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Inhibition of the Tissue Factor/Factor VIIa Complex - Lead Optimisation Using Combinatorial Chemistry

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Abstract:
Following a high throughput screen (HTS) for the inhibition of the tissue factor/factor VIIa complex and the identification of a number of original hits a lead optimisation programme was initiated to improve their potency. This necessitated the development of an amidine based linker which allowed the generation of a library of amidinonaphthols prepared both by multiple parallel synthesis (MPS) and split and mix methods. The most active compound had an IC_{50} of 4μM some 10x more potent than the original lead compound.

Keywords: amidines; combinatorial; solid phase; library

Introduction

Factor VII is a circulating plasma serine protease zymogen which plays a pivotal role in the initiation of blood coagulation [1]. Damage to the normal integrity of the vascular system or activation of monocytes or endothelial cells results in exposure of tissue factor (TF) to the blood. Factor VII is activated to factor VIIa principally by factor Xa [2] and binds to tissue factor which acts as a co-factor, dramatically amplifying its proteolytic activation of factor IX and X [3]. The net result of this activation of the coagulation cascade is the production of thrombin which serves both to activate platelets and to form insoluble fibrin from soluble fibrinogen. Both of these are important events in the process of hemostasis and its pathological counterpart, thrombosis. This critical position within the coagulation cascade combined with predicted benefits with respect to tolerability [4] makes the tissue factor/factor VIIa complex an attractive target for antithrombic drug discovery. Despite the attractions of this avenue of research, little has been published concerning low molecular weight inhibitors.

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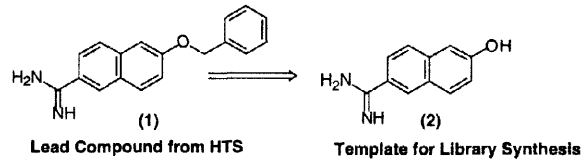
From random screening (>100,000 compounds) the amidinonaphthyl compound (**1**) was identified as a weak inhibitor of tissue factor/factor VIIa (IC_{50} 60μM).



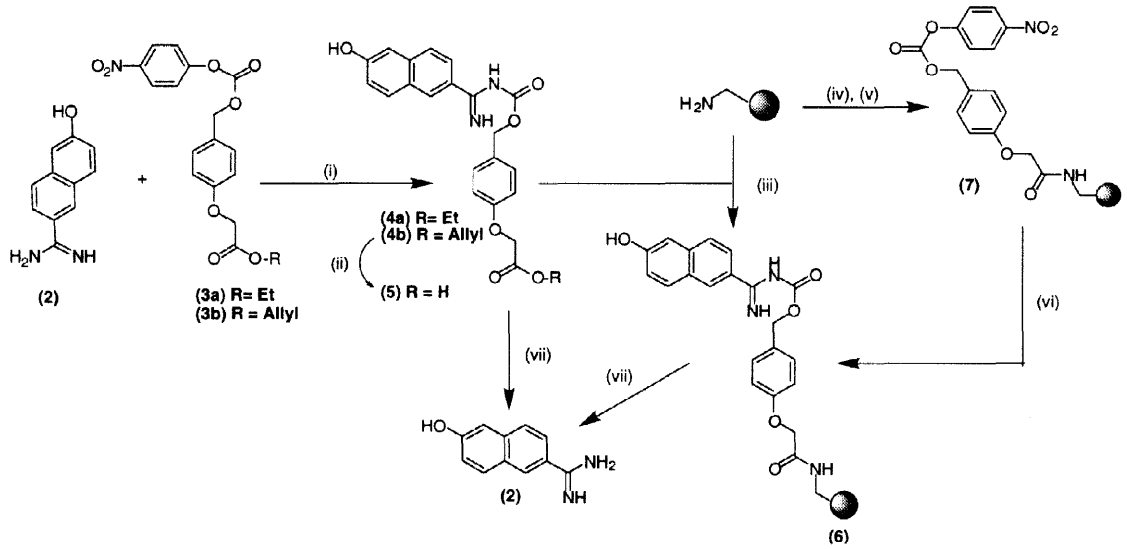
A number of amidinonaphthol compounds have previously been shown to act as serine protease inhibitors [5] the more active derivatives tending to be esters [6] which acylate the active site serine residue. However, a recent study by workers from Daiichi led to stable, potent and specific amidinonaphthyl containing compounds as a new class of factor Xa inhibitors [7] with claimed oral activity. In the present study, we developed a novel solid phase method using the amidinonapthol (**2**) as the template for a solid phase synthesis campaign, arising from the hit (**1**). Although an X-ray structure of the protease was not available at the time the structures of related proteins were used to direct synthetic activity and this allowed the rapid construction of a library of aromatic amidinonaphthyl ethers which would fit within the active site of the protease. Screening for tissue factor/VIIa inhibitory activity led to the discovery of two inhibitors with low μM activity, an approximately 10 fold increase in potency over the original hit (**1**).

Results and Discussion:

Based on our experiences in the polyamine area [8], the known protection of amidines with chloroformates and following our previously published work on solid phase amidine synthesis [9], we decided to immobilise the amidinonaphthol (**2**) using the Wang type carbonates (**3**) and (**7**) [8,10]. This was initially optimised in solution to give (**4a**) and proceeded in good to excellent yield with no evidence of trans-carbonification occurring (scheme 1). The resulting carbamate was observed to be stable for prolonged periods without decomposition and the amidine was cleanly cleaved from the linker using a mixture of DCM/TFA/H_2O (45/50/5).

Following these solution trials, the amidinonapthol was coupled *via* the linker to the resin using two methods (scheme 1): (i) pre-forming the amidine-linker (**4b**) followed by allyl ester removal [11] to give (**5**), then coupling to the resin to give (**6**) or (ii) pre-forming the resin based "Wang carbonate" (**7**) and subsequent solid phase amidine coupling. Both methods were carried out on relatively large scales (10g of resin) and both methods proceeded efficiently. Method one was hampered by the slow but noticeable decomposition of the free acid (**5**) which necessitated allyl deprotection and immediate coupling to aminomethyl resin, while the reaction time for method two was much longer and required an exhaustive washing process for the complete removal of nitrophenol. Both methods resulted in good to excellent loading of the amidine onto the solid support (HPLC quantification) but the pre-formed amidine linker method gave better results. It should be noted in passing, that in our hands, attachment of the "Wang" type linker *via* an amide bond (using 4-

hydroxymethyl phenoxyacetic acid) rather than the benzylic ether led to fewer side products. We attribute this phenomenon to the relative acid stabilities of the amide and benzylic ether moieties. Amidine solid phase chemistry commenced with the efficient Mitsunobu reaction [12], initially using 5eq of 43 commercially available benzylic type alcohols (**7i-xliii**, see experimental), PBu_3 and TMAD (tetramethylazodicarboxamide) (Scheme 2).

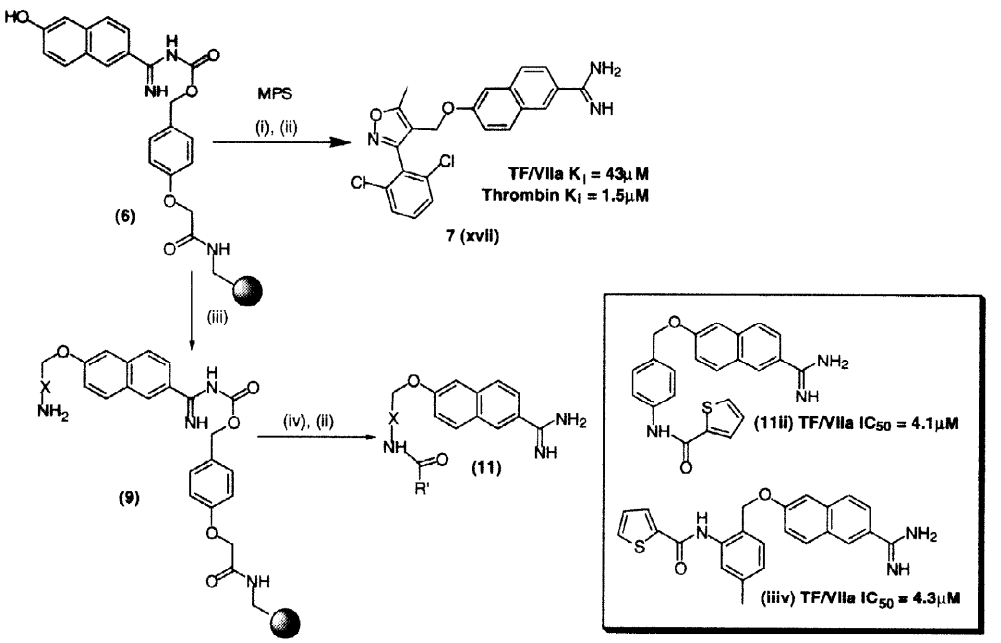


Scheme 1. (i) (**2**), K_2CO_3, dioxane (42%); (ii) R = Allyl, $Pd(PPh_3)_4$, dimedone, DCM (53%); (iii) DIC, HOBt, DCM (quant); (iv) $HOCH_2C_6H_4pOCH_2CO_2H$, DIC, HOBt, DCM (quant); (v) $ClCOOPhpNO_2$, pyridine, DCM; (vi) (**2**), K_2CO_3, dioxane, DMF; (vii) TFA/DCM/H_2O (quant).

The 43 single compounds prepared directly from the Mitsunobu couplings were analysed by RP-HPLC and ESMS. They all gave the expected product by ESMS and had an average HPLC purity of 60%. Biological testing revealed no improvement over that observed for the lead structure (**1**) except for one case, the product from the coupling of alcohol (**7xvii**), which gave a slightly improved binding for TF/VIIa (IC_{50} 43μM) over the lead compound but displayed preferential binding against thrombin (IC_{50} 1.5μM). In the absence of any real improvement in binding affinity from the original hit, small compound mixtures were prepared from (**6**) in a two step process (a): Mitsunobu reaction with a variety of aminoalcohols followed by (b): acylation with a range of carboxylic acids (Scheme 2).

11 aminoalcohols (**8i-xi**) were *O*-alkylated with the amidinonapthol template (**6**) using a double Mitsunobu coupling to give (**9i-xi**). Most of the reactions proceeded to greater than 80% completion as judged by HPLC and MS analysis. However the three couplings using the monomers pyridoxime, (2-aminophenyl)methanol and 3-indolemethanol failed or proceeded poorly [13], and these resin samples were discarded, leaving eight compounds in the final mixed resin sample. Figure 1 shows the HPLC-MS data for the material cleaved following resin mixing, with all the desired compounds clearly present, in approximately equimolar ratios and as the major components. The library of acylation products was generated using 18 acids (**10i-xviii**). Coupling was carried out until no blue coloration was observed by the ninhydrin test [14] using 1-2 mg samples of resin. Sub-libraries were analysed by ES MS and

in one case by HPLC MS which showed that all the expected compounds were present as the major components.



Scheme 2. (i) ROH, Bu_3P, TMAD, THF, DCM; (ii) TFA/DCM/H_2O; (iii) x= aminoalcohol (**8i-xi**), Bu_3P, TMAD, NEt_3, THF, DCM, (iv) RCO_2H (**10i-xviii**), DIC, HOBt, DCM.

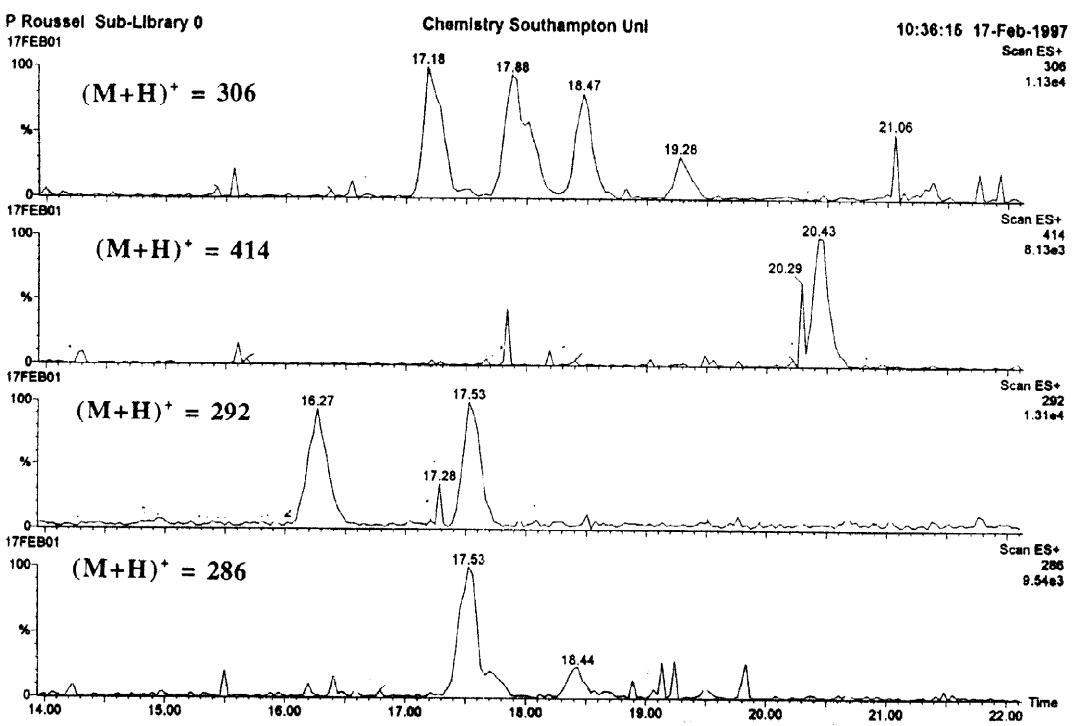


Figure 1. HPLC MS data for the mixture of eight amidinonaphtholethers.

The 18 sub-libraries were screened in the TF/VIIa assay at an initial concentration of 50μM per component (900μM total) and gave the results shown below:

Sub-Library	*Capping Acid Group*	*% Inhibition*
(i)	**4-chlorobenzoic acid**	**91**
(ii)	2-phenoxyacetic acid	27
(iii)	4-sulfamoylbenzoic acid	55
(iv)	2,2-dichloro-1-methylcyclopropane carboxylic acid	57
(v)	2-naphthoxyacetic acid	13
(vi)	2-thiopheneacetic acid	40
(vii)	**benzoic acid**	**93**
(viii)	**2-thiophenecarboxylic acid**	**98**
(ix)	5-chloro-2-nitrobenzoic acid	41
(x)	2,6-dimethoxybenzoic acid	56
(xi)	**3,4-methylene-dioxybenzoic acid**	**93**
(xii)	1-piperidinopropionic acid	21
(xiii)	2,4-dichloro-5-sulfamoylbenzoic acid	-16
(xiv)	6-chloronicotinic acid	54
(xv)	4-chloro-3-sulfamyl benzoic acid	-11
(xvi)	**5-bromofuroic acid**	**98**
(xvii)	5-bromovaleric acid	31
(xviii)	3,5-dihydroxy-2-naphthoic acid	22

Five sub-libraries (i,vii,viii,xi and xvi) were more fully analysed to give the following IC_{50} values for the compounds terminating in the following acid functionalities: (i) 4-chlorobenzoic acid (1.8μM); (vii) benzoic acid (2.9μM); (viii) 2-thiophenecarboxylic acid (0.13μM); (xi) 3,4-methylenedioxybenzoic acid (1.3μM); (xvi) 5-bromofuroic acid (1.1μM). The most potent hit (2-thiophenecarboxylic acid) was deconvoluted by the synthesis, purification and testing of the individual components **(11i-viii)** as well as the phenol esterification product **(12)** to give the compounds shown in Figure 2.

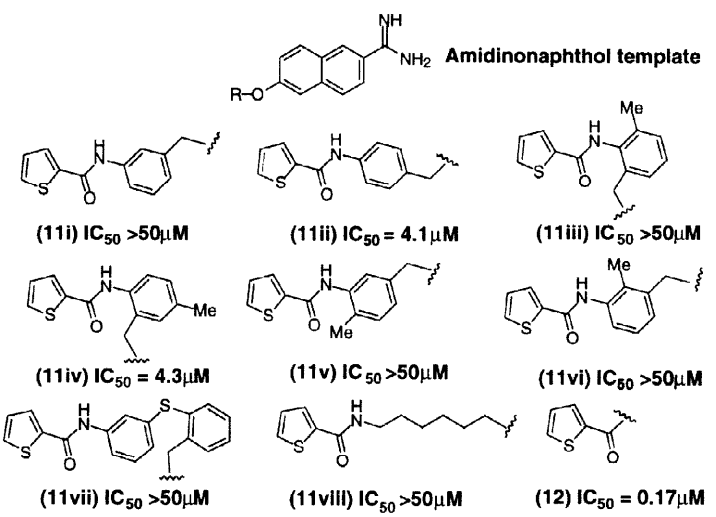


Figure 2. The deconvolution and screening of the sub-library (viii).

The two most potent, non ester based compounds were (**11ii** and **iv**) which had IC_{50} values of 4.1μM and 4.3μM respectively, some 10 fold more potent than the original lead compound, although another order of magnitude is obviously needed to take this compound into the therapeutically useful arena. The thiophene moiety certainly seems to be playing a valuable role in the terminating position of the inhibitor. The potent inhibition seen in the original compound mixture was undoubtedly due to the presence of an amidinonaphthol ester which are known to be very potent acylating agents. This hypothesis was verified by screening (**12**) which had an IC_{50} of 0.17μM and demonstrates the importance of purifying the library elements prior to screening and the advantages of single compound synthesis and screening.

Conclusion

We have demonstrated the reversible immobilisation of amidines onto a solid support and their use in solid phase chemistry in both a multiple parallel synthesis (MPS) and a split and mix manner. The approach lead to the successful synthesis, screening and deconvolution of small compound library and gave a compound with an IC_{50} of 4μM against TF/VIIa. Although not potent enough in its own right it is a promising lead for future developments and the first cited example of a non-acylating amidinonapthol compound with activity against TF/VIIa.

Experimental

Biological assays:

Stock solutions were prepared by dissolving sub-libraries to a concentration of 10mM in DMSO/H_2O (1:1). Stock solutions were then diluted in the same solvent to give doubling dilutions in the range 1:1 to 1:1028. Solutions were dispensed with chromogenic substrate solution (in house copy of Chromogenix S-2288, 400μM) to give a final concentration range of 195nM to 400μM. The reactions were initiated by the addition of pre-formed TF/VIIa complex. TF/VIIa complex was recombinant human TF (American Diagnostica) 120ng + recombinant human VIIa (Novo Nordisc, 240ng) in TRIS buffered saline (0.05M), pH 7.4 in the presence of $CaCl_2$, Triton-X100 and BSA, (2mg/mL). Assay plates were incubated at 37°C until reasonable levels of colour had developed in the solvent control wells. OD values were read at 405nm. % inhibition is calculated compared to solvent controls (0% inhibition). Dilution curve values were plotted as % inhibition against Log[Inhibitor] and IC_{50} values were determined from these plots. In all assays it was ensured that the DMSO concentration was kept below 0.5%. TF/VIIa enzyme complex K_i determinations were performed using a series of different substrate concentrations in the range 0.25 to 2.5mM and with three different concentrations of experimental compound (as determined in a range finding experiment). Reaction rates were measured on a Thermomax Kinetic plate reader. The inhibition constant (K_i) for TF/VIIa complex was derived from a Hanes plot of [substrate]/rate against [substrate] using the following formula: $K_i = [I]/((K_p/K_m)-1)$, where [I] is the concentration of inhibitor in the well, K_m is the negative intercept on the X-axis in the

control and K_p is the "apparent K_m" in the presence of inhibitor at a concentration [I] (negative intercept on the X-axis).

4-Nitrophenyl-4'-hydroxymethylphenyl-1'-oxyallylacetate carbonate (3a).
To 4-hydroxybenzaldehyde (20 g, 164 mmol), K_2CO_3 (2.5 eq, 410 mmol, 56 g) and KI (5%, 8.2 mmol) in MeCN (150 mL) was added dropwise allylchloroacetate (1.2 eq, 197 mmol, 22.8 mL) over a period of 20 min at room temperature. The reaction was then heated at reflux overnight. The reaction mixture was filtered, concentrated *in vacuo*, dissolved in ethyl acetate, filtered and concentrated *in vacuo* and the process repeated. The crude material was dissolved in ethanol (150 mL) and a trace of bromocresol green added. $NaBH_3CN$ was added portion wise at 0°C with 10% HCl to keep the reaction at approximately pH 4. Upon completion the reaction mixture was diluted with brine (300 mL) and the ethanol evaporated. After extraction with ethyl acetate (3x200 mL), the organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo*. The crude product was dissolved in pyridine (1.5 eq, 225 mmol, 18 mL) and DCM (200 mL) to which was added a solution of 4-nitrophenylchloroformate (1 eq, 165 mmol, 33 g) in DCM (75 mL) at 0°C. The reaction was stirred at room temperature for 3 h. This was followed by the addition of an aqueous saturated solution of $CuSO_4$ (200 mL), followed by extraction with DCM (2x200 mL). The organic phases were washed with an aqueous saturated solution of $CuSO_4$ (200 mL), brine (2x200 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. Purification by flash chromatography on silica gel (eluting with DCM) and recrystallisation gave the title material asa white solid in an overall yield of 50% (32 g). Mpt 54°C; υmax./cm^{-1} (Solid) 1760, 1613, 1523, 1348; δ_H (300MHz, $CDCl_3$): 8.26 and 7.40 (4 H, 2xd, *J* 9, H-2,3,5,6 of phenyl), 7.40 and 6.95 (4 H, 2xd, *J* 9, H-2,3,5,6 of benzyl), 5.90 (1 H, m, C<u>H</u>=CH_2), 5.36 and 5.29 (2 H, 2xd, *J* 17 and 12, CH=C<u>H</u>$_2$), 5.23 (2 H, s, Ar-C<u>H</u>$_2$), 4.70 (2 H, d, *J* 7, C<u>H</u>$_2$CH=CH_2), 4.68 (2 H, s, OC<u>H</u>$_2$$CO_2$); δ_C (75.5 MHz, $CDCl_3$): 168.5 (<u>C</u>O_2), 158.5, 155.7 and 152.6 (2 Ar<u>C</u>O, O<u>C</u>O_2), 145.5 (<u>C</u>NO_2), 131.5 (<u>C</u>H=CH_2), 130.9, 125.7, 121.9 and 115.0 (8x ArCH C-2, C-3, C-5, C-6 benzyl and phenyl), 127.6 (Ar<u>C</u>CH_2), 119.4 (CH=<u>C</u>H_2), 70.8(Ar<u>C</u>H_2O), 66.1 (<u>C</u>H_2CH=CH_2), 65.5 (O<u>C</u>$H_2$$CO_2$); HRMS (FAB, MNOB matrix) Found 387.0942, $C_{19}H_{17}NO_8$ Requires 387.0954.

N-(4-Benzyloxycarbonyl-1-oxyacetic acid)-6-amidinonaphth-2-ol (5).
To amidino naphthol **2** (3.12 mmol, 0.88 g) and K_2CO_3 (7.5 eq, 4.7 mmol, 0.65 g) in dioxane (15 mL) was added a solution of the allyl ester carbonate (**3**) (1.1 eq, 3.12 mmol, 1.2 g) in dioxane (30 mL). The reaction was stirred at room temperature for 20 h before completion (tlc, Rf = 0.15; (ethyl acetate/hexane = 1/1)). The reaction mixture was diluted with water (200 mL) and acidified with 2M $KHSO_4$ to pH 7.0. After partial evaporation of the solvents the reaction was extracted with DCM (3x200 mL) and the aqueous phase re-extracted with ethyl acetate (3x200 mL). The organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography on silica gel (eluting with petroleum ether/ethyl acetate = 70/30 to 60/40) gave the crystalline urethane allyl ester (**4b**)

in 42% yield (0.57 g). **(4b)** was treated with $Pd(PPh_3)_4$ (10%, 0.13 mmol, 0.15 g) and 3,3-dimethyl 1,5-cyclohexadione (1.1 eq, 1.44 mmol, 0.20 g) and the reaction was stirred at room temperature for 4 h in degassed DCM (15 mL). Concentration *in vacuo* and purification by direct column chromatography on silica gel (eluting with 5-25% methanol in DCM) gave the title material as an oil in 53% yield, which was used immediately in the subsequent coupling reaction; $\upsilon_{max.}/cm^{-1}$ (Solid) 1722, 1613, 1512; δ_H (300MHz, CD_3SOCD_3): 8.50 (1 H, s, H-5 naphthyl), 7.95 (1 H, d, *J* 8, H-8 naphthyl), 7.88 (1 H, d, *J* 8, H-7 naphthyl), 7.72 (1 H, d, *J* 8, H-4 naphthyl), 7.35 and 6.88 (4 H, 2xd, *J* 7, AB system, H-2, 3, 5, 6 benzyl), 7.18 (2 H, overlapping aromatic signals, H-1, 3 naphthyl), 5.00 (2 H, s, $ArC\underline{H}_2O$), 4.25 (2 H, s, $OC\underline{H}_2CO_2H$); δ_C (75.5MHz, CD_3SOCD_3): 172.1($\underline{C}O_2H$), 166.9 and 163.4 ($O\underline{C}ONH$ and $\underline{C}$=NH), 158.7 and 157.8 (2xAr$\underline{C}$O), 136.7 (Ar$\underline{C}$-C=NH), 130.8, 129.7, 128.5, 125.9, 124.5, 119.8, 114.4 and 108.7 (8xArCH), 129.7 (Ar$\underline{C}CH_2O$), 128.1 and 126.6 (Ar C-9,10 of napthyl), 67.4 (Ar$\underline{C}H_2O$), 65.9 ($O\underline{C}H_2CO_2H$); m/z (ESI +ve): 789.3 (dimer, $[M\text{-}M + H]^+$, 89%), 395.2 ($[M + H]^+$, 70.4%), 125.8 (base peak); HRMS (FAB, MNOB matrix) Found 395.1233, $C_{21}H_{19}N_2O_6$ Requires 395.1243. This carboxylic acid (1.0 eq. followed by 0.5 eq.) was coupled to aminomethyl polystyrene resin using a standard 12h HOBt/DIC coupling in DCM/DMF (1:1) to give a negative ninhydrin test. Loading was quantified by analysis of cleaved material by reverse phase HPLC and gave a quantitative yield with respect to the starting aminomethyl resin, with only a single peak visible by reverse phase HPLC.

4-Nitrophenyl-4'-hydroxymethylpheny-1'-oxyacetamide-resin

4-Hydroxymethylphenoxyacetic acid (1.5eq, 2.2 g), DIC (1.5eq, 1.9 mL) and HOBt (3eq, 3.2 g) were dissolved in DCM (40 mL) and DMF (10 mL) and stirred for 15 min before addition to aminomethyl polystyrene resin (8 mmol, 10 g) previously preswollen in DCM. After stirring for 12h the reaction mixture was filtered and the reaction repeated before washing the resin with DCM(10x100 mL), DMF (10x100 mL), DCM (10x100 mL), MeOH (10x100 mL) and Et_2O (10x100 mL). A ninhydrin test was negative.The resin was swollen in DCM and to this were added 4-nitrophenylchloroformate (2.5eq, 4.0 g), in DCM (40 mL) and pyridine (5eq, 3.25 mL). The reaction was shaken for 12 h after which time the resin was washed with DCM(10x100 mL), DMF (10x100 mL), DCM (10x100 mL), MeOH (10x100 mL) and Et_2O (10x100 mL). Amidinonapthol.HCl (2 eq, 2.66 g) was dissolved in dioxane (130 mL) and DMF(20 mL) and K_2CO_3 (3eq, 2.5 g) added. This mixture was added to the Wang-4-nitrophenylcarbonate resin (7.5 g) which had been preswollen in DCM. The suspension, which rapidly became yellow, was shaken for 48h at which time the resin was thoroughly washed with DCM (10x100 mL), DMF (10x100 mL), DCM (10x100 mL), MeOH (10x100 mL), water (10x100 mL), MeOH (10x100 mL), DCM (10x100 mL) and Et_2O (10x100 mL). Loading was quantified by reverse phase HPLC analysis of cleaved material and gave a value of 68% with respect to the starting aminomethyl resin. Extensive washing was needed to remove all traces of nitrophenol from the resin.

Mitsunobu Couplings

43 aliquots of resin-bound 6-amidino-2-naphthol (0.10 g, 0.133 mmol) were swollen in dry DCM (2 mL). To these were added under N_2 various amino alcohols (5 eq) in solution in dry THF (2 mL) with Et_3N (15 eq), Bu_3P (5 eq). The vessels was cooled to 0°C before addition (portion wise) of TMAD (5 eq). The reaction vessels were sealed under nitrogen and shaken for 15 h. Following filtration the resins were washed sequentially with DCM (5 mL), THF (5 mL), DMF (5 mL), MeOH (5 mL), Ether (5 mL), MeOH (5 mL), DMF (5 mL), THF (5 mL), DCM (5 mL) and Ether (5 mL) and then dried *in vacuo*. For the split and mix library synthesis the reactions were then repeated as above but with 3eq of reagents. Compounds (**7i-viil**) were cleaved by treatment with TFA/DCM/Water, (50/45/5, 2 mL, 4h), the resin filtered through a plug of glass wool and rinsed with DCM (2 mL) and MeOH (2 mL). The solvents were removed under a stream of nitrogen and the residue triturated with Et_2O. The residue was dried *in vacuo* before analysis by HPLC and ESMS. For library analysis 10mg of resin were removed from each reaction vessel and material cleaved (TFA/DCM/Water, 50/45/5, 1mL, 4h) and the solvents removed *in vacuo* prior to analysis.

Multiple Parallel Synthesis Alcohols

(% HPLC purity, K_I (μM) if less than 150μM)

(i) (2-Amino-3-methylphenyl)methanol (42%), (ii) [2-(2-aminomethyl)phenysulfanyl)-phenyl]methanol (52%), (iii) 1-naphthylmethanol (40%), (iv) (2,5-dichlorophenyl)methanol (>80%) (v) (2-phenoxyphenyl)methanol (38%), (vi) 3-furanylmethanol (53%), (vii) [3-(benzyloxy)phenyl]methanol (23%), (viii) [4-(methylsulfanyl)phenyl]methanol (>80%), (ix) hycanthone (78%), (x) methyl 2-[8-(hydroxymethyl)-1-naphthyl]sulfinylbenzoate 59%), (xi) 4-(styryl(E)phenyl)methanol (51%), (xii) 3-pyridylmethanol (82%), (xiii) 4-[(3,4,dichlorobenzyl)oxy]phenyl)methanol (52%), (88μM), (xiv) 4-[(4-nitrobenzyl)oxy]-phenylmethanol (45%), (92μM), (xv) (2-benzylphenyl)methanol 1 (60%), (141μM), (xvi) (6-chloro-1,3-benzodioxol-5-yl)methanol (86%), (53μM), (xvii) [3-(2,6 dichlorophenyl)-5-methyl-4-isoxazolyl]methanol (82%), (43μM), (xviii) (2,3,5,6 tetramethylphenyl)methanol (57%), (56μM), (xix) [5,3'-dimethyl-[3,5]bi-4-isoxazolyl]methanol (144μM), (xx) [3-(dimethylamino)phenyl]methanol (70%),(xxi) [2-fluoro-4-(trifluoromethyl)phenyl]methanol (92%), (53μM), (xxii) 4pyridylmethanol (90%), (50μM), (xxiii) 4-[(4-fluorbenzyl)oxy)]phenylmethanol (40%), (49μM), (xxiv) (3-phenoxyphenyl)methanol (84%), (52μM), (xxv) [3-(4-chlorophenoxy)phenyl]methanol (80%), (48μM), (xxvi) 4-phenyl(phenyl)methanol (38%), (54μM), (xxvii) (2,4-dichlorophenyl)methanol (86%), (54μM), (xxviii) (2,3,4-trimethoxyphenyl)methanol (50%), (52μM), (xxix) [4-(tertiarybutyl)phenyl]methanol (53%), (56μM), (xxx) (2-amino-3-methylphenyl)methanol (35%), (47μM), (xxxi) (2-amino-5-chlorophenyl)methanol (30%), (45μM), (xxxii) 4-hydroxymethylpyridine-*N*-oxide (81%), (xxxiii) (4-n-butyl-phenyl)methanol (38%), (xxxiv) (2-methoxyphenyl)methanol (22%), (xxxv) (2,5 dimethoxyphenyl)methanol (22%), (xxxvi) (4-methoxyphenyl)methanol (22%), (xxxvii) [2-(benzyloxy)phenyl]methanol (51%),

(xxxviii) 3-thienylmethanol (62%), (xxxix) (3,4,5 trimethoxyphenyl)methanol, (45%), (xl) (4-aminophenyl)methanol (66%), (xli) [3-(trifluoromethoxy)phenyl]methanol (88%), (xlii) (3-ethoxyphenyl)methanol (87%), (xliii) 2-phenyl-phenyl-methanol (54%).

Split and Mix Library Synthesis - Coupling of 11 amino alcohols to amidinonapthol template: Amino Alcohol, % Conversion (HPLC), % Purity (HPLC) Aminoalcohols (**8**) (i) (3-aminophenyl)methanol, 100%,85%; (ii) (4-aminophenyl)methanol, 80%,65%; (iii) (2-amino-3-methylphenyl)methanol, 66%,55%; (iv) (2-amino-5-methylphenyl)methanol, 95%,85%; (v) (3-amino-4-methylphenyl)methanol, 100%,95%; (vi) (3-amino-2-methylphenyl)methanol, 100%,95%; (vii) 6-aminohexanol, 85%,80%; (viii) 2-(2-aminomethyl)phenylthiobenzyl alcohol 90%,85%; (ix) pyridoxamine, 0%,0%; (x) 3-indolemethanol, 40%,<50%; (xi) (2-amino-phenyl)-methanol 66%,<50%.

Acylations with Resin Bound 6-amidino-2-naphthol-ethers

Eight (i-viii) of the eleven batches of resin were chosen for the next step after considerations of conversion and purity of the ether product. The 8 chosen resins were mixed and from that mix 18 batches (50 mg) were prepared. HPLC and ES MS, analysis as well as HPLC-MS, confirmed that the mixed resin was composed of 8 components in approximately the same ratios (from expected absorbances). Some starting 6-amidino-2-naphthol was also present but to a lesser amount. To each batch of resin, swollen in DCM (2mL), was added a mixture of DIC (15eq) and HOBt (15 eq) and the corresponding carboxylic acid (the carboxylic acids used for library synthesis are given below (**10i-xviii**)), in DCM/DMF (9/1) (2 mL). The reaction vessels were shaken for 5 h before the solvents were removed under vacuum. The resin samples were washed and dried as above. The couplings were repeated using the same conditions for 12 h. Amidino materials were cleaved from the resin with (TFA/water/DCM) (50/5/45) (2 mL) and the solvents were evaporated *in vacuo* yielding 18 sublibraries of 5-10 mg of amidino-based material. Sublibrary 7, which had been obtained by coupling the mixed resin with benzoic acid, was chosen for analysis by HPLC, ES MS and HPLC-MS. All the desired products had been synthesised and formed the major components of the sublibrary, which also included acylated aminoether and di-acylated material as a result of acylation taking place at the amidino moiety.

Library Deconvolution

Each of the 8 resin-bound 6-amidino-2-naphthol-ethers (10 mg resin, 6 μmol), (which had been put to one side before the mixing step) were swollen in dry DCM (0.5 mL) to which was added a previously activated mixture of DIC (15 eq), HOBt (15 eq) and 2-thiophene carboxylic acid (15 eq) and DMAP (1 eq) in dry DMF (0.5mL). The reaction vessels were then agitated for 2h. The process was then repeated using a 12 h coupling. The resin samples were washed with DCM (5 mL), DMF (5 mL), MeOH (5 mL), ether (5 mL), MeOH (5 mL), DMF (5 mL), DCM (5 mL) and ether (5 mL), and subsequently dried *in vacuo*. Cleavage was performed using TFA/water/DCM (50/5/45) (2.5 mL) for 3 h. The solvents were

evaporated *in vacuo* and each sample purified by RP-HPLC (C_{18} 250x25mm, 2.5 mL/min, gradient from 10% MeCN to 100% over 1 h) and charaterised by ES MS prior to screening which gave the following results:

(11i) *N*2-3-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]phenyl-2-thiophenecarboxamide >50μM.

(11ii) *N*2-4-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]phenyl-2-thiophenecarboxamide 4.1μM.

(11iii) *N*2-2-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]-6-methylphenyl-2-thiophenecarboxamide >50μM.

(11Iv) *N*2-2-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]-4-methylphenyl-2-thiophenecarboxamide 4.3μM.

(11v) *N*2-5-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]-2-methylphenyl-2-thiophenecarboxamide >50μM.

(11vi) *N*2-3-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]-2-methylphenyl-2-thiophenecarboxamide >50μM.

(11vii) *N*2-[2-(2-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]phenylsulfanyl)benzyl]-2-thiophenecarboxamide >50μM

(11viii) *N*2-[6-(6-[amino(imino)methyl]-2-naphthyloxy)hexyl]-2-thiophenecarboxamide >50μM.

(12) 6-[amino(imino)methyl]-2-naphthyl 2-thiophenecarboxylate 0.17μM.

*N*2-4-[(6-[amino(imino)methyl]-2-naphthyloxy)methyl]phenyl-2-thiophenecarboxamide (11ii).

8.5% overall yield from the aminomethyl resin, 17 mg of a white lyophilised powder (TFA salt); δ_H (300MHz, CD_3OD): 8.37 (1H, d, *J* 2.2, H-5 naphthyl), 8.08 (2 H, 2 overlapping signals, *J* 9.6, H-8 naphthyl), 7.91 (1 H, d, *J* 8.9, H-7 naphthyl), 7.88 (1 H, dd, *J* 3.8, *J* 1.5, H-3 or H-5 thiophene), 7.73 (1 H, dd, *J* 5.0, *J* 1.5, H-3 or H-5 thiophene), 7.71 (1 H, dd, *J* 9.0, *J* 2.2, H-4 naphthyl), 7.52 and 7.41 (4 H, 2xd, AB system, *J* 8.8, H-2,3,5,6 benzyl), 7.28-7.18 (3 H, overlapping signals, H-1 and H-3 naphthyl, H-4 thiophene), 4.50 (2 H, s, CH_2 benzyl); δ_C (75.5MHz, CD_3OD): 165.9 (C=NH), 162.6 (C=O amide), 157.5 (ArC-O), 138.8, 137.9, 137.1 (ArC-C=NH, S-C-CONH, ArC-NH), 132.4, 131.2, 130.8, 129.9, 129.6, 128.9, 125.8, 124.2, 122.4, 120.7 (13 aromatic CH signals with 3 overlapped signals), 125.8, 122.6, 119.8 (C-9 and C-10 naphthyl, Ar-C-CH_2), 30.7 (CH_2); m/z (ESI +ve): 402.2 ($[M + H]^+$ = 100%); HRMS (FAB, MNOBA matrix) Found 402.1253, $C_{23}H_{20}N_2O_2S$ Requires 402.1276.

Aminoalcohols used (8).

(i) (3-aminophenyl)methanol, (ii) (4-aminophenyl)methanol, (iii) (2-amino-3-methylphenyl)methanol, (iv) (2-amino-5-methylphenyl)methanol, (v) (3-amino-4-methylphenyl)methanol, (vi) (3-amino-2-methylphenyl)methanol, (vii) 6-aminohexanol, (viii) 2-(2-aminomethyl)phenylthiobenzyl alcohol, (ix) pyridoxamine, (x) (2-aminophenyl)methanol, (xi) 3-indolemethanol.

Carboxylic acids used (10).

(i) 4-chlorobenzoic acid, (ii) 2-phenoxyacetic acid, (iii) 4-sulfamoylbenzoic acid, (iv) 2,2-dichloro-1-methyl-cyclopropane carboxylic acid, (v) 2-naphthoxyacetic acid, (vi) 2-thiopheneacetic acid, (vii) benzoic acid, (viii) 2-thiophenecarboxylic acid, (ix) 5-chloro-2-nitrobenzoic acid, (x) 2,6-dimethoxybenzoic acid, (xi) 3,4-methtlene-dioxybenzoic acid, (xii) 1-piperidinopropionic acid, (xiii) 2,4-dichloro-5-sulfamoylbenzoic acid, (xiv) 6-chloronicotinic acid, (xv) 4-chloro-3-sulfamylbenzoic acid, (xvi) 5-bromofuroic acid, (xvii) 5-bromovaleric acid, (xviii) 3,5-dihydroxy-2-naphthoic acid.

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