Bruker Daltonics. Purity was determined using two diverse HPLC systems using, respectively, a mass and UV-detector. Water (A) and CH_3CN (B) were used as eluents. LC–MS spectra were recorded on an Agilent 1100 Series HPLC system using a Alltech Prevail C18 column (2.1 × 50 mm, 3 μm) coupled with an Esquire 3000plus as MS detector and a 5–100% B, 20 min gradient was used with a flow rate from 0.2 mL/min. Formic acid 0.1% was added to solvents A and B. Reversed phase HPLC was run on a Gilson instrument equipped with an Ultrasphere ODS column (4.6 × 250 mm, 5 μm). A 10–100% B, 35 min gradient was used with a flow rate from 1 mL/min. Trifluoroacetic acid 0.1% was added to solvents A and B. A wavelength of 214 nm was used. The products were purified with flash chromatography on a Flashmaster II (Jones chromatography), with a 30 min gradient of 0–30% EtOAc in hexane or 0–10% MeOH in EtOAc in necessary cases. The synthesis of compounds **13**, **14**, **16**, **17**, **9a**, **3a**, **9j, 37, 39** was already reported.[15,25,18d] Modified procedure was used to make the compounds **3b** and **3c**.[22]

5.1.1. 6-Carbamimidoyl-*N*-phenyl-2-naphthamide (3a)

To a solution of **9a** (0.3 g, 1.1 mmol) in 20 mL of dry THF was added $LiN(TMS)_2$ (1 M solution in THF, 6.6 mL, 6.6 mmol), stirred at room temperature for 72 h, treated with 2 M HCl (20 mL), stirred for another 24 h and basified with saturated Na_2CO_3 and extracted with EtOAc (3 × 75 mL). The extracts were washed with brine, dried (Na_2SO_4), filtered and concentrated. Evaporation of solvent afforded yellow color solid. The crude mixture was chromatographed using EtOAc followed by 10% MeOH in EtOAc to provide 40 mg (13%) as light brown sticky powder, which was lyophilized. ^{1}H NMR (DMSO-d_6) δ 7.16–7.20 (m, 1H), 7.37–7.42 (m, 2H), 7.74 (d, 2H, J = 8.6 Hz), 7.88 (dd, 1H, J = 8.6 Hz), 8.11 (dd, 1H, J = 8.6 Hz), 8.17 (d, 1H, J = 8.6 Hz), 8.22 (d, 1H, J = 8.6 Hz), 8.47 (br s, 1H), 8.58 (s, 1H); HPLC (214 nm) t_r 14.5 min, 100%; LC–MS (214 nm) t_r 11.2 min, 100%; MS (ESI) m/z 290 $(M+H)^+$.

5.1.2. *N*-Phenyl-2-naphthamide (9b)

To a solution of **19** (170 mg, 1 mmol) in 7 mL DMF was added iPr$_2$NEt (0.7 mL, 4 mmol) and TBTU (350 mg, 1.1 mmol), stirred for 15 min, treated with aniline (0.1 mL, 1.1 mmol) and stirred for 24 h. The mixture was poured into water (50 mL), extracted with EtOAc (2 × 75 mL), washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to afford a light brown powder which was washed with methanol to provide 90 mg of **9b** as white powder. Yield 36%, mp180–181 °C; ^{1}H NMR (DMSO-d_6) δ 7.11 (t, 1H, J = 7.4 Hz), 7.37 (t, 2H, J = 7.6 Hz), 7.59–7.66 (m, 2H), 7.81 (d, 2H, J = 7.8 Hz), 7.99–8.09 (m, 4H), 8.57 (s, 1H), 10.39 (s, 1H); HPLC (214 nm) t_r 24.8 min, 100%; LC–MS (214 nm) t_r 16.8 min, 100%; MS (ESI) m/z 248 $(M+H)^+$.

5.1.3. 6-Bromo-*N*-phenyl-2-naphthamide (9c)

Prepared from **20** using the procedure described for the preparation of **9b**, White powder, Yield 67%, mp 213–214 °C; ^{1}H NMR (DMSO-d_6) δ 7.11 (t, 1H, J = 7.4 Hz), 7.36 (t, 2H, J = 7.6 Hz), 7.73 (dd, 1H, J = 8.8 Hz), 7.80 (d, 2H, J = 8.4 Hz), 8.04–8.07 (m, 3H), 8.30 (s, 1H), 8.58 (s, 1H), 10.42 (s, 1H); HPLC (214 nm) t_r 27.8 min, 95%; LC–MS (214 nm) t_r 18.4 min, 100%; MS (ESI) m/z 330 $(M+H)^+$.

5.1.4. 6-Amino-*N*-phenyl-2-naphthamide (9d)

Prepared from **21** using the procedure described for the preparation of **9b**. The crude product was flash chromatographed on silica gel eluting with 50% EtOAc in hexanes to provide **9d**. Brown powder, Yield 46%, mp 196–197 °C; ^{1}H NMR (DMSO-d_6) δ 5.69 (s, 2H), 6.84 (s, 1H), 6.99–7.09 (m, 2H), 7.34 (t, 2H, J = 7.7 Hz), 7.56 (d, 1H, J = 8.7 Hz), 7.72 (d, 1H, J = 8.8 Hz), 7.78–7.83 (m, 3H), 8.3 (s, 1H), 10.13 (s, 1H); HPLC (214 nm) t_r 14.7 min, 100%; LC–MS (214 nm) t_r 14.1 min, 100%; MS (ESI) m/z 263 $(M+H)^+$.

5.1.5. 6-Methoxy-*N*-phenyl-2-naphthamide (9e)

Prepared from **22** using the procedure described for the preparation of **9b**. White powder, Yield 54%, mp 195–196 °C; ^{1}H NMR

(DMSO-d_6) δ 3.9 (s, 3H), 7.09–7.12 (m, 1H), 7.25 (dd, 1H, J = 8.9 Hz), 7.34–7.41 (m, 3H), 7.80 (dd, 2H, J = 8.6 Hz), 7.91–8.00 (m, 3H), 8.50 (s, 1H), 10.30 (s, 1H); HPLC (214 nm) t_r 24.6 min, 94%; LC–MS (214 nm) t_r 17.0 min, 100%; MS (ESI) m/z 278 $(M+H)^+$.

5.1.6. Methyl 6-(phenylcarbamoyl)-2-naphthoate (9f)

Prepared from **13** using the procedure described for the preparation of **9b**. White powder, Yield 38%, mp 222–223 °C; ^{1}H NMR (DMSO-d_6) δ 3.93 (s, 3H), 7.12 (t, 1H, J = 7.4 Hz), 7.38 (t, 2H, J = 8.0, 7.6 Hz), 7.81 (d, 2H, J = 7.8 Hz), 8.05–8.09 (m, 2H), 8.19 (d, 1H, J = 8.6 Hz), 8.27 (d, 1H, J = 8.5 Hz), 8.62 (s, 1H), 8.70 (s, 1H), 10.47 (s, 1H); HPLC (214 nm) t_r 24.8 min, 100%; LC–MS (214 nm) t_r 17.2 min, 100%; MS (ESI) m/z 306 $(M+H)^+$.

5.1.7. 6-(Phenylcarbamoyl)-2-naphthoic acid (9g)

Prepared from **9f** using the procedure described for the preparation of **15**. White powder, Yield 40%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.12 (t, 1H, J = 7.4 Hz), 7.37 (t, 2H, J = 7.6 Hz), 7.81 (d, 2H, J = 7.7 Hz), 8.05–8.11 (m, 2H), 8.17 (d, 1H, J = 8.6 Hz), 8.23 (t, 1H, J = 8.7 Hz), 8.61 (s, 1H), 8.67 (s, 1H), 10.5 (s, 1H), 13.18 (s, 1H); HPLC (214 nm) t_r 20.2 min, 100%; LC–MS (214 nm) t_r 15.1 min, 100%; MS (ESI) m/z 292 $(M+H)^+$.

5.1.8. N^2-Phenylnaphthalene-2,6-dicarboxamide (9h)

Prepared from **15** using the procedure described for the preparation of **9b**. White powder, Yield 24%, mp 296–297 °C; ^{1}H NMR (DMSO-d_6) δ 7.12 (t, 1H, J = 7.4 Hz), 7.35–7.40 (m, 2H), 7.51 (s, 1H), 7.82 (dd, 2H, J = 8.1 Hz), 8.02–8.28 (m, 5H), 8.54–8.64 (m, 2H), 10.46 (s, 1H); HPLC (214 nm) t_r 17.7 min, 97%; LC–MS (214 nm) t_r 14.5 min, 99%; MS (ESI) m/z 291 $(M+H)^+$.

5.1.9. N^2-(3,5-Dimethoxyphenyl)naphthalene-2,6-dicarboxamide (9i)

Prepared by coupling **23** with **15** using the procedure described for the preparation of **9b**. White powder, Yield 11%, mp 280–281 °C; ^{1}H NMR (DMSO-d_6) δ 3.75 (s, 6H), 6.28 (s, 1H), 7.12 (s, 2H), 7.51 (s, 1H), 8.03 (d, 2H, J = 8.6 Hz), 8.11–8.17 (m, 3H), 8.54 (s, 1H), 8.58 (s, 1H), 10.38 (s, 1H); HPLC (214 nm) t_r 18.6 min, 93%; LC–MS (214 nm) t_r 14.8 min, 95%; MS (ESI) m/z 351 $(M+H)^+$.

5.1.10. 6-Cyano-*N*-(3,5-dimethoxyphenyl)-2-naphthamide (9j)

Prepared by coupling **23** with **17** using the procedure described for the preparation of **9b**. White powder, Yield 60%, mp 206–207 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 6H), 6.29 (s, 1H), 7.10–7.13 (m, 2H), 7.88 (dd, 1H, J = 8.5 Hz), 8.11 (dd, 1H, J = 8.6 Hz), 8.19 (d, 1H, J = 8.7 Hz), 8.26 (d, 1H, J = 8.6 Hz), 8.63–8.66 (m, 2H), 10.43 (s, 1H); LC–MS: t_r 17.2 min, 80%; MS (ESI) m/z 333 $(M+H)^+$.

5.1.11. N^2-(4-(Aminomethyl)phenyl)naphthalene-2,6-dicarboxamide trifluoroacetate (9k)

Prepared by deprotection of **25** which was obtained by coupling of **24b** with **15**. White powder, Yield 10%, mp >300 °C; ^{1}H NMR (MeOH-d_4) δ 4.13 (br s, 2H), 7.48 (d, 2H, J = 8.4 Hz), 7.87 (d, 2H, J = 8.4 Hz), 8.02–8.17 (m, 4H), 8.51–8.55 (m, 2H); HPLC (214 nm) t_r 10 min, 88%; LC–MS (214 nm) t_r 9.3 min, 87%; MS (ESI) m/z 318 $(M-H)^-$.

5.1.12. 6-Carbamimidoyl-*N*-(3,5-dimethoxyphenyl)-2-naphthamide (3b)

To a solution of **9j** (100 mg, 0.3 mmol) in 5 mL of 1,2-dimethoxyethane was added iPr$_2$NEt (0.15 mL, 0.03 mmol) and H_2O (0.3 mL) and then diethyl dithiophosphate (0.25 mL, 1.5 mmol) was added and the reaction mixture was refluxed for 24 h. Solvents were evaporated and the crude mixture was washed with water, filtered and dried to get the thioamide **26a**. To the resulting **26a** in acetone (10 mL) was added CH_3I (2 mL) and the mixture stirred for 5 h, after which the precipitated imidate thioester HI salt **26b** was collected by filtration. The salt was dissolved in MeOH (10 mL), to which was added NH_4OAc (0.31 g, 4.0 mmol) and the reaction mixture was stirred overnight. After evaporation of the solvent the crude mixture was chromatographed using EtOAc followed by 5% MeOH in EtOAc to provide 40 mg (42%) of **3b** as light brown sticky powder, which was lyophilized. ^{1}H NMR (MeOH-d_4) δ 2.34 (s, 6H), 4.86 (s, 1H), 5.57 (s, 2H), 6.42 (dd, 1H, J = 8.6 Hz), 6.65–6.68 (m, 2H), 6.73 (d, 1H, J = 8.7 Hz), 6.80 (d, 1H, J = 8.6 Hz), 7.04 (s, 1H), 7.14 (s, 1H); HPLC (214 nm) t_r 15.8 min, 85%; LC–MS (214 nm) t_r 11.7 min, 82%; MS (ESI) m/z 350 $(M+H)^+$.

5.1.13. *N*-(4-(Aminomethyl)phenyl)-6-carbamimidoyl-2-naphthamide trifluoro acetate (3c)

Prepared by deprotection of **27d** which was prepared from **24b** using the procedure described for the preparation of **3b**. Lyophilized light brown powder, Yield 30%; ^{1}H NMR (MeOH-d_4) δ 4.13 (s, 2H), 7.49 (d, 2H, J = 8.6 Hz), 7.85–7.91 (m, 3H), 8.14 (dd, 1H, J = 8.6 Hz), 8.21 (d, 1H, J = 8.7 Hz), 8.26 (d, 1H, J = 8.7 Hz), 8.51 (s, 1H), 8.64 (s, 1H); HPLC (214 nm) t_r 8.1 min, 83%; MS (ESI) m/z 319 $(M+H)^+$.

5.1.14. Methyl 4′-(2-naphthamido)biphenyl-3-carboxylate (10a)

To a solution of **19** (170 mg, 1 mmol) in 7 mL of DMF was added iPr$_2$NEt (0.7 mL, 4 mmol) and TBTU (350 mg, 1.1 mmol), stirred for 15 min, treated with **32a** (0.1 mL, 1.1 mmol) and stirred for 48 h. The mixture was poured into water (50 mL), extracted with EtOAc (2 × 75 mL), washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to afford dark brown solid which was washed with methanol to provide 110 mg of **10a** as white powder, Yield 29%, mp 234–235 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H), 7.60–7.66 (m, 3H), 7.74 (d, 2H, J = 8.6 Hz), 7.93 (d, 1H, J = 7.7 Hz), 7.97 (d, 3H, J = 8.6 Hz), 8.01 (d, 1H, J = 9.1 Hz), 8.03–8.11 (m, 3H), 8.21 (s, 1H), 8.6 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 29.6 min, 100%; LC–MS (214 nm) t_r 19.6 min, 100%; MS (ESI) m/z 382 $(M+H)^+$.

5.1.15. *N*-(3′-Methoxybiphenyl-4-yl)-2-naphthamide (10b)

Prepared from **35b** using the procedure described for the preparation of **10a**. White powder, Yield 71%, mp 206–207 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 6.90 (dd, 1H, J = 8.2 Hz), 7.20 (s, 1H), 7.24 (d, 1H, J = 8.0 Hz), 7.36 (t, 1H, J = 7.9 Hz), 7.61–7.66 (m, 2H), 7.69 (d, 2H, J = 8.7 Hz), 7.92 (d, 2H, J = 8.7 Hz), 8.00–8.10 (m, 4H), 8.59 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 29.8 min, 100%; LC–MS (214 nm) t_r 19.4 min, 100%; MS (ESI) m/z 354 $(M+H)^+$.

5.1.16. Methyl 4′-(6-carbamoyl-2-naphthamido)biphenyl-3-carboxylate (10c)

Prepared by coupling of **32a** with **15** using the procedure described for the preparation of **10a**. White powder, Yield 12%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3H), 7.51 (s, 1H), 7.61 (t, 2H, J = 7.7 Hz), 7.74 (d, 3H, J = 8.5 Hz), 7.92–8.25 (m, 8H), 8.54 (s, 1H), 8.62 (s, 1H), 10.60 (s, 1H); HPLC (214 nm) t_r 23.0 min, 92%; LC–MS (214 nm) t_r 17.4 min, 95%; MS (ESI) m/z 425 $(M+H)^+$.

5.1.17. N^2-(3′-Methoxybiphenyl-4-yl)naphthalene-2,6-dicarboxamide (10d)

Prepared by coupling of **35b** with **15** using the procedure described for the preparation of **10a**. White powder, Yield 10%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 6.90 (dd, 1H, J = 8.1 Hz), 7.20 (s, 1H), 7.25 (d, 1H, J = 8.1 Hz), 7.36 (t, 1H, J = 7.9, 6.1 Hz), 7.50 (s, 1H), 7.71 (dd, 2H, J = 8.6 Hz), 7.91 (dd, 2H, J = 8.6 Hz), 8.02–8.23 (m, 5H), 8.54–8.66 (m, 2H), 10.59 (s, 1H); HPLC (214 nm) t_r 23.3 min, 100%; LC–MS (214 nm) t_r 17.2 min, 100%; MS (ESI) m/z 397 $(M+H)^+$.

5.1.18. N^2-(3′-(Trifluoromethyl)biphenyl-4-yl)naphthalene-2,6-dicarboxamide (10e)

Prepared by coupling of **35d** with **15** using the procedure described for the preparation of **10a**. White powder, Yield 9%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.52 (s, 1H), 7.69 (d, 2H, J = 5.1 Hz), 7.81 (dd, 2H, J = 8.7 Hz), 7.96–8.26 (m, 9H), 8.50–8.68 (m, 2H), 10.61 (s, 1H); HPLC (214 nm) t_r 26.4 min, 93%; LC–MS (214 nm) t_r 19.0 min, 100%; MS (ESI) m/z 435 $(M+H)^+$.

5.1.19. N^2-(3′-Chlorobiphenyl-4-yl)naphthalene-2,6-dicarboxamide (10f)

Prepared by coupling of **35f** with **15** using the procedure described for the preparation of **10a**. Light brown powder, Yield 8%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.39 (d, 1H, J = 8.0 Hz), 7.50 (t, 2H, J = 8.0 Hz), 7.66 (d, 1H, J = 7.8 Hz), 7.73–7.75 (m, 3H), 7.93–8.23 (m, 7H), 8.54–8.67 (m, 2H), 10.60 (s, 1H); HPLC (214 nm) t_r 25.8 min, 95%; LC–MS (214 nm) t_r 18.6 min, 97%; MS (ESI) m/z 399 $(M-H)^-$.

5.1.20. N^2-(2,4′-Dimethoxybiphenyl-4-yl)naphthalene-2,6-dicarboxamide (10g)

Prepared by coupling of **35i** with **15** using the procedure described for the preparation of **10a**. Light brown powder, Yield 7%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 3.77 (s, 6H), 6.95 (d, 2H, J = 8.7 Hz), 7.25 (dd, 1H, J = 8.3 Hz), 7.42 (d, 2H, J = 8.6 Hz), 7.52 (dd, 2H, J = 8.2 Hz), 7.65 (s, 1H), 8.02–8.18 (m, 5H), 8.51–8.65 (m, 2H), 10.54 (s, 1H); HPLC (214 nm) t_r = 23.1 min, 100% and LC–MS (214 nm) t_r 17.2 min, 100%; MS (ESI) m/z 425 $(M-H)^-$.

5.1.21. Methyl 6-(3′-(methoxycarbonyl)biphenyl-4-ylcarbamoyl)-2-naphthoate (10h)

Prepared by coupling of **32a** with **13** using the procedure described for the preparation of **10a**. Light brown powder, Yield 20%, mp 233–234 °C; ^{1}H NMR (DMSO-d_6) δ 3.88 (s, 3H), 3.93 (s, 3H), 7.62 (d, 1H, J = 7.8 Hz), 7.74 (d, 2H, J = 8.6 Hz), 7.92 (d, 1H, J = 7.7 Hz), 7.97 (d, 3H, J = 8.6 Hz), 8.06–8.12 (m, 2H), 8.21 (t, J = 8.2 Hz, 2H), 8.29 (d, 1H, J = 8.6 Hz), 8.6 (s, 1H), 8.7 (s, 1H), 10.6 (s, 1H); HPLC (214 nm) t_r 30.2 min, 98%; LC–MS (214 nm) t_r 20.6 min, 99%; MS (ESI) m/z 440 $(M+H)^+$.

5.1.22. 6-Cyano-*N*-(3′-methoxybiphenyl-4-yl)-2-naphthamide (10i)

Prepared by coupling of **35b** with **17** using the procedure described for the preparation of **10a**. White powder, Yield 41%; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 6.90 (d, 1H, J = 8.1 Hz), 7.20 (s, 1H), 7.24 (d, 1H, J = 7.6 Hz), 7.36 (t, 1H, J = 7.9 Hz), 7.70 (d, 2H, J = 8.5 Hz), 7.90 (t, 3H, J = 8.5 Hz), 8.20 (dd, 2H, J = 8.5, 8.7 Hz), 8.29 (d, 1H, J = 8.5 Hz), 8.68 (s, 2H), 10.6 (s, 1H); HPLC (214 nm) t_r 28.5 min, 96%; LC–MS (214 nm) t_r 19.3 min, 100%; MS (ESI) m/z 379 $(M+H)^+$.

5.1.23. Methyl 4′-(6-cyano-2-naphthamido)biphenyl-3-carboxylate (10j)

Prepared by coupling of **32a** with **17** using the procedure described for the preparation of **10a**. White powder, Yield 44%, mp 228–229 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3H), 7.61 (t, 1H, J = 7.8 Hz), 7.75 (d, 2H, J = 8.6 Hz), 7.88–7.98 (m, 5H), 8.14–8.22 (m, 3H), 8.28 (d, 1H, J = 8.5 Hz), 8.68 (s, 2H), 10.60 (s, 1H); HPLC (214 nm) t_r 28.7 min, 90%; LC–MS (214 nm) t_r 19.4 min, 90%; MS (ESI) m/z 407 $(M+H)^+$.

5.1.24. 4′-(6-Cyano-2-naphthamido)biphenyl-3-carboxylic acid (10k)

Prepared by hydrolysis of **10j** using the procedure described for **15**. White powder, Yield 71%, mp >300 °C; ^{1}H NMR (DMSO-d_6) δ 7.59 (t, 1H, J = 7.8 Hz), 7.74 (d, 2H, J = 8.5 Hz), 7.88–7.96 (m, 5H), 8.19 (dd, 3H, J = 8.4 Hz), 8.29 (d, 1H, J = 8.6 Hz), 8.69 (s, 2H), 10.60 (s, 1H), 13.0 (br s, 1H); HPLC (214 nm) t_r 24.3 min, 96%; LC–MS (214 nm) t_r 17.6 min, 100%; MS (ESI) m/z 393 $(M+H)^+$.

5.1.25. Methyl 4′-(6-methoxy-2-naphthamido)biphenyl-3-carboxylate (10l)

Prepared by coupling of **32a** with **22** using the procedure described for the preparation of **10a**. White powder, Yield 27%, mp 229–230 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3H), 3.91 (s, 3H), 7.26 (dd, 1H, J = 8.9 Hz), 7.42 (s, H), 7.61 (t, H, J = 7.8 Hz), 7.73 (d, 2H, J = 8.7 Hz), 7.92–8.02 (m, 7H), 8.21 (s, 1H), 8.53 (s, 1H), 10.5 (s, H); HPLC (214 nm) t_r 29.7 min, 100%; LC–MS (214 nm) t_r 20.3 min, 100%; MS (ESI) m/z 412 $(M+H)^+$.

5.1.26. 6-Methoxy-*N*-(3′-methoxybiphenyl-4-yl)-2-naphthamide (10m)

Prepared by coupling of **35b** with **22** using the procedure described for the preparation of **10a**. White powder, Yield 24%, mp 223–224 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 3.9 (s, 3H), 6.90 (dd, 1H, J = 8.3 Hz), 7.19–7.28 (m, 3H), 7.36 (t, 1H, J = 8.0 Hz), 7.41 (s, 1H), 7.68 (d, 2H, J = 8.6 Hz), 7.92 (t, 3H, 8.8 Hz), 7.98–8.02 (m, 2H), 8.52 (s, 1H), 10.40 (s, 1H); HPLC (214 nm) t_r 29.6 min, 100%; LC–MS (214 nm) t_r 19.6 min, 100%; MS (ESI) m/z 384 $(M+H)^+$.

5.1.27. 6-Methoxy-*N*-(3′-nitrobiphenyl-4-yl)-2-naphthamide (10n)

Prepared by coupling of **35c** with **22** using the procedure described for the preparation of **10a**. Light brown powder, Yield 30%, mp 253–254 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H), 7.26 (dd, 1H, J = 9.0 Hz), 7.42 (s, 1H), 7.75 (t, 1H, J = 8.0 Hz), 7.82 (d, 2H, J = 8.7 Hz), 7.93–8.02 (m, 5H), 8.17–8.20 (m, 2H), 8.45 (s, 1H), 8.53 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 29.3 min, 100%; LC–MS (214 nm) t_r 19.7 min, 100%; MS (ESI) m/z 399 $(M+H)^+$.

5.1.28. 6-Methoxy-*N*-(3′-(trifluoromethyl)biphenyl-4-yl)-2-naphthamide (10o)

Prepared by coupling of **35d** with **22** using the procedure described for the preparation of **10a**. White powder, Yield 38%, mp 232–233 °C; ^{1}H NMR (DMSO-d_6) δ 3.91 (s, 3H), 7.26 (dd, 1H, J = 9.0 Hz), 7.41 (s, 1H), 7.68 (d, 2H, J = 5.0 Hz), 7.78 (d, 2H, J = 8.7 Hz), 7.93–8.02 (m, 7H), 8.53 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 32.2 min, 100%; LC–MS (214 nm) t_r 20.7 min, 100%; MS (ESI) m/z 422 $(M+H)^+$.

5.1.29. 6-Methoxy-*N*-(4′-methoxybiphenyl-4-yl)-2-naphthamide (10p)

Prepared by coupling of **35g** with **22** using the procedure described for the preparation of **10a**. Brown powder, Yield 11%, mp 280–281 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H), 3.91 (s, 3H), 7.0 (d, 2H, J = 8.6 Hz), 7.25 (dd, 1H, J = 8.9 Hz), 7.41 (s, 1H), 7.61 (dd, 4H, J = 8.6 Hz), 7.87 (d, 2H, J = 8.6 Hz), 7.93 (d, 1H, J = 8.6 Hz), 7.99 (s, 2H), 8.52 (s, 1H), 10.37 (s, 1H); HPLC (214 nm) t_r 29.0 min, 100%; LC–MS (214 nm) t_r 19.6 min, 94%; MS (ESI) m/z 384 $(M+H)^+$.

5.1.30. *N*-(3′,4′-Dimethoxybiphenyl-4-yl)-6-methoxy-2-naphthamide (10q)

Prepared by coupling of **35h** with **22** using the procedure described for the preparation of **10a**. Brown powder, Yield 36%, mp 238–239 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 3H), 3.84 (s, 3H), 3.90 (s, 3H), 7.01 (d, 1H, J = 8.4 Hz), 7.19 (dd, 2H, J = 10.3 Hz), 7.25 (dd, 1H, J = 9.0 Hz), 7.41 (s, 1H), 7.64 (d, 2H, J = 8.6 Hz), 7.86 (d, 2H, J = 8.6 Hz), 7.92 (d, 1H, J = 8.6 Hz), 7.98 (dd, 2H, J = 9.0 Hz), 8.50 (s, 1H), 10.37 (s, 1H); HPLC (214 nm) t_r = 27.5, 95%; LC–MS (214 nm) t_r = 18.3, 93%; MS (ESI) m/z 414 $(M+H)^+$.

5.1.31. *N*-(2,4′-Dimethoxybiphenyl-4-yl)-6-methoxy-2-naphthamide (10r)

Prepared by coupling of **35i** with **22** using the procedure described for the preparation of **10a**. White powder, Yield 58%, mp 224–225 °C; ^{1}H NMR (DMSO-d_6) δ 3.78 (s, 6H), 3.92 (s, 3H), 6.95 (d, 2H, J = 8.3 Hz), 7.24–7.28 (m, 2H), 7.41 (br s, 3H), 7.53 (d, 1H, J = 8.1 Hz), 7.65 (br s, 1H), 7.94 (d, 1H, J = 8.2 Hz), 8.00 (d, 2H, J = 8.9 Hz), 8.52 (s, 1H), 10.39 (s, 1H); HPLC (214 nm) t_r 29.2 min, 100%; LC–MS (214 nm) t_r 19.0 min, 100%; MS (ESI) m/z 414 (M+H)$^+$.

5.1.32. Methyl 4′-(6-chloro-2-naphthamido)biphenyl-3-carboxylate (10s)

Prepared by coupling of **32a** with **37** using the procedure described for the preparation **10a**. Brown powder, Yield 22%, mp 210–211 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3H), 7.59–7.65 (m, 3H), 7.74 (d, 3H, J = 8.5 Hz), 7.91–7.98 (m, 4H), 8.07 (dd, 1H, J = 8.6 Hz), 8.15 (d, 1H, J = 8.7 Hz), 8.20 (s, 1H), 8.62 (s, 1H), 10.60 (s, 1H); HPLC (214 nm) t_r 31.8 min, 95%; LC–MS (214 nm) t_r 21.9 min, 88%; MS (ESI) m/z 416 (M+H)$^+$.

5.1.33. Methyl 4′-(6-bromo-2-naphthamido)biphenyl-3-carboxylate (10t)

Prepared by coupling of **32a** with **20** using the procedure described for the preparation of **10a**. Brown powder, Yield 33%, mp 231–232 °C; ^{1}H NMR (DMSO-d_6) δ 3.89 (s, 3H), 7.61 (t, 1H, J = 7.8 Hz), 7.75 (d, 3H, J = 8.6 Hz), 7.92–7.99 (m, 4H), 8.04–8.09 (m, 3H), 8.21 (s, 1H), 8.30 (s, 1H), 8.61 (s, 1H), 10.61 (s, 1H); HPLC (214 nm) t_r 32.4 min, 100%; LC–MS (214 nm) t_r 21.6 min, 100%; MS (ESI) m/z 462 (M+H)$^+$.

5.1.34. Methyl 4′-(6-amino-2-naphthamido)biphenyl-3-carboxylate (10u)

Prepared by coupling of **32a** with **21** using the procedure described for the preparation of **10a**. Brown powder, Yield 40%, mp 233–234 °C; ^{1}H NMR (DMSO-d_6) δ 3.90 (s, 3H), 5.7 (s, 2H), 6.85 (s, 1H), 7.01 (dd, 1H, J = 8.7 Hz), 7.57–7.64 (m, 2H), 7.73 (dd, 3H, J = 8.8 Hz), 7.83–8.09 (m, 5H), 8.20 (s, 1H), 8.34 (s, 1H), 10.60 (s, 1H); HPLC (214 nm) t_r 20.8 min, 83%; LC–MS (214 nm) t_r 17.7 min, 79%; MS (ESI) m/z 397 (M+H)$^+$.

5.1.35. Methyl 4′-(naphthalene-2-sulfonamido)biphenyl-3-carboxylate (11a)

To a solution of **32a** (230 mg, 1 mmol) in 3 mL pyridine was added **38** (230 mg, 1 mmol) and the reaction mixture was stirred overnight. The mixture was acidified to pH 2 and then extracted with EtOAc (2 × 75 mL), washed with water and brine, dried (Na_2SO_4), filtered, and concentrated to afford brown solid which was washed with methanol to provide 220 mg of **11a**. White powder, Yield 53%, mp 172–173 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 7.24 (d, 2H, J = 8.5 Hz), 7.51 (d, 1H, J = 7.8 Hz), 7.55 (d, 2H, J = 8.5 Hz), 7.62–7.69 (m, 2H), 7.80 (d, 2H, J = 8.4 Hz), 7.86 (d, 1H, J = 7.7 Hz), 7.98 (d, 1H, J = 7.8 Hz), 8.05–8.14 (m, 3H), 8.47 (s, 1H), 10.5 (s, 1H); HPLC (214 nm) t_r 27.8 min, 100%; LC–MS (214 nm) t_r 19.4 min, 100%; MS (ESI) m/z 418 (M+H)$^+$.

5.1.36. Methyl 4′-(naphthalene-2-sulfonamido)biphenyl-4-carboxylate (11b)

Prepared by treatment of **32b** with **38** using the procedure described for the preparation of **11a**. White powder, Yield 34%, mp 189–190 °C; ^{1}H NMR (DMSO-d_6) δ 3.82 (s, 3H), 7.24 (d, 2H, J = 8.6 Hz), 7.59–7.70 (m, 6H), 7.80 (dd, 1H, J = 8.7 Hz), 7.93 (d, 2H, J = 8.4 Hz), 7.98 (d, 1H, J = 7.9 Hz), 8.10 (dd, 2H, J = 8.8, 7.9 Hz), 8.49 (s, 1H), 10.60 (s, 1H); HPLC (214 nm) t_r 27.6 min, 100%; LC–MS (214 nm) t_r 18.4 min, 100%; MS (ESI) m/z 418 (M+H)$^+$.

5.1.37. *N*-(3′-Methoxybiphenyl-4-yl)naphthalene-2-sulfonamide (11c)

Prepared by treatment of **35b** with **38** using the procedure described for the preparation of **11a**. White powder, Yield 49%, mp 101–102 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 6.84 (dd, 1H, J = 8.2 Hz), 7.04 (s, 1H), 7.08 (d, 1H, J = 7.8 Hz), 7.19 (d, 2H, J = 8.5 Hz), 7.27 (t, 1H, J = 7.9 Hz), 7.50 (d, 2H, J = 7.5 Hz), 7.61–7.69 (m, 2H), 7.80 (dd, 1H, J = 8.7 Hz), 7.98 (d, 1H, J = 7.9 Hz), 8.08 (d, 1H, J = 8.8 Hz), 8.12 (d, 1H, J = 8.0 Hz), 8.46 (s, 1H), 10.5 (s, 1H); HPLC (214 nm) t_r 28.2 min, 100%; LC–MS (214 nm) t_r 18.6 min, 100%; MS (ESI) m/z 390 (M+H)$^+$.

5.1.38. *N*-(Biphenyl-4-yl)-6-methoxynaphthalene-2-sulfonamide (11d)

Prepared by treatment of **35a** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 56%, mp 180–181 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 7.19–7.40 (m, 7H), 7.49–7.53 (m, 4H), 7.73 (dd, 1H, J = 8.7 Hz), 7.95 (d, 1H, J = 8.8 Hz), 8.03 (d, 1H, J = 9.0 Hz), 8.38 (s, 1H), 10.45 (s, 1H); HPLC (214 nm) t_r 28.0 min, 100%; LC–MS (214 nm) t_r 18.4 min, 100%; MS (ESI) m/z 390 (M+H)$^+$.

5.1.39. Methyl 4′-(6-methoxynaphthalene-2-sulfonamido)biphenyl-3-carboxylate (11e)

Prepared by treatment of **32a** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 34%, mp 156–157 °C; ^{1}H NMR (DMSO-d_6) δ 3.84 (s, 3H), 3.87 (s, 3H), 7.22 (d, 2H, J = 8.5 Hz), 7.25 (dd, 1H, J = 9.0, 9.1 Hz), 7.38 (s, 1H), 7.53 (t, 3H, J = 8.7 Hz), 7.74 (dd, 1H, J = 8.7 Hz), 7.81 (d, 1H, J = 7.9 Hz), 7.86 (d, 1H, J = 7.7 Hz), 7.94 (d, 1H, J = 8.8 Hz), 8.01–8.05 (m, 2H), 8.38 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 27.6 min, 100%; LC–MS (214 nm) t_r 18.6 min, 100%; MS (ESI) m/z 448 (M+H)$^+$.

5.1.40. 4′-(6-Methoxynaphthalene-2-sulfonamido)biphenyl-3-carboxamide (11f)

Prepared by treatment of **32c** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 28%, mp 229–230 °C; ^{1}H NMR (DMSO-d_6) δ 3.86 (s, 3H), 7.20 (d, 2H, J = 8.6 Hz), 7.26 (dd, 1H, J = 9.0 Hz), 7.39 (s, 1H), 7.44 (t, 1H, J = 7.7 Hz), 7.56 (d, 2H, J = 8.6 Hz), 7.67 (d, 1H, J = 7.8 Hz), 7.78 (t, 1H, J = 7.8 Hz), 7.95 (d, 1H, J = 8.8 Hz), 8.02 (t, 3H, J = 9.0 Hz), 8.39 (s, 1H), 10.48 (s, 1H); HPLC (214 nm) t_r 21.2 min, 93%; LC–MS (214 nm) t_r 16.0 min, 93%; MS (ESI) m/z 433 (M+H)$^+$.

5.1.41. 6-Methoxy-*N*-(3′-(trifluoromethyl)biphenyl-4-yl)naphthalene-2-sulfonamide (11g)

Prepared by treatment of **35d** with **39** using the procedure described for the preparation of **11a**. Brown powder, Yield 53%, mp 162–163 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 7.22 (d, J = 8.7 Hz, 2H), 7.26 (dd, 1H, J = 9.0 Hz), 7.39 (s, 1H), 7.58–7.64 (m, 4H), 7.74 (dd, 1H, J = 8.7 Hz), 7.82–7.86 (m, 2H), 7.95 (d, 1H, J = 8.8 Hz), 8.03 (d, 1H, J = 9.0 Hz), 8.40 (s, 1H), 10.54 (s, 1H); HPLC (214 nm) t_r 30.0 min, 96%; LC–MS (214 nm) t_r 19.8 min, 92%; MS (ESI) m/z 458 (M+H)$^+$.

5.1.42. *N*-(3′-Fluorobiphenyl-4-yl)-6-methoxynaphthalene-2-sulfonamide (11h)

Prepared by treatment of **35e** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 30%, mp 161–162 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 7.10 (t, 1H, J = 7.2 Hz), 7.20 (d, 2H, J = 8.6 Hz), 7.26 (d, 1H, J = 9.0 Hz), 7.35–7.41 (m, 4H), 7.54 (d, 2H, J = 8.6 Hz), 7.74 (dd, 1H, J = 8.7 Hz), 7.94 (d, 1H, J = 8.7 Hz), 8.02 (d, 1H, J = 9.0 Hz), 8.38 (s, 1H), 10.47 (s, 1H); HPLC (214 nm) t_r 28.4 min, 95%; LC–MS (214 nm) t_r 18.9 min, 96%; MS (ESI) m/z 406 (M−H)$^-$.

5.1.43. *N*-(3′-Chlorobiphenyl-4-yl)-6-methoxynaphthalene-2-sulfonamide (11i)

Prepared by treatment of **35f** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 24%, mp 141–142 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 7.20 (d, 2H, J = 8.6 Hz), 7.25 (dd, 1H, J = 9.0 Hz), 7.32 (d, 1H, J = 7.9 Hz), 7.38 (t, 2H, J = 7.9 Hz), 7.48–7.57 (m, 4H), 7.74 (dd, 1H, J = 8.7 Hz), 7.94 (d, 1H, J = 8.7 Hz), 8.02 (d, 1H, J = 9.0 Hz), 8.38 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 31.2 min, 96%; LC–MS (214 nm) t_r 19.7 min, 94%; MS (ESI) m/z 422 $(M-H)^-$.

5.1.44. 6-Methoxy-*N*-(3′-methoxybiphenyl-4-yl)naphthalene-2-sulfonamide (11j)

Prepared by treatment of **35b** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 60%, mp 77–78 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 3.87 (s, 3H), 6.84 (dd, 1H, J = 8.1 Hz), 7.04 (s, 1H), 7.08 (d, 1H, J = 7.8 Hz), 7.17 (d, 2H, J = 8.6 Hz), 7.26–7.30 (m, 2H), 7.39 (s, 1H), 7.50 (d, 2H, J = 8.6 Hz), 7.73 (dd, 1H, J = 8.8 Hz), 7.94 (d, 1H, J = 8.7 Hz), 8.03 (d, 1H, J = 9.1 Hz), 8.38 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 27.7 min, 100%; LC–MS (214 nm) t_r 18.4 min, 100%; MS (ESI) m/z 420 $(M+H)^+$.

5.1.45. 6-Methoxy-*N*-(3′-nitrobiphenyl-4-yl)naphthalene-2-sulfonamide (11k)

Prepared by treatment of **35c** with **39** using the procedure described for the preparation of **11a**. Light brown powder, Yield 51%, mp 190–191 °C; ^{1}H NMR (DMSO-d_6) δ 3.87 (s, 3H), 7.26–7.29 (m, 3H), 7.39 (s, 1H), 7.62–7.69 (m, 3H), 7.75 (dd, 1H, J = 8.7 Hz), 7.95 (d, 1H, J = 8.8 Hz), 8.00–8.14 (m, 3H), 8.30 (s, 1H), 8.40 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 27.5 min, 100%; LC–MS (214 nm) t_r 18.6 min, 95%; MS (ESI) m/z 435 $(M+H)^+$.

5.1.46. *N*-(3′-Aminobiphenyl-4-yl)-6-methoxynaphthalene-2-sulfonamide (11l)

Prepared by reduction of **11k** using the procedure described for the preparation of **32a**. Brown powder, Yield 69%, mp 97–98 °C; ^{1}H NMR (DMSO-d_6) 3.87 (s, 3H), 7.17–7.28 (m, 5H), 7.39–7.48 (m, 5H), 7.74 (dd, 1H, J = 8.7 Hz), 7.94 (d, 1H, J = 8.7 Hz), 8.02 (d, 1H, J = 9.0 Hz), 8.39 (s, 1H), 10.50 (s, 1H); HPLC (214 nm) t_r 18.1 min, 100%; LC–MS (214 nm) t_r 15.0 min, 95%; MS (ESI) m/z 405 $(M+H)^+$.

5.1.47. Methyl 4′-(6-methoxynaphthalene-2-sulfonamido)biphenyl-4-carboxylate (11m)

Prepared by treatment of **32b** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 31%, mp 225–226 °C; ^{1}H NMR (DMSO-d_6) δ 3.83 (s, 3H), 3.86 (s, 3H), 7.22 (d, 2H, J = 8.9 Hz), 7.26 (dd, 1H, J = 9.0 Hz), 7.39 (s, 1H), 7.59 (d, 2H, J = 8.7 Hz), 7.68 (d, 2H, J = 8.4 Hz), 7.74 (dd, 1H, J = 8.7 Hz), 7.95 (dd, 3H, J = 8.8, 8.4 Hz), 8.03 (d, 1H, J = 9.1 Hz), 8.40 (s, 1H), 10.56 (s, 1H); HPLC (214 nm) t_r 27.4 min, 100%; LC–MS (214 nm) t_r 18.6 min, 100%; MS (ESI) m/z 448 $(M+H)^+$.

5.1.48. 6-Methoxy-*N*-(4′-methoxybiphenyl-4-yl)naphthalene-2-sulfonamide (11n)

Prepared by treatment of **35g** with **39** using the procedure described for the preparation of **11a**. White powder, Yield 67%, mp 213–214 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 3.88 (s, 3H), 6.92 (d, 2H, J = 8.7 Hz), 7.15 (d, 2H, J = 8.6 Hz), 7.25 (dd, 1H, J = 9.0 Hz), 7.39 (s, 1H), 7.44 (t, 4H, J = 9.0 Hz), 7.73 (dd, 1H, J = 8.7 Hz), 7.94 (d, 1H, J = 8.7 Hz), 8.01 (d, 1H, J = 9.0 Hz), 8.36 (s, 1H), 10.32 (s, 1H); HPLC (214 nm) t_r 27.4 min, 100%; LC–MS (214 nm) t_r 18.6 min, 100%; MS (ESI) m/z 420 $(M+H)^+$.

5.1.49. *N*-(3′,4′-Dimethoxybiphenyl-4-yl)-6-methoxynaphthalene-2-sulfonamide (11o)

Prepared by treatment of **35h** with **39** using the procedure described for the preparation of **11a**. Brown powder, Yield 42%, mp 152–153 °C; ^{1}H NMR (DMSO-d_6) δ 3.74 (s, 3H), 3.76 (s, 3H), 3.88 (s, 3H), 6.93 (d, J = 8.3 Hz, 1H), 7.03–7.08 (m, 2H), 7.15 (d, 2H, J = 8.5 Hz), 7.25 (dd, 1H, J = 9.0 Hz), 7.39 (s, 1H), 7.46 (d, 2H, J = 8.5 Hz), 7.73 (dd, 1H, J = 8.7 Hz), 7.94 (d, 1H, J = 8.8 Hz), 8.02 (d, 1H, J = 9.0 Hz), 8.36 (s, 1H), 10.32 (s, 1H); HPLC (214 nm) t_r 25.6 min, 97%; LC–MS (214 nm) t_r 17.8 min, 98%; MS (ESI) m/z 450 $(M+H)^+$.

5.1.50. *N*-(2,4′-Dimethoxybiphenyl-4-yl)-6-methoxynaphthalene-2-sulfonamide (11p)

Prepared by treatment of **35i** with **39** using the procedure described for the preparation of **11a**. Brown powder, Yield 33%, mp 87–88 °C; ^{1}H NMR (DMSO-d_6) δ 3.62 (s, 3H), 3.72 (s, 3H), 3.88 (s, 3H), 6.72 (dd, 1H, J = 8.1 Hz), 6.84–6.88 (m, 2H), 7.05 (d, 1H, J = 8.2 Hz), 7.25–7.29 (m, 3H), 7.39 (s, 1H), 7.70–7.78 (m, 2H), 7.96 (d, 1H, J = 8.7 Hz), 8.04 (d, 1H, J = 9.0 Hz), 8.40 (s, 1H), 10.36 (s, 1H); HPLC (214 nm) t_r 27.4 min, 95%; LC–MS (214 nm) t_r 18.4 min, 88%; MS (ESI) m/z 450 $(M+H)^+$.

6. uPA Inhibition

6.1. In vitro evaluation

Enzymatic activity was measured at 37 °C in a Spectramax 340 (Molecular Devices) microtiter plate reader using the chromogenic substrate S-2444 (*pyro*Glu-Gly-Arg-*p*-NA.HCl), with a K_m of 80 μM. The substrate was obtained from Nodia. The human enzyme was obtained from Sigma–Aldrich. The reaction was monitored at 405 nm, and the initial rate was determined between 0 and 0.25 absorbance units in 10 min. The reaction mixture contained 200 μM of substrate and approximately 1 mU of enzyme in 145 μL of buffer in a final volume of 200 μL. A 50 mM Tris buffer, pH 8.8, was used. From each inhibitor concentration, 5 μL was added, obtaining a final concentration from 0 to 250 μM in a total volume of 0.2 mL. Activity measurements were routinely performed in duplicate. The IC_{50} value is defined as the concentration of inhibitor required to reduce the enzyme activity to 50% after a 15-min preincubation with the enzyme at 37 °C before addition of the substrate. IC_{50} values were obtained by fitting the data with the four-parameter logistics equation using Grafit 5.

$$v = (v \text{ range})/(1 + \exp(s \times \ln(\text{abs}(I_0/\text{IC}_{50})))) + \text{background}$$

where s = slope factor, v = rate, I_0 = inhibitor concentration, and range = the fitted uninhibited value minus the background. The equation assumes the y falls with increasing x.

Inhibitor stock solutions were prepared in DMSO and stored at −20 °C.

6.2. Determination of the selectivity for uPA

The IC_{50} values for plasmin (from human plasma, Sigma), tPA (recombinant, Boehringer Ingelheim) and thrombin (from human plasma, Sigma) were determined in the same way as for uPA. S-2288 (H-D-Ile-Pro-Arg-*p*NA.2HCl) for tPA (K_m: 1 mM), S-2366 (*pyro*Glu-Pro-Arg-*p*-NA.HCl) for plasmin (K_m: 400 μM) and thrombin (K_m: 150 μM) were used as substrates. The mixture contained 580 μM substrate for thrombin and plasmin, 1.25 mM for tPA and approximately 5 mU of enzyme and 145 μL of buffer. For tPA and thrombin, Tris buffer, pH 8.3, was used and for plasmin, Tris buffer, pH 7.4, was used.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2011.12.040.

References and notes

1. Duffy, M. J. *Curr. Pharm. Des.* **2004**, *10*, 39.
2. Ilies, M. A.; Scozzafava, A.; Supuran, C. T. *Expert Opin. Ther. Patents* **2002**, *12*, 1181.
3. (a) Saksela, O. *Biochim. Biophys. Acta* **1985**, *823*, 35; (b) Testa, J. E.; Qiugley, J. P. *Cancer Metastasis Rev.* **1990**, *9*, 353.
4. Mignatti, P.; Robbins, E.; Rifkin, D. B. *Cell* **1986**, *47*, 487.
5. Jan, W. A. S.; Van der Pluijm, G.; Hans, A. R.; Lowik, C. W. G. M.; Hans, M.; Bernard, M. G. *Thyroid* **1999**, *9*, 913.
6. Ge, Y.; Elghetany, T. *Lab. Hematol.* **2003**, *9*, 67.
7. Ulisse, S.; Baldini, E.; Sorrenti, S.; D'Armiento, M. *Curr. Cancer Drug Targets* **2009**, *9*, 32.
8. (a) Rabbani, S. A.; Xing, R. H. *J. Int. Oncol.* **1998**, *12*, 911; (b) Andersen, P. A.; Kjoller, L.; Christensen, L.; Duffy, M. J. *Int. J. Cancer* **1997**, *72*, 1; (c) Achbarou, A.; Kaiser, S.; Tremblay, G.; Sainte Marie, L.; Brodt, P.; Goltzman, D.; Rabbani, S. A. *Cancer Res.* **1994**, *54*, 2372; (d) Duffy, M. J. *Biochem. Soc. Trans.* **2002**, *30*, 207.
9. (a) Pappot, H.; Brunner, N. *Lung Cancer* **1995**, *12*, 1; (b) Schmitt, M.; Wilhelm, O. G.; Reuning, U.; Kruger, A.; Harbeck, N.; Lengyel, E.; Graeff, H.; Gansbacher, B.; Kessler, H.; Burgle, M.; Sturzebecher, J.; Sperl, S.; Magdolen, V. *Fibrinol. Proteol.* **2000**, *14*, 114; (c) Tsatas, D.; Kaye, A. H. *J. Clin. Neurosci.* **2003**, *10*, 139.
10. Rockway, T. W.; Nienaber, V.; Giranda, V. L. *Curr. Pharm. Des.* **2002**, *8*, 2541.
11. Nienaber, V. L.; Wang, J.; Davidson, D.; Henkin, J. *J. Biol. Chem.* **2000**, *10*, 7239.
12. Schweinitz, A.; Steinmetzer, T.; Banke, I. J.; Arlt, M. J. E.; Sturzebecher, A.; Schuster, O.; Geissler, A.; Giersiefen, H.; Zeslawska, E.; Jacob, U.; Kruger, A.; Sturzebecher, J. *J. Biol. Chem.* **2004**, *279*, 33613.
13. Setyono-Han, B.; Sturzebecher, J.; Schmalix, W. A.; Muehlenweg, B.; Sieuwerts, A. M.; Timmermans, M.; Magdolen, V.; Schmitt, M.; Klijn, J. G. M.; Foekens, J. A. *Thromb. Haemost.* **2005**, *93*, 779.
14. (a) Schmitt, M.; Harbeck, N.; Brünner, N.; Jänicke, F.; Meisner, C.; Mühlenweg, B.; Jansen, H.; Dorn, J.; Nitz, U.; Kantelhardt, E. J.; Thomssen, C. *Expert Rev. Mol. Diagn.* **2011**, *11*, 617; (b) Goldstein, L. J.; Stemmer, S. M.; Schmalfeldt, B.; Gottschalk, N.; Cardoso, F.; Dushkin, H.; Mala, C.; Uebler, N.; Bevan, P.; Harbeck, N. ASCO Annual Meeting, Abstract No: TPS131, 2010.; (c) Heinemann, V.; Ebert, M. P.; Pinter, T.; Bevan, P.; Neville, N. G.; Mala, C. ASCO Annual Meeting, Abstract No: 4060, 2010.
15. Wendt, M. D.; Rockway, T. W.; Geyer, A.; McClellan, W.; Weitzberg, M.; Zhao, X.; Mantei, R.; Nienaber, V. L.; Stewart, K.; Klinghofer, V.; Giranda, V. L. *J. Med. Chem.* **2004**, *47*, 303.
16. Joossens, J.; Ali, O. M.; Sayed, I. E.; Surpateanu, G.; Van der Veken, P.; Lambeir, A. M.; Han, B. S.; Foekens, J. A.; Schneider, A.; Schmalix, W.; Haemers, A.; Augustyns, K. *J. Med. Chem.* **2007**, *50*, 6638.
17. Zhu, M.; Gokhale, V. M.; Szabo, L.; Munoz, R. M.; Baek, H.; Bashyam, S.; Hurley, L. H.; Von Hoff, D. D.; Han, H. *Mol. Cancer Ther.* **2007**, *6*, 1348.
18. (a) Renatus, M.; Bode, W.; Huber, R.; Sturzebecher, J.; Stubbs, M. *J. Med. Chem.* **1998**, *41*, 5445; (b) Mackman, R.; Katz, B.; Breitenbucher, J.; Hui, H.; Verner, E.; Luong, C.; Liu, L.; Sprengeler, P. *J. Med. Chem.* **2001**, *44*, 3856; (c) Sperl, S.; Jacob, U.; Arroyo de Prada, N.; Sturzebecher, J.; Wilhelm, O.; Bode, W.; Magdolen, V.; Huber, R.; Moroder, L. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 5113; (d) Wendt, M. D.; Geyer, A.; McClellan, W. J.; Rockway, T. W.; Weitzberg, M.; Zhao, X.; Stewart, K. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 3063; (e) Fish, P. V.; Barber, C. G.; Brown, D. G.; Butt, R.; Collis, M. G.; Dickinson, R. P.; Henry, B. T.; Horne, V. A.; Huggins, J. P.; King, E.; Gara, M.; McCleverty, D.; McIntosh, F.; Phillips, C.; Webster, R. *J. Med. Chem.* **2007**, *50*, 2341.
19. (a) Frederickson, M.; Callaghan, O.; Chessari, G.; Congreve, M.; Cowan, S. R.; Matthews, J. E.; McMenamin, R.; Smith, D. M.; Vinković, M.; Wallis, N. G. *J. Med. Chem.* **2008**, *51*, 183; (b) West, C. W.; Adler, M.; Arnaiz, D.; Chen, D.; Chu, K.; Gualtieri, G.; Ho, E.; Huwea, C.; Light, D.; Phillips, G.; Pulk, R.; Sukovich, D.; Whitlow, M.; Yuan, S.; Bryant, J. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5712.
20. (a) Roehrig, S.; Straub, A.; Pohlmann, J.; Lampe, T.; Pernerstorfer, J.; Schlemmer, K. H.; Reinemer, P.; Perzborn, E. *J. Med. Chem.* **2005**, *48*, 5900; (b) Mathias, B.; Christian, T.; Joachim, R.; Dirk, G. WO2005068456, 2005.; (c) Paris, J. A.; Christopher, R. E. *Pharmacotherapy* **2009**, *29*, 167.
21. Boere, R. T.; Oakley, R. T.; Reed, R. W. *J. Organomet. Chem.* **1987**, *331*, 161.
22. Cui, J.; Crich, D.; Wink, D.; Lam, M.; Rheingold, A. L.; Case, D. A.; Fu, W.; Zhou, Y.; Rao, M.; Olson, A. J.; Johnson, M. E. *Bioorg. Med. Chem.* **2003**, *11*, 3379.
23. Geoffrey, W.; Wilson, H. P. WO2005085177, 2005.
24. Marco, M.; Silvia, R.; Alessio, L.; Pier Vincenzo, P.; Giorgio, T.; Andrea, D.; Andrea, T.; Giovanni, P.; Satish, K.; Daniele, P. *J. Med. Chem.* **2004**, *47*, 4998.
25. Capkova, K.; Yoneda, Y.; Dickerson, T. J.; Janda, K. D. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6463.
26. Loewenthal, H. J. E.; Gottlieb, L. *J. Org. Chem.* **1992**, *57*, 2631.
27. Rang, H. P.; Dale, M. M.; Ritter, J. M.; Moore, P. K. Haemostasis and Thrombosis. In *Pharmacology*, 5th ed.; Elsevier: New York, 2003; p 314.
28. Wilhelm, O.; Magdolen, V.; Sturzebecher, J.; Foekens, J.; Lutz, V. WO2000004954, 2000.