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Oxygenated analogues of UK-396082 as inhibitors of activated thrombin activatable fibrinolysis inhibitor

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ABSTRACT

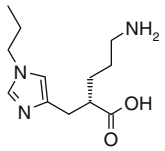
A suitable inhibitor of activated thrombin activatable fibrinolysis inhibitor (TAFIa) has the potential to be a novel treatment for thrombosis. The TAFIa inhibitor UK-396082 (**1**) was used as a starting point to seek more potent analogues. With knowledge of encouraging human pharmacokinetics and toleration for the clinical candidate (**1**), the programme continued to seek structure–activity relationships (SAR) that could positively impact on both potency and half-life, and therefore the projected dose of any future nominated clinical agent. A series of oxygenated analogues based on compound **1** were prepared to evaluate changes in pharmacology, selectivity and pharmacokinetics.

Thrombosis is defined as the obstruction of a blood vessel through the formation of a clot (thrombus). Given risk factors for thrombosis such as diet, exercise and smoking (as opposed to genetic or natural imbalances), it is not surprising that thrombotic diseases represent one of the most common causes of mortality and morbidity in the developed world. Thrombin activatable fibrinolysis inhibitor (TAFI) is an inactive 60-kDa glycoprotein zymogen found in human plasma.[1] Through the action of the enzyme thrombin (from the coagulation cascade), TAFI is converted from its inactive form to the activated TAFIa. Activated TAFIa is an unstable, zinc dependent basic carboxypeptidase that significantly inhibits fibrinolysis. Inhibition of activated TAFI represents a potentially subtle modulation of the clotting and lysis balance, without resorting to direct action on the coagulation cascade (as traditionally tackled by inhibitors of thrombin or factor Xa). Ideally, this subtlety may imply a reduced risk of side effects when using a TAFIa inhibitor, in comparison to other potential mechanisms for treating thrombotic disease. The role of the zinc metalloprotease TAFIa in coagulation is to remove C-terminal lysine residues from the surface of fibrin—a key clot constituent. This reduction in C-terminal lysine concentrations has the downstream effect of reducing the local concentration of plasmin at the site of the clot. Plasmin is anticoagulant in its properties. The action of TAFIa serves to limit plasmin recruitment at the site of the forming thrombus, thus an inhibitor of TAFIa should be antithrombotic.

The TAFIa inhibitor UK-396082 **1** (Fig. 1) has previously been disclosed as a clinical candidate from these laboratories.[2] It was rationally designed as a C-terminal lysine mimetic and unsurprisingly possesses structural elements common to that amino acid (position of the side-chain basic centre, free carboxylic acid).

The imidazole serves as a rare example of the functional group being used as a ligand for zinc in a metalloprotease inhibitor.[3] The zinc ion is a key component of the enzyme in its role during peptide cleavage and hence small molecule inhibitors of TAFIa are likely to feature some sort of zinc ligating functionality.[4]

The attractive characteristics of compound **1** are its small size and polarity. Alongside the pharmacological design requirements of seeking to mimic a C-terminal lysine, we deliberately sought to stay within low molecular weight, polar drug space. This strat-



UK-396082
1

TAFIa Ki = 16nM
Ligand Efficiency (LE) 0.62
MWt = 239

i.v. PK	Cl(ml/min/kg)	Vd(L/kg)	$T_{1/2}$ (h)
Human	1.5	0.5	4
Dog	3.1	0.35	1.4
Rat	10.2	1.9	2.1

Figure 1. UK-396082 (**1**) profile.

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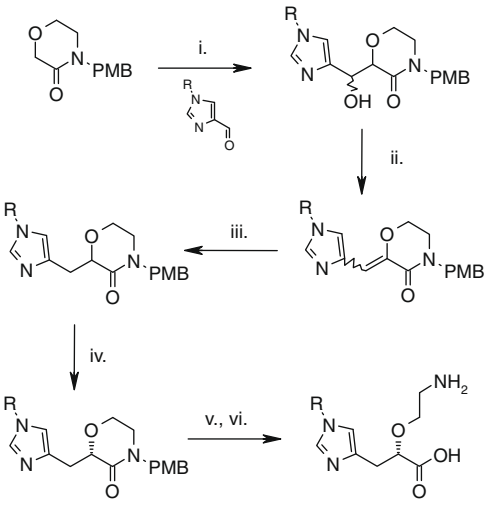
egy successfully translated to paracellular oral absorbtion, passive renal clearance and limited any hepatic metabolic processes for compound **1**. This drug design space has further attractions. The physicochemical properties of the compound also limit the likelihood of off-target pharmacology, hERG activity and potentially, compound-mediated toxicity.[5] Compound **1** had no plasma protein binding and a volume of distribution around that of body water in vivo. Given this chemotype physicochemistry, design hypotheses beyond **1** were limited to improving TAFIa inhibition and compound pharmacokinetics. Small changes in either parameter would likely have a significant impact on any future candidate's predicted dose.

The ligand efficiency of compound **1** is notably high (0.62).[6] Despite 'only' being 16 nM in its primary pharmacology,[7] this is achieved using just seventeen heavy atoms. However, with such an efficient lead and some molecular weight in hand before the small, polar drug space characteristics were compromised, there appeared to be sufficient flexibility in as yet, unexplored design space for the template. The hypotheses investigated in this Letter are around the effects of changes in the basic side-chain and the imidazole N-1 substituent of **1**. We initially sought to explore the effect of an oxygen linking atom through compound **2** (Fig. 2). Compound **1** was initially discovered by moving from an N-linked basic side-chain (**3**) to a C-linked alternative (Fig. 2).[2] This change had achieved the vital 10-fold increase in potency, making the nomination of **1** as a clinical candidate possible.

Undeterred by the apparently negative effect of a heteroatom at this position in the side-chain (based on our knowledge of the SAR relating to nitrogen), compound **2** was synthesised by the route shown in Scheme 1. The general synthetic strategy allowed for a variety of imidazole carboxaldehydes to be condensed in an aldol/dehydration sequence with a protected morpholinone enolate. Alkene reduction, deprotection and ring-opening liberated the ethanolamine side-chain and carboxylic acid. Single enantiomers were prepared by chiral HPLC separation of intermediate morpholinone racemates, before the deprotection/ring-opening sequence.

This O-linked change covered several sub-hypotheses. Lacking the H-bond donor of the previously studied secondary amine (**3**), oxygen was thought to be more carbon-like in its properties. In contrast to carbon, oxygen did offer the potential for intramolecular hydrogen bonding to the terminal primary amine through acting as a weak acceptor, thus bringing in the potential for a conformationally-driven optimisation of potency. It also had a role to play in moderating the pK_a of local ionisable groups, primarily the carboxylic acid and the side-chain primary amine. In placing the oxygen as a heteroatom with a β-relationship to the primary amine, the pK_a of this group could be reduced by two units. Equally having a heteroatom-α to a carboxylic acid should render the



Scheme 1. Reagents and conditions: (i) LDA, THF, −78 °C; (ii) MsCl, Et_3N, THF, 0–40 °C; (iii) 10% Pd/C, 50 psi, EtOH; (iv) Chiral HPLC; (v) CAN, CH_3CN/H_2O; (vi) 6 N HCl, 110 °C.

group more acidic. This oxygenated analogue (**2**) was therefore calculated to be less basic and more acidic, but with a similar δ-pK_a between the zwitterionic functionality, when compared to **1**.[8] The oxygenated analogue **2** proved to be 2.5 times more potent than the equivalent methylene containing lead compound **1**. This is clearly reflected in its improved ligand efficiency as the potency enhancement has cost no extra heavy atoms. With a molecular weight of only 241 used in attaining a 6.7 nM compound, it was felt that up to another 60 mass units could be used in seeking out further potency enhancement without compromising the small, polar status of the series and the potential for paracellular oral absorbtion.

Phenylation of the N-1 position of the imidazole (**4**) did achieve a further potency enhancement when replacing the *n*-propyl group of **2**. This proved to be the most potent compound made in the whole oxygenated series at 3.5 nM (Fig. 3). Addition of further lipophilicity at the *para* position of the phenyl group in compound **5** maintained, but did not improve potency.

Cyclic (**6**), or homologated (**7**) aliphatic lipophilicity at the imidazole N-1 added nothing over the initial prototype **2**. Changing the torsional angle of the phenyl-imidazole bond through an *ortho*-substituent as in compound **8** cost around 10-fold in activity over compound **4**. In the case of **6**, as with arylated examples **4** and **5**, the structural changes only served to erode selectivity over human pancreatic carboxypeptidase B (Human pCPB). This was the only

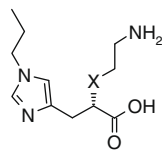
Template	Entry	X	TAFIa Ki (nM) [7]	LE	Human CPB (nM)	Amine pKa (calc.) [8]	Acid pKa (calc.) [8]	δ-pKa (calc.) [8]
	**1**	CH_2	16	0.62	206	10.7	3.9	6.8
	2	O	6.7	0.65	238	8.9	2.2	6.7
	3	NH	150	0.55	1160	9.8	1.5	8.3

Figure 2. The effect of O and NH insertion into compound **1** (UK-396082).

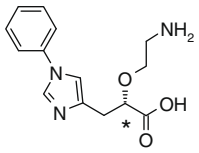
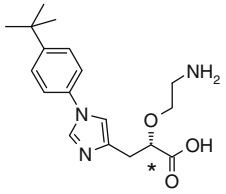
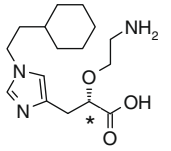
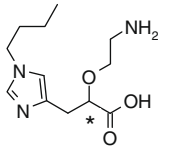
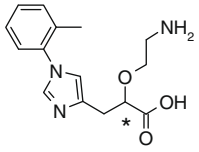
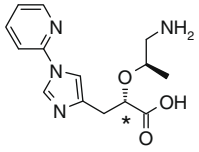
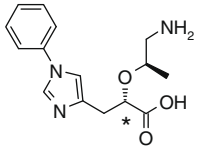
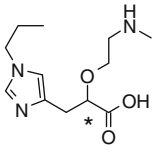
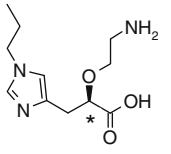
Compound	Entry	TAFIa Ki (nM) [7]	Ligand Efficiency [6]	Human pCPB Ki (nM)	Configuration *	MWt
	**4**	3.5	0.58	27	*S*	275
	**5**	5.7	0.47	49	*S*	331
	**6**	8.2	0.50	27	*S*	309
	**7**	15	0.59	360	+/-	255
	**8**	65	0.47	2720	+/-	289
	**9**	5.8	0.53	265	*S*	290
	**10**	7.5	0.53	240	*S*	289
	**11**	407	0.48	>10000	+/-	255
	**12**	>1000	-	>10000	*R*	241

Figure 3. Structure–activity relationships within the series.

selectivity monitored for the series as Cerep BioPrint® screening had indicated no other selectivity issues for this polar chemotype. The loss of Human pCBP selectivity could be countered relatively efficiently through the stereo-defined positioning of a methyl group in the basic ethanolamine side-chain. Compounds **9** and **10** illustrate the point with TAFIa inhibition maintained at sub-10 nM while disfavouring human pCPB activity. The methyl group was responsible for improving selectivity over human pCPB >30-fold. The toleration of a pyridyl group in compound **9** is also of note as a more polar isostere of the phenyl N-1 substituent in **4**. Changes

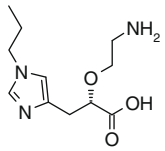
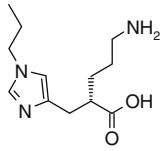
Structure	Entry	Study	Dose (mg/kg)	Cl (ml/min/kg)	Vd (L/kg)	$T_{1/2}$ (h)	ppb	F_{oral}
	**2**	Rat *i.v.*	2	13.4	3.1	2.7	0%	-
		Rat oral	5	-	-	2.1		9.8%
	**1**	Rat *i.v.*	2	10.2	1.9	2.1		-
		Rat oral	5	-	-	2.4	0%	10.6%
		Hu. *i.v.*	10	1.5	0.5	4		-

Figure 4. Pharmacokinetic comparison of compounds **1** and **2**.

to the primary amine at the side-chain terminus were costly. A simple methylation reduced activity almost 100-fold. Absolute stereochemistry was also important as seen in compound **12**. This enantiomer of the leading oxygenated analogue **2** was completely inactive against TAFIa.

The somewhat surprising conclusion to this SAR study was that we were unable to significantly enhance potency beyond 6 nM despite having up to six lipophilic heavy atoms to use, before compromising our small, polar drug properties. On assessing the optimal combination of potency, selectivity and the potential for good oral bioavailability/pharmacokinetics, the programme decided to profile compound **2** further.

Compound **2** was assessed in both iv and oral rat pharmacokinetic studies (Fig. 4). In the iv study (2 mg/kg), the compound had a relatively low clearance of 13.4 ml/min/kg and had no plasma protein binding. The clearance was higher than expected. It was three times higher than the glomerular filtration rate for rat that would have indicated passive renal clearance for the compound. However, the volume of distribution of 3.1 l/kg was also higher than expected for such a small, polar zwitterion. The rat iv half-life was 2.7 h. Oral pharmacokinetic studies at a dose of 5 mg/kg revealed an oral bioavailability of ~10% for compound **2**. This overall profile compared favourably with the equivalent rat data for clinical candidate UK-396082 (**1**) which had a lower volume of distribution, lower clearance and an iv half-life of 2.1 h.

The carbon to oxygen switch in going from **1** to **2** had been designed to potentially moderate the primary amine basicity and alter the carboxylic acid pK_a. This could potentially affect the compound's half-life through small changes in volume of distribution, but with minimal impact on clearance and oral bioavailability. Although exemplified in a very different chemotype, the ethanolamine side-chain has previously been employed by these laboratories to extend compound half-life in the discovery of the calcium channel antagonist amlodipine.[9] From the rat iv pharmacokinetic data, compound **2** has the potential for a comparable human half-life to C-linked compound **1**. Given the chemotype physicochemistry, dramatic changes in half-life are unlikely through the structural changes made (compounds have a low volume of distribution, low clearance and no plasma protein binding). The measured change in the rat pharmacokinetic data for compound **2** compared to compound **1** falls within experimental error.

In conclusion, compound **2** has a comparable pre-clinical profile to previously disclosed clinical candidate **1**. The apparent small improvements made in primary pharmacology and pharmacokinetics through the design of compound **2** fall within experimental error for those assays and models. Small changes in pharmacokinetics and primary pharmacology would have a significant effect on clinical dose for these particular compounds. These would likely manifest themselves in further clinical trials. Surprisingly, efforts to enhance potency through addition of lipophilicity at the N-1 position of the imidazole made little impact, although addition of a methyl group in the side-chain proved to be a good strategy for regaining human CPB selectivity when required. These compounds represent some of the most potent and selective TAFIa inhibitors disclosed to date.[4] In conjunction with reliable rat oral bioavailability and a useful rodent half-life, compound **2** has significant potential as a pre-clinical tool for further understanding the utility of TAFIa inhibition as a mechanism for treating thrombosis or for other potential emerging uses in non-cardiovascular indications.[10]

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7. Details of assay for TAFIa inhibition (i) TAFI activation: Human TAFI (recombinant or purified) was activated by incubating 20 μl of stock solution (360 μg/ml) with 10 μl of human thrombin (10NIH units/ml), 10 μl of rabbit thrombomodulin (30 μg/ml), 6 μl calcium chloride (50 mM) in 50 μl of 20 mM HEPES (N-10 [2-hydroxyethyl]piperazine-*N*-[2-ethanesulfonic acid]) buffer containing 150 mM sodium chloride and 0.01% TWEEN 80 (polyoxyethylene-sorbitan monooleate) pH 7.6 for 20 min at 22 °C. At the end of the incubation period, thrombin was neutralised by the addition of 10 μl of PPACK (D Phe-Pro-Arg chloromethyl ketone) (100 nM). The resulting TAFIa solution was stored on ice for 5 min and finally diluted with 175 μl of HEPES buffer. (ii) K_i determination (TAFIa): A number of different dilutions of the test compound in water were made up. To 20 μl of each dilution were added 150 μl of HEPES buffer and 10 μl of TAFIa, which was then pre-incubated for 15 min at 24 °C. To each dilution was then added 20 μl furylacryloyl-alanyl-lysine (FAAL) at a standard concentration. Substrate turnover was measured by reading the absorbance of the reaction mixture at 330 nm every 15 s for 30 min. The reaction was performed at 24 °C and samples were mixed for 3 s prior to each absorbance reading. A graph of %inhibition against test compound concentration was then plotted; from which was calculated the IC_{50} value. The K_i value was then calculated using the Cheng–Prusoff equation. Two controls, positive and negative, were used to check the accuracy of the results in each case. For the first control, the assay was performed as above, but with 20 μl of water rather than a dilution of the test compound. This showed minimal inhibition. For the second control, the assay was performed as above, but with an effective amount of a non-specific carboxypeptidase inhibitor rather than a dilution of the test compound. This showed maximal inhibition. When the two controls did not demonstrate minimal and maximal inhibition

respectively, the results were discounted and the test compound was reanalysed. Using the above assay, the compounds of the examples illustrated in the Letter were found to be potent and selective inhibitors of TAFIa.

8. Calculated pK_a's quoted in the Letter were derived from a commercially available software package. ACD/pK_a DB is a programme that calculates accurate acid-base ionisation constants (pK_a) at 25 °C and zero ionic strength in aqueous solutions for organic structures. The accuracy of calculations is usually better than ±0.2 pK_a units except for very complex structures or poorly-characterised substituents, where the accuracy is usually better than ±0.5 pK_a units. In order to achieve this accuracy, ACD/pK_a DB uses its own internal databases and algorithms. For structures with important through conjugation, sometimes larger differences from experimental values are observed. Each calculation is supported by its ±95% confidence limits and a detailed report of how it has been carried out, including Hammett-type equation(s), substituent constants, and the literature references where available.
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