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Parallel Liquid Synthesis of *N*,*N*′-Disubstituted 3-Amino Azepin-2-ones as Potent and Specific Farnesyl Transferase Inhibitors☆

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Abstract—A rapid structure–activity study was performed by parallel liquid synthesis on *N*,*N*′-disubstitution of 3-amino azepin-2-one to afford potent and specific farnesyl transferase inhibitors with low nM enzymatic and cellular activities. The activities of the selected compounds were validated in vivo, and compounds **41a** and **44a** presented significant antitumour activity.

Introduction

Protein farnesyl transferase (FTase) is a zinc-dependent enzyme that catalyses the attachment of a farnesyl lipid group to the sulfur atom of a cysteine residue of numerous proteins involved in cell signalling, including the oncogenic H-Ras protein.[1] This lipid side chain is critical for the cell membrane anchoring of these proteins,[2] and therefore FTase inhibition has been recognized as a valuable antitumour therapeutic approach,[3] although the target proteins whose farnesylation is inhibited by FTase inhibitors are not all identified. Another more ubiquitous prenylation of proteins can occur via geranylgeranyl transferase (GGTase-1) processing.[1] Indeed the RhoB protein, another putative FTase target, may be geranylgeranylated to an inactive form after FTase inhibition.[4] The issue of selectivity of the inhibition of FTase versus GGTase has been addressed and the optimal degree of selectivity is still debatable. However, access to specific inhibitors would help to understand the relationship between the various enzymes and different physiological pathologies. The substrates of these enzymes present a consensus sequence of four amino acid residues (CAAX) at the carboxylic terminal of the protein, with the cysteine in fourth, two aliphatic residues and the terminal residue X, being preferably methionine for FTase and leucine for GGTase.[5] In the design of FTase inhibitors, histidine was found to be a good bioisosteric replacement of the cysteine residue, and numerous non-thiol CVIM (**1**) peptidomimetics were developed around an imidazol function.[6] Therefore, among the compounds in clinical development we can distinguish those containing an imidazole associated to a benzodiazepine (BMS-214662);[7] a piperazinone (L-778,123),[8] or a quinolinone (R115777)[9] scaffold and the tricyclic SCH66336,[10] containing a piperidine obtained from HTS. Crystal structures of the complex between FTase and **1** are in favour of an extended conformation of **1** in the active site cavity.[11] The caprolactam derivatives **2** are proposed as the core of constrained CVIM bioisosteres in an elongated conformation including an imidazole zinc-complexing function. This scaffold can be substituted by R_3 (and/or eventually R_2) on the amine function N-1 (and/or eventually N-4) to mimic part of the Ras protein R (and/or

☆Supplementary data associated with this article can be found at doi:10.1016/S0968-0896(03)00218-9 [Tables 7–9].

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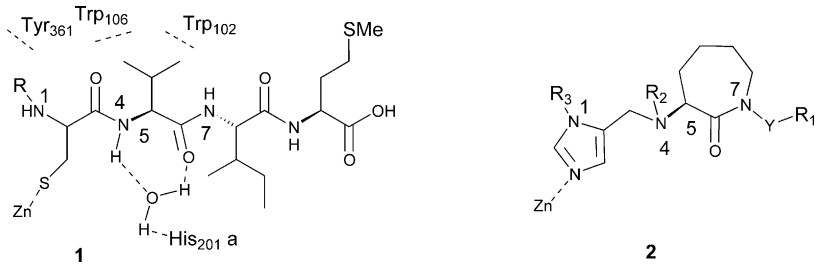
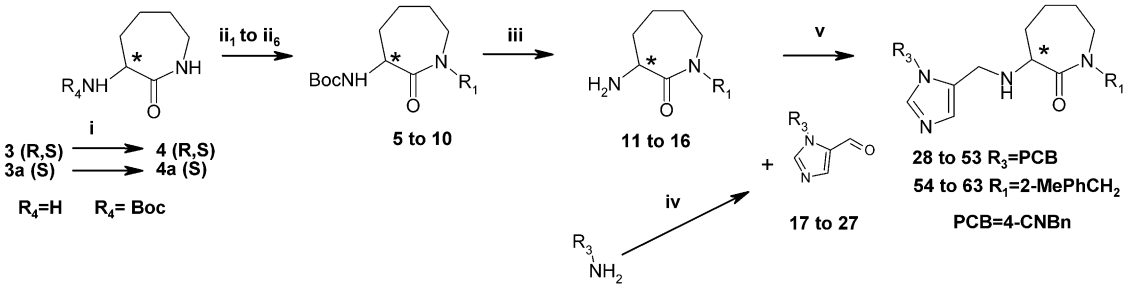


Figure 1. Interactions of compound **1** with FTase and structures of compounds **1** and **2**.



Scheme 1. Synthesis of compounds **28**–**63**. (i): $(Boc)_2O$, NaOH, H_2O/tBuOH, rt, 12 h, (**4**, 88%); (ii_1) (a) $BiPh_3$, pyridine, $Cu(OAc)_2$, CH_2Cl_2, rt, 24 h (**5**, 77%); (ii_2) (a) NaH THF/DMF, 0 °C, 30 min; (b) $PhCH_2Br$, $N(Bu)_4$, I, rt, 24 h (**6**, 97%). (ii_3) (a) NaH, DMF, 0 °C, 30 min; (b) $Ph(CH_2)_2OTs$, 0 °C, 12 h (**7**, 75%); (ii_4) (a) NaH DMF, 0 °C, 30 min, rt, 2 h; (b) $Ph(CH_2)_3OTs$, rt, 12 h (**8**, 86%); (ii_5) (a) LiHMDS, 0 °C, 15 min; (b) $PhSO_2Cl$, 0 °C, rt, 2 h (**9**, 77%); (ii_6) (a) $PhSCH_2Cl$, KOH, $N(Bu)_4I$, THF, rt, 12 h; (b) dimethyldioxirane, acetone, 0 °C, rt, 2 h, (**10**, 40%); (iii) TFA, CH_2Cl_2, 0 °C, rt, 2 h or 4 N HCl/dioxane, rt (**11**–**16**); (iv) (a) dihydroxyacetone dimer, KSCN, MeCN/$EtCO_2H$, 60 °C, 2 h; (b) H_2O_2, H_2O, 40 °C, 2 h; (c) DMSO, SO_3/pyridine, NEt_3, rt, 2 h (**17**–**27**: Tables 4 and 6); (v) (a) **17**–**27**, $NaBH(OAc)_3$, CH_2Cl_2, 0 °C, rt, 12 h; (b) HCl, ether, or fumaric acid, EtOH (**28**–**33**: Table 1; **34**–**53**: Tables 2 and 7; **54** to **63**: Tables 4 and 8).

the farnesyl co-substrate) and R_1 separated by a spacer Y on the lactam nitrogen atom N-7 to replace the lipophilic non polar side chains of the isoleucine/methionine residue. Moreover, a synthesis of **2** by a methodology applicable to liquid parallel synthesis was performed to allow an extensive and rapid investigation of the $Y/R_1/R_2/R_3$ parts of the molecule (Fig. 1).

Results and Discussion

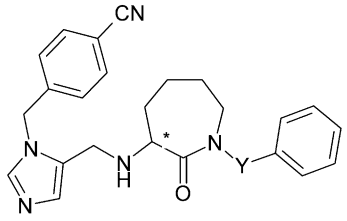
Investigation on the *N*-lactam substitution R_1

A straightforward retro-synthetic analysis links **2** to 2-aminocaprolactam (**3**) by two selective *N*-alkylations. The primary amino function of **3** was protected as a Boc derivative to give the intermediate **5** ready for *N*-lactam substitution and further substitution after deprotection. In order to validate the caprolactam core hypothesis and investigate the *N*-lactam substitution, the proximal *N*-*p*-cyanobenzyl function (PCB) was used as the imidazole substituent R_3, as a well described cysteine bioisostere.[12] Then the replacement of the isoleucine/methionine by a lipophilic side chain was first considered by using various spacer Y and a phenyl group as R_1. When Y was a bond, the *N*-arylation was achieved by treatment of **4** with triphenylbismuth to give **5**.[13] The *N*-alkylation was obtained by lactam deprotonation and addition of the benzyl chloride to give **6**. Based on the same strategy, various alkyl spacers were also considered to complete the study to give the corresponding compounds **7** and **8**. A sulfonyl spacer was also introduced either by lactam deprotonation and addition of the corresponding sulfonyl chloride to give **9**, or by sequential alkylation of the chloromethyl sulfide and oxidation to give **10**. Then, they were deprotected quantitatively under acidic conditions to give the corresponding primary amines **11**–**16**, and the (N-1-PCB) imidazol-5-yl-methyl substituent was introduced by reductive amination with the corresponding aldehyde **17** (R_3 = PCB), to afford the compounds **28** to **33** (Scheme 1, Table 1).

The inhibitory activities of these compounds were examined against FTase and against GGTase-1 and the results were compared to the various clinical references (Table 1). Their cellular activities for FTase inhibition were determined on an H-Ras based assay.

In the series of compounds **28**–**33**, the introduction on the caprolactam moiety of an aryl substituent to replace the two terminal amino acids of CVIM afforded very potent inhibitors of FTase when the junction was made either by a bond or a methylene group in compounds **28** and **29**, respectively. Further chain elongation decreased the potency in the case of an ethyl group (**30**) and even abolished the activity in the case of the propyl spacer (**31**). The sulfone spacer, previously used in the case of diaryl as VIM surrogate,[14] was detrimental to inhibition in our case, either alone (**32**) or with a methyl group (**33**). These results suggest a different binding mode for the caprolactam scaffold, possibly in a conformation closer to that of the peptide ligand. The stereospecificity of the enzyme inhibition was validated by using the enantiomers of **29**, respectively **29a** and **29b**, separated by chiral HPLC, **29a** being significantly more active than

Table 1. Structures and biological data of compounds **28–33**



Compd (conf.)	Y	FTase IC_{50} nM	GGTase IC_{50} nM	Ras Cell IC_{50} nM	Compd (conf.)	Y	FTase IC_{50} nM	GGTase IC_{50} nM	Ras Cell IC_{50} nM
BMS-214662[7]		1.3	2300	25	**29a** (*S*)	CH_2	9	16,200	10
found		16	7600	29	**29a** (*S*)[a]		5		
L-778,123[8]		1.6		70	**29b** (*R*)[a]	CH_2	2800	12,000	ND
found		8	13,200	31					
SCH66336[10]		1.9	> 50,000	10	**30** (*RS*)	$(CH_2)_2$	69	5200	288
R115777[9]		K_i 0.5	> 50,000	1.7	**30a** (*S*)	$(CH_2)_2$	39	5900	160
found		4		2					
28 (RS)	Bond	28	11,200	32	**31** (*RS*)	$(CH_2)_3$	1000	6200	4400
28a (S)	Bond	17	3300	17	**32** (*RS*)	SO_2	293	10,600	260
29 (RS)	CH_2	8	10,600	ND	**33** (*RS*)	CH_2SO_2	168	9700	3800

[a]Enantiomer obtained from the racemate by separation on chiral HPLC.

29b. To assess the C-5 configuration, a stereospecific synthesis of the *S* enantiomer for the most active compounds was carried out from the *S* caprolactam **3a**. The retention of the configuration during the synthesis was confirmed by the synthesis of the *S* enantiomer of **28**, **29** and **30**, respectively **28a**, **29a** and **30a**, with an enantiomeric excess up to 85%. The conservation of activity was observed for each of these *S* enantiomers versus their racemates, which corroborated the stereospecificity of the enzymatic and the cellular inhibition, with activities already in the same range as those of the reference compounds (Table 1). The active configuration *S* of these inhibitors is also in agreement with the hypothesis of a close mimicry of the peptide ligand.

Further investigation on the primary amine substitution R_1

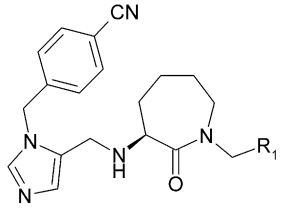
Since the best enzymatic and especially cellular activities were observed for compounds **28a** and **29a**, they could be considered for further lead optimization, with a slight advantage for the latter. Moreover, the benzyl derivative **28a** was also the most attractive in terms of molecular diversity for R_1 optimization, with a large commercial access to benzylic reagents compared to triarylbismuth derivatives. Therefore, solution phase parallel synthesis, was performed by sequential alkylation of the *S* enantiomer **4a** with a series of benzyl derivatives to give, after acidic deprotection, the amines. These intermediates were condensed, without purification, by reductive amination with **17**, to afford compounds **34a** to **53a**. Purification of these compounds by simple automated SPE, led average yield, but complied with the high criteria purity necessary for the accuracy of the biological tests (Scheme 1, Table 2).

In this second selection, the majority of the random substitution of the aromatic moiety in **29a** maintained or even improved the enzymatic activity with an IC_{50} in the low nM range (Table 2). Some exceptions included its replacement by a 4-pyridyle or substitution by a *para* trifluoromethyl group (compounds **35a** and **38a**, respectively) with a 10-fold decrease in potency and the *para* substitution by an ester leading to an inactive derivative (compound **37a**). However, the cellular assay was even more discriminating, with a 3- to 5-fold decrease in potency between enzymatic and cellular activities for a naphtyl, cyclohexyl and dimethyl substitution (compounds **34a**, **36a**, **40a**, respectively) and more than a 10-fold decrease in potency for *para* and poly substitution by various halogen atoms (compounds **43a** and **46a–53a**). No simple explanation to this phenomenon could be provided, but differences in cellular potency versus the isolated enzyme may originate at the level of membrane crossing, protein binding or chemical stability. However, the *ortho* or *meta* mono and disubstitution by a methyle or an halogen maintained the cellular activity at the low nM range IC_{50} (compounds **39a**, **41a**, **42a**, **44a**, **45a** and **51a**).

Investigation on the amine substitution R_{2xv}

In order to investigate the importance of the N-1 substituent on the imidazole, the unsubstituted derivative **65a** ($R_2 = R_3 = H$) was synthesized by reductive amination of the benzyl caprolactam **12a** with the 1-trityl-4-carboxaldehyde imidazole. Since this compound was devoid of any enzyme inhibitory activity (Table 3), the bis PCB ($R_2 = R_3 = PCB$) **66a**, and the mono PCB derivatives ($R_2 = H$, $R_3 = PCB$) **67a** were synthesized by mono and dialkylation of **64a**. Surprisingly, these compounds were equipotent, but with an IC_{50} one order of magnitude higher that than of **29a.** To assess the potential of this substitution R_2, a series of N-4 substituted derivatives of **65a** was rapidly generated by the

Table 2. Structures and biological data of compounds **34a–53a**



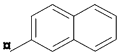
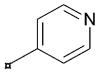
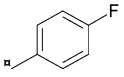
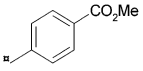
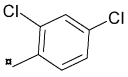
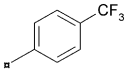
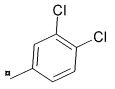
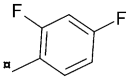
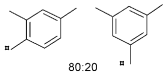
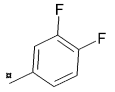
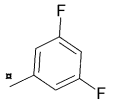
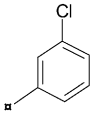
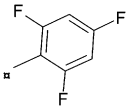
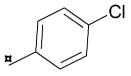
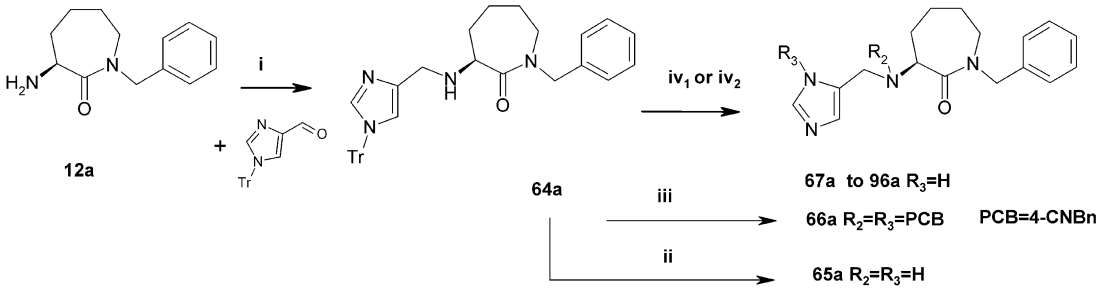
Compd	R_1	FTase IC_{50} (nM)	GGTase IC_{50} (nM)	Ras cell IC_{50} (nM)	Compd	R_1	FTase IC_{50} (nM)	GGTase IC_{50} (nM)	Ras cell IC_{50} (nM)
29a		9	16,200	10					
34a		43	2500	156	**44a**		3	5600	2
35a		87	20,500	10^3	**45a**		5	19,800	4
36a		13	18,400	70	**46a**		2	5100	64
37a		9000	3600	$>10^4$	**47a**		9	ND	160
38a		113	4200	350	**48a**		20	ND	370
39a		5	5500	4	**49a**		2	ND	120
40a	 80:20	7	24,500	46	**50a**		2	9300	44
41a		3	7400	7	**51a**		1	25,800	14
42a		6	10,500	2	**52a**		5	ND	210
43a		19	ND	184	**53a**		41	ND	465

Table 3. Biological data of compounds **65a–96a**

Compd	FTase IC_{50} (nM)	Compd	FTase IC_{50} (nM)
65a	$>10^4$	**67a**	255
66a	298	**68a–96a**	$>10^4$

parallel liquid phase procedure by either aminoreduction with various aldehydes to give compounds **67a** to **82a**, or by *N*-acylation with various acylchlorides to give compounds **83a** to **96a** (Scheme 2, Table 3).

In this third selection, the random amino substitution of **65a** was produced, but did not allow recovery of any significant FTase inhibitory activity (compounds **68a–96a**, Table 3). These data also diverged from the results



Scheme 2. Synthesis of compounds **65a–96a**. (i) $NaBH(OAc)_3$, CH_2Cl_2, 0 °C to rt 12 h, (**64a**, 88%); (ii) (a) TFA, Et_3SiH, CH_2Cl_2, rt, 12 h; (b) fumaric acid, EtOH (**65a**); (iii) (a) $pCNPhCH_2Br$, AcOEt, 60 °C, 12 h; (b) MeOH, 60 °C, 12 h; (c) fumaric acid, EtOH (**66a**); (iv_1) (a) $NaBH(OAc)_3$, R_2CHO, CH_2Cl_2, rt 12 h; (b) TFA, Et_3SiH, CH_2Cl_2, rt, 12 h (**67a–82a**: Fig. 2, Table 9); (iv_2) (a) R_2COCl polyvinylpyridine, CH_2Cl_2, rt 12 h; (b) TFA, Et_3SiH, CH_2Cl_2, rt, 18 h; (c) HCl, ether, or fumaric acid, EtOH (**83a–96a**: Fig. 2, Table 9).

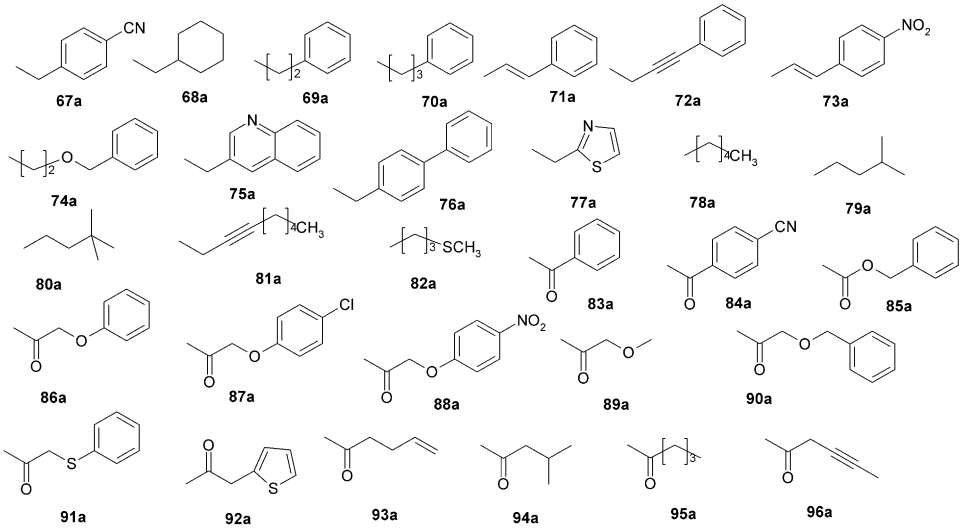


Figure 2. Substitutents of componud **67a–96a** R_3=H R_2=

reported with the aryl scaffold where the N substitution of the imidazol-5-yl-methylamine function either on the imidazole ring or the amine function afforded equipotent compounds.[12] In this case, the authors proposed a nearby second binding pocket at this cysteine region. A plausible explanation, in absence of X-ray data, is a different recognition of the two scaffolds, the caprolactam following the peptide side chain and the aryl being perpendicular allowing the fit of substituents in the various amino acid pocket sites of the enzyme.

Investigation on the imidazol ring substitution R_3

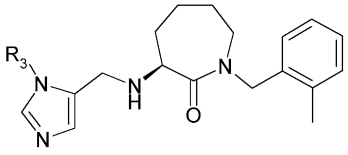
In the fourth selection, the effect of the modification of the N-1 substitution of the imidazole side chain on **39a** was investigated. For this purpose, a series of N-1 substituted imidazol-5-ylcarboxaldehydes **18** to **27** were synthesized from various primary amines by a modification of the method described by Aulaskari.[15] They were then condensed by reductive amination with the amine intermediate precursor of **39a**, to afford compounds **54a** to **63a** (Scheme 1, Table 4).

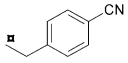
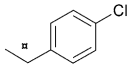
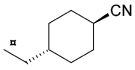
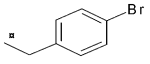
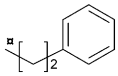
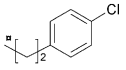
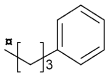
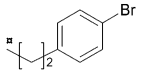
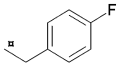
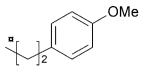
In this selection, the removal of the cyano substituent or the reduction of the aromatic ring (compounds **54a** and **55a**, respectively) led to a 10-fold loss of enzymatic inhibition and a 100-fold loss in cellular potency compared to **39a**. In addition, the spacer elongation by one or two carbons, led to inactive compounds despite further substitution (compounds **56a**, **57a** and **61a–63a**). The replacement of the cyano substituent by various halogen (compounds **58a–60a**) kept the enzymatic activity almost at the same level as those of **39a**, but induced a one order of magnitude loss in cellular potency in spite of an increase in their CLogP. Therefore, no better substitution for the PCB group was found so far.

Pharmacological Evaluation

As a preliminary evaluation of antitumour activity, selected compounds, with low nM IC_{50} in the enzymatic and cellular assays, were tested in an in vivo model. H-Ras transfected fibroblasts were grafted sc into nude mice, and compounds were administered at 100 and 200 mg/kg po for 5 days, starting from day 6 after tumour implantation. Among these compounds, **41a, 42a** and **44a** showed significant activity with a marked reduction of the tumour volume (39, 85 and

Table 4. Structures of compounds **17**–**27** and **54a**–**63a** and biological data of compounds **54a**–**63a**



Compd	Compd	R_3	FTase IC_{50} (nM)	Ras cell IC_{50} (nM)	Compd	Cpmpd	R_3	FTase IC_{50} (nM)	Ras cell IC_{50} (nM)
17	**39a**		5	4					
18	**54a**		48	430	**23**	**59a**		16	180
19	**55a**		81	730	**24**	**60a**		27	190
20	**56a**		3000	ND	**25**	**61a**		3800	ND
21	**57a**		1700	ND	**26**	**62a**		5300	ND
22	**58a**		19	210	**27**	**63a**		2700	ND

40%, respectively, at 200 mg/kg). However, these activities remained lower than those of the reference compound BMS-214662 which inhibited tumour growth by 50 and 80% at 100 and 200 mg/kg, respectively, in this model. Moreover, since signs of toxicity occurred with **42a** at the higher dose, only **41a** and **44a** were selected for further pharmacological evaluation (Table 5).

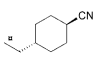
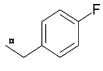
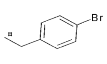
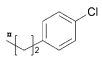
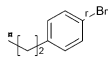
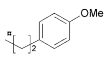
Table 5. In vivo antitumour activity

No.	% Control 100 mg/kg po	% Control 200 mg/kg po
BMS-214662	48	22
29a	83	72
41a	66	61
42a	51	15 (Tox.)
44a	99	60
45a	103	71
51a	126	76

Conclusion

An efficient synthetic access was developed from *N*,*N*′-disubstituted 2-amino caprolactam to generate a new class of inhibitors of FTase selective versus GGTase-1. A rapid lead optimization by liquid parallel synthesis allowed independent investigation of the substituents and afforded very potent compounds with low nM IC_{50} in the enzymatic and cellular assays. It is interesting to note that a differently substituted caprolactam scaffold was previously used to design FTase inhibitors as reverse-turn mimics, but led to almost inactive compounds (IC_{50} in the μM range).[16] The activities of the selected compounds were validated in vivo, and compounds **41a** and **44a** presented significant antitumour activity.

Table 6. Yields and analytical data of compounds **18**–**27**

Compd	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
R_3										
Yield%	33	27	60	37	51	46	52	18	32	26
Analysis	RMN/IR	RMN/IR	RMN/IR	RMN/IR	RMN/IR	RMN/IR	RMN/IR	RMN/IR	RMN/IR	RMN/IR

Experimental

Chemistry general techniques

All reactions were carried out under nitrogen or argon atmosphere under anhydrous conditions unless otherwise noted.

Parallel synthesis was performed on STEM Blocks, Workup was performed on Zymate. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogenous material unless otherwise stated. All reagents were purchased at highest commercial quality and used without further purification unless otherwise stated. All reactions were monitored by thin-layer chromatography carried out on 0.2 mm Merck silicagel plates using UV light and ethanol phosphomolybdic acid or *p*-anisaldehyde and heat as developing agent.

Preparative flash chromatography separations were carried out on Kiesegel 60 (0.04–0.063 mm) Merck silicagel. Normal-phase solid-phase extraction (SPE) were carried out on cartridges loaded with 8 g of Kiesegel 60 (0.04–0.063×mm) Merck silicagel. Reversed-phase chromatography were carried out on columns loaded with of Kiesegel C18 (LiChroprep RP-18; 25–40 μm) Merck. Mobile phase $H_2O/CH_3CN + 0.5\%$ TFA. Reverse-phase HPLC analysis was performed on an Agilent 1100 instrument using a Waters Xterra MS C18 column with detection at 210 nm using a $H_2O/CH_3CN + 0.1\%$ CH_3SO_3H gradient over 10 min. NMR spectra were recorded on a Brucker PPX 200 instrument as indicated, calibrated using TMS as an internal reference. The following abbreviations were used to explain multiplicities: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet; b, broad. IR spectra were recorded on a Brucker Vector 22 spectrophometer.

Electrospray mass spectra were recorded in a positive mode on a Finnigan TSQ 7000 spectrophometer, by infusion at 15 μL/min of a 0.1 mg/mL sample solution in a mixture of CH_3CN/H_2O (75:25).

Preparation of (*R*,*S*) 1-phenyl-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (5). To a solution of **4** (1.6 g, 7 mmol), $Cu(OAc)_2$ (1.36 g, 7 mmol) and pyridine (0.6 mL, 7 mmol) in CH_2Cl_2 (20 mL) was added dropwise triphenylbismuth (3.4 g, 7.6 mmol), at room temperature. The reaction mixture was stirred 24 h and then filtrated over Celite and washed several times with AcOEt. The filtrate was concentrated in vacuo, and the residue was purified by flash chromatography (silica gel, petroleum ether/AcOEt; 60:40) to give **5** (0.84 g, 38%) as an oil. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.73 (m, 5H, *Ph*), 3.85–3.65 (m, 3H, CH_2N, NC*H*CO), 2.2–1.7 (m, 6H, (CH_2)$_3$), 1.5 (s, 9H, *tBu*). IR: V_{max} 3375–3317, 1647 cm^{-1}

Preparation of (*S*) 1-phenyl-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (5a). Compound **5a** (1.2 g, 43%) was prepared by the same procedure used for **5** with compound **4a** (2 g, 8.7 mmol), $Cu(OAc)_2$ (1.7 g, 8.7 mmol) and pyridine (0.7 mL, 8.7 mmol) and triphenylbismuth (4.2 g, 9.6 mmol) in CH_2Cl_2 (22 mL). RMN; IR similar to **5**.

Preparation of (*R*,*S*) 1-benzyl-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (6). To a suspension of NaH (60% in oil, 1.9 g, 47 mmol) in THF (20 mL) was added a solution of compound **4** (9.8 g, 43 mmol) in THF (90 mL) at room temperature. Then this mixture was stirred for 1 h and a solution of benzyl bromide (7.6 mL, 64 mmol) in THF (20 mL) was added. After an additional stirring of 2 h the reaction mixture was cooled down to 0 °C, treated with a satured solution of NH_4Cl and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, heptane/AcOEt; 70:30) to give compound **6** as a solid (12.7 g, 93%). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.25 (m, 5H, *Ph*), 4.85 (d, 1H, *CH*Ph), 4.4 (m, 2H, *CH*Ph, *CH*NH), 3.6–3.15 (2dd, 2H, CH_2N), 2.2–1.8 (m, 6H, (CH_2)$_3$) 1.5 (s, 9H, *tBu*). IR: V_{max} 3413, 1711, 1648 cm^{-1}. Anal. ($C_{18}H_{26}N_2O_3$): C, H, N calcd: 67.90; 8.23; 8.80. Found: 67.82; 8.21; 8.81.

Preparation of (*S*) 1-benzyl-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (6a). Compound **6a** (0.615 g, 94%) was prepared by the same procedure used for **6** with compound **4a** (0.5 g, 2.2 mmol), NaH (60% in oil, 0.096 g, 2.4 mmol) and benzyl bromide (0.46 mL, 3.6 mmol) in THF (5 mL). RMN; IR similar to **6**.

Preparation of (*R*,*S*) 1-(2-phenylethyl)-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (7). Compound **7** (0.45 g, 41%) was prepared by the same procedure used for **6** with compound **4** (1 g, 4.3 mmol), NaH (60% in oil, 0.2 g, 4.8 mmol) and 2-phenylethyltosylate (1.3 g, 4.8 mmol) in DMF (5 mL). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.3 (m, 5H, *Ph*), 4.25 (m, 1H, *CH*NH), 3.5–3.25 (m, 4H, CH_2NCH_2), 2.7 (d, 2H, CH_2Ph), 2.1–1.7 (m, 6H, (CH_2)$_3$) 1.4 (s, 9H, *tBu*). IR: V_{max} 3422, 1703, 1640 cm^{-1}

Preparation of (*S*) 1-(2-phenylethyl)-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (7a). Compound **7a** (5.1 g, 47%) was prepared by the same procedure used for **6** with compound **4a** (7.4 g, 32.3 mmol), NaH (60% in oil, 1.4 g, 35 mmol) and 2-phenylethyltosylate (10.7 g, 38.6 mmol) in DMF (130 mL). RMN; IR similar to **7**.

Preparation of (*R*,*S*) 1-(3-phenylpropyl)-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (8). Compound **8** (2.6 g, 86%) was prepared by the same procedure used for **6** with compound **4** (2 g, 8.7 mmol), NaH (60% in oil, 0.384 g, 9.5 mmol) and 3-phenylpropyltosylate (3 g, 10.3 mmol) in DMF (43 mL). ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.4 (m, 5H, *Ph*), 4.4 (dd, 1H, *CH*NH), 3.8–3.3 (m, 4H, CH_2NCH_2), 2.7 (d, 2H, CH_2Ph), 2.1–1.7 (m, 6H, 2(CH_2)$_2$) 155 (s, 9H, *tBu*). IR: V_{max} 3411, 1711, 1642 cm^{-1}.

Preparation of (*R*,*S*) 1-(benzenesulfonyl)-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (9). Compound **9** (6 g, 77%) was prepared by a similar procedure used for **6** with compound **4** (5 g, 21.8 mmol), LiHMDS (1.3 M in THF, 20 mL, 26 mmol) and benzenesulfonyl chloride (3.3 mL, 26 mmol) in THF (50 mL) at 0 °C. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.95–7.55 (m, 5H, *Ph*),

4.5–4.3 (m, 2H, *CHa*N, NC*H*CO), 3.75–4.3 (dd, 1H, *CHa'*N), 2.0–1.4 (m, 6H, $(CH_2)_3$), 1.35 (s, 9H, *tBu*). IR: V_{max} 3426, 1702 cm^{-1}.

Preparation of (*R*,*S*) 1-(benzenesulfonylmethyl)-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine (10). Preparation of (*R*,*S*) 1-(benzenesulfinylmethyl)-2-oxo-3-(*tert*-butoxycarbonylamino)-azepine. To a mixture a solution of compound **4** (1 g, 4.36 mmol), phenylsulfinylmethyl chloride (1.7 mL, 13 mmol) and NBu_4I (0.24 g, 0.64 mmol) in THF (10 mL) was added KOH in powder (0.73 g, 13 mmol) at room temperature. Then this mixture was stirred overnight at room temperature and treated with a saturated solution of NH_4Cl and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, petroleum ether/AcOEt; 85:15) to give the title compound as an oil (0.745 g, 49%). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.8–7.4 (m, 5H, *Ph*), 5.1–4.8 (AB, 2H, SCH_2), 4.25 (m, 1H, *CH*NH), 3.55 (m, 2H, CH_2N), 2.7 (d, 2H, CH_2Ph), 1.8 (m, 6H, $(CH_2)_3$), 1.3 (s, 9H, *tBu*). IR: V_{max} 3415, 1711, 1654 cm^{-1}.

Preparation of (10). To a solution of compound described in step a (0.46 g, 1.3 mmol), in acetone (3 mL) was added dropwise a solution of dimethyldioxirane (0.05 M, in acetone, 34 mL, 1.7 mmol) at 0 °C. The reaction mixture was stirred 2 h at room temperature and then concentrated in vacuo to give **10** (0.32 g, 81%) as a white solid. ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.8–7.4 (m, 5H, *Ph*), 5.1–4.8 (AB, 2H, SCH_2), 4.25 (m, 1H, *CH*NH), 3.55 (m, 2H, CH_2N), 2.7 (d, 2H, CH_2Ph), 1.8 (m, 6H, $(CH_2)_3$), 1.3 (s, 9H, *tBu*). IR: V_{max} 3408, 1707, 1653, 1383, 1148 cm^{-1}. Anal. ($C_{18}H_{26}N_2O_5S$): C, H, N, S calcd: 56.53; 6.85; 7.32, 8.38. Found: 56.53; 6.86; 7.04, 8.21.

Preparation of (*R*,*S*) 1-phenyl-2-oxo-3-amino-azepine (11). To a solution of **5** (0.69 g, 2.3 mmol) in CH_2Cl_2 (14 mL) was added dropwise trifluoroacetic acid (1.7 mL) at room temperature. The reaction mixture was stirred 2 h and then concentrated in vacuo. The residue was purified by flash chromatography (silica gel, CH_2Cl_2/MeOH; 95:5) to give **11** (0.32 g, 70%) as an oil. ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.3 (m, 5H, *Ph*), 3.85–3.65 (m, 3H, CH_2N, NC*H*CO), 2.2–1.7 (m, 6H, $(CH_2)_3$). IR: V_{max} 3375–3317, 1647 cm^{-1}.

Preparation of (*S*) 1-phenyl-2-oxo-3-amino-azepine (11a). Compound **11a** (0.53 g, 69%) was prepared by the same procedure used for **11** with compound **5a** (1.2 g, 3.9 mmol) and trifluoroacetic acid (3 mL). RMN; IR similar to **11**.

Preparation of (*R*,*S*) 3-amino-2-oxo-1-benzyl-azepine (12). To a solution of **6** (12.7 g, 40 mmol) in dioxane (20 mL) was added dropwise a solution of 4 N HCl in dioxane (100 mL) at room temperature. The reaction mixture was stirred 3 h and then concentrated in vacuo. The residue was triturated in ether to give **12** (10.6 g, 100%) which was used for the next step without further purification. ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.3 (m, 5H, *Ph*), 4.7–4.5 (2d, 2H, CH_2Ph), 4.35 (m, 1H, *CH*NH), 3.6–3.3 (m, 2H, CH_2N), 2.2–1.2 (m, 6H, $(CH_2)_3$). IR: V_{max} 3414, 2937, 1657 cm^{-1}. Anal. ($C_{13}H_{18}N_2O$) HCl, $2H_2O$: C, H, N calcd: 53.70; 9.63; 11.9. Found: 53.14; 7.94; 9.2.

Preparation of (*S*) 1-benzyl-2-oxo-3-amino-azepine (12a). Compound **12a** (5.05 g, 95%) was prepared by the same procedure used for **11** with compound **6** (7.74 g, 24.3 mmol) and trifluoroacetic acid (19 mL). RMN; IR similar to **12**.

Preparation of (*R*,*S*) 1-(2-phenylethyl)-2-oxo-3-amino azepine (13). Compound **13** (1.4 g, 95%) was prepared by the same procedure used for **11** with compound **7** (2 g, 6.05 mmol) and trifluoroacetic acid (4.7 mL). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.3 (m, 5H, *Ph*), 4.15 (m, 1H, *CH*NH), 3.4–3.25 (m, 4H, CH_2NCH_2), 2.6 (d, 2H, CH_2Ph), 2–1.3 (m, 6H, $(CH_2)_3$). IR: V_{max} 3362, 3297, 1645 cm^{-1}.

Preparation of (*S*) 1-(2-phenylethyl)-2-oxo-3-amino-azepine (13a). Compound **13**a (3.2 g, 91%) was prepared by the same procedure used for **11** with compound **7a** (5.1 g, 15.2 mmol) and trifluoroacetic acid (12 mL). RMN; IR similar to **13**. Anal. ($C_{14}H_{20}N_2O$) $0.25H_2O$: C, H, N calcd: 70.93; 8.65; 11.82. Found: 70.71; 8.53; 11.49.

Preparation of (*R*,*S*) 3-amino-2-oxo-1-(3-phenylpropyl)-azepine (14). Compound **14** (1.2 g, 100%) was prepared by the same procedure used for **11** with compound **8** (1.3 g, 3.74 mmol) and trifluoroacetic acid (2.9 mL). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.25 (m, 5H, *Ph*), 4.1 (dd, 1H, *CH*NH), 3.7–3.3 (m, 4H, CH_2NCH_2), 2.55 (d, 2H, CH_2Ph), 1.9–1.25 (m, 6H, $2(CH_2)_2$). IR: V_{max} 3500–3000, 1668 cm^{-1}.

Preparation of (*R*,*S*) 3-amino-2-oxo-1-(benzenesulfonyl)-azepine (15). Compound **15** (3.45 g, 79%) was prepared by the same procedure used for **11** with compound **9** (6 g, 16.2 mmol) and trifluoroacetic acid (12.5 mL). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.95–7.6 (m, 5H, *Ph*), 4.4 (m, 1H, *CHa*N, NC*H*CO), 3.95 (d, 1H, NC*H*CO), 3.7 (dd, 1H, *CHa'*N), 2.1–1.8 (m, 6H, $(CH_2)_3$). IR: V_{max} 3600–2300, 1700 cm^{-1}.

Preparation of (*R*,*S*) 3-amino-2-oxo-1-(benzenesulfonylmethyl)-azepine (16). Compound **16** (0.31 g, 100%) was obtained as a yellow gum by the same procedure used for **11** with compound **10** (0.4 g, 1.04 mmol) and trifluoroacetic acid (0.8 mL). ^1H NMR (200 MHz, $CDCl_3$): δ (ppm) 7.85 (d, 2H, o-*Ph*), 7.75–7.5 (m, 3H, m, p-*Ph*), 5.0 (AB, 2H, SCH_2), 3.7 (m, 1H, *CH*NH), 3.5–3.3 (m, 2H, CH_2N), 2.7 (d, 2H, CH_2Ph), 1.8–1.2 (m, 6H, $(CH_2)_3$). IR: V_{max} 3367–3100, 1670 cm^{-1}.

Preparation of [3-(4-cyanobenzyl)-3-H-imidazol-4-yl]-carboxaldehyde (17). Step 1: Preparation of [3-(4-cyanobenzyl)-3-H-2-thioimidazol-4-yl]-methyl alcohol. To a mixture of 4-cyanobenzylamine (18 g, 100 mmol), and propionic acid (30 mL) in H_2O/CH_3CN (97:3, 300 mL)

was added sequentially KSCN (11 g, 115 mmol) and dihydroxyacetone (dimer, 11.2 g, 115 mmol). This mixture was stirred for 2 h at 60 °C and overnight at room temperature. Then the precipitate was filtrated off and was washed with CH_3CN and then H_2O. The solid was dried in vacuo to give the title compound (24 g, 98%) which was used for the next step without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 12.2 (s, 1H, *SH*), 7.8–7.35 (2d, 4H, *Ph*), 6.9 (s, 1H, N*CH*N), 5.4 (s, 2H, Ph*CH*$_2$N), 4.15 (s, 1H, *CH*$_2$O), 5.25 (s, 2H, *OH*). IR: V_{max} 3600–3200, 2300, 2222, 1025 cm^{-1}. MH$^+$ (246).

Step 2: Preparation of [3-(4-cyanobenzyl)-3-H-imidazol-4-yl]-methyl alcohol. To a solution of the thioalcohol obtained in step 1 (23 g, 100 mmol) in diluted acetic acid (H_2O/AcOH; 80:20, 250 mL) was added dropwise an aqueous solution of H_2O_2 (50%, 20 mL, 700 mmol) during 10 min and the temperature was maintained below 40 °C. Then this mixture was stirred for 3 h at room temperature and treated with an aqueous solution of Na_2SO_3 (20%, 450 mL) and filtrated. The filtrate was neutralized with aqueous ammonia (5%) and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtrated, concentrated in vacuo and dried to give the title compound (14 g, 74%) which was used for the next step without further purification. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.75–7.3 (2d, 4H, *Ph*), 7.7 (s, 1H, N*CH*=N), 6.85 (s, 1H, N*CH*=C), 5.35 (s, 2H, Ph*CH*$_2$N), 5.15 (s, 2H, *OH*), 4.3 (s, 1H, *CH*$_2$O). IR: V_{max} 3200, 2232, 1025 cm^{-1}.

Step 3: Preparation of 17. To a solution of the alcohol obtained in step 2 (10 g, 47 mmol) and TEA (26 mL, 200 mmol) in DMSO (230 mL) was added portion wise pyridine sulfur trioxide (50%, 18.6 g, 117 mmol) during 10 min at room temperature. Then this mixture was stirred for 3 h at room temperature, treated with a saturated solution of NH_4Cl and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtrated and concentrated in vacuo. The residue was triturated in diethyl ether to give **17** (9.6 g, 95%) as a white solid. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.75 (s, 1H, *CH*O) 7.9–7.8 (2s, 2H, N*CH*=C, N*CH*=N), 7.65–7.25 (2d, 4H, *Ph*), 5.6 (s, 2H, Ph*CH*$_2$N). IR: V_{max} 2229 cm^{-1}.

Preparation of compounds [3-(R_3)-3-H-imidazol-4-yl]-carboxaldehyde 18–27. The aldehydes **18** to **27** listed in Table 6 were obtained by the same procedure used for **17** by using the corresponding amine in step 1.

Preparation of (*R*,*S*) 4-{5-[(1-phenyl-2-oxo-azepan-3-yl-amino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (28). To a mixture of compounds **11** (0.28 g, 1.4 mmol) and **17** (0.29 g, 1.4 mmol) in DCE (5 mL), was added NaBH(OAc)$_3$ (0.435 g, 2.1 mmol) at 0 °C. Then this mixture was stirred overnight at room temperature and concentrated in vacuo. The residue was diluted with CH_2Cl_2 washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography (silica gel, CH_2Cl_2/MeOH; 95:5) gave compound **28** (0.28 g, 50%) as an oil which was crystallized in diethylether as the fumarate. ^{1}H NMR (200 MHz, $CDCl_3$): δ (ppm) 9.3 (s, 1H, N*CH*N), 7.85–7.35 (m 9H, *Ph*), 3.85–3.65 (m, 3H, *CH*$_2$N, NC*H*CO), 2.2–1.7 (m, 6H, (C*H*$_2$)$_3$). IR: V_{max} 2800, 2229, 1702, 1655, 1561 cm^{-1}. Anal. ($C_{24}H_{25}$ N_5O) 1.4 C_4H_4 O_4, 1H_2O. C, H, N calcd: 61.72; 5.62; 12.06. Found: 61.40; 5.64; 11.74.

Preparation of (*S*) 4-{5-[(1-phenyl-2-oxo-azepan-3-yl-amino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (28a). Compound **28a** (0.22 g, 65%) was prepared by the same procedure used for **28** with compound **11a** (0.2 g, 0.98 mmol), **17** (0.21 g, 0.98 mmol) and NaBH(OAc)$_3$ (0.31 g, 1.5 mmol). Mp: 75 °C (decomposed); RMN; IR similar to **28**. Anal. ($C_{24}H_{25}$ N_5O) 1.2 C_4H_4 O_4, 1H_2O: C, H, N calcd: 61.82; 5.69; 12.5. Found: 64.15; 6.05; 11.80. The enantiomeric excess was determined by HPLC using a Chiralcel OD column (eluant/heptane/isopropanol:DEA; 500:500:0.5) and a UV detector at 230 nm (ee 89%).

Preparation of (*R*,*S*) 4-{5-[(1-benzyl-2-oxo-azepan-3-yl-amino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (29). Compound **29** was prepared by the same procedure used for **28** with compound **12** (10.6 g, 40 mmol), **17** (11.05 g, 51.6 mmol), and NaBH(OAc)$_3$ (8.9 g, 43 mmol). Flash chromatography (silica gel, CH_2Cl_2/MeOH; 95:5) gave compound **29** as an oil which was crystallized in diethylether as the dihydrochloride (10.2 g, 52%). ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.25 (s, 1H, N*CH*N), 7.85–7.3 (m 9H, *PhPh*), 5.85 (s, 2H, *CH*$_2$Ph), 4.8–4.4 (m, 3H, *CH*$_2$Ph, *CH*NH), 3.7–3.25 (m, 2H, *CH*$_2$N), 2.35–1.2 (m, 6H, (C*H*$_2$)$_3$). IR: V_{max} 3317, 2226, 1651, 1538 cm^{-1}. Anal. ($C_{25}H_{27}$ N_5O) 2HCl, 0.5H_2O: C, H, N calcd: 60.60; 6.26; 14.14. Found: 61.06; 5.93; 14.18.

Preparation of (*S*) 4-{5-[(1-benzyl-2-oxo-azepan-3-yl-amino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (29a). Compound **29a** was prepared as the dihydrochloride (0.135 g, 26%) by the same procedure used for **28** with compound **12a** (0.25 g, 1.14 mmol), **17** (0.24 g, 1.14 mmol) and NaBH(OAc)$_3$ (0.34 g, 1.6 mmol). RMN; IR similar to **29**. Anal. ($C_{25}H_{27}$ N_5O) 2HCl, 1H_2O: C, H, N calcd: 59.46; 6.14; 13.87. Found: 59.58; 6.17; 13.45. The enantiomeric excess was determined by HPLC using a Chiralcel OD column (eluant: methanol/DEA; 1000:1) and a UV detector at 230 nm. (ee 90%).

Preparation of the enantiomers of 2[2-(4′-chlorobiphenyl-thio)]-5-[2-(4-oxo-4H-benzo[d] [1,2,3]triazin-3-ylmethyl)]-cyclopentanecarboxylic acid (29a/29b). The enantiomeric mixture **29** (1.6 g) was separated by preparative HPLC using a Chiralpack AD 250*4.6 mm column (eluant: methanol/DEA; 1000:1) and a UV detector at 230 nm to give: Pic 1: **29a** (0.6 g, elution time 7.75 min), ee >98%, $[\alpha]_D^{20} = +6.69$ (*c* 0.99; MeOH); ^{1}H NMR RMN; IR similar to **29**. Anal. ($C_{25}H_{27}$ N_5O) 2HCl, 1H_2O: C, H, N calcd: 59.46; 6.14; 13.87. Found: 59.68; 6.43; 13.90. Pic 2**: 29b** (0.6 g, elution time 11.5 min), ee >99%, $[\alpha]_D^{20} = -6.52$ (*c* 0.89; MeOH); ^{1}H NMR RMN; IR similar to **29**. Anal. ($C_{25}H_{27}$ N_5O) 2HCl, 1H_2O: C,

H, N calcd: 59.46; 6.14; 13.87. Found: 59.67; 6.49; 13.88.

Preparation of (*R*,*S*) 4-{5-[(1-(2-phenylethyl)-2-oxo-azepan-3-ylamino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (30). Compound **30** was prepared as the dihydrochloride (0.155 g, 31%) by the same procedure used for **28** with compound **13** (0.25 g, 1.1 mmol), **17** (0.23 g, 1.1 mmol) and $NaBH(OAc)_3$ (0.34 g, 1.6 mmol). Mp: 227 °C; ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.2 (s, 1H, N*CH*N), 7.95–7.25 (m, 10H, N*CH*, *PhPh*), 5.8 (AB, 2H, CH_2Ph), 4.55 (m, 1H, *CH*NH), 4.25–3.85 (AB, 2H, CH_2N), 3.8–3.3 (m, 4H, CH_2N, CH_2N), 2.75 (t, 2H, CH_2Ph), 2.3–1.2 (m, 6H, $(CH_2)_3$). IR: V_{max} 3600–2400, 2236, 1659, 1606 cm^{-1}. Anal. ($C_{25}H_{27}N_5O$) 2HCl, 1.5H_2O: C, H, N calcd: 59.14; 5.87; 13.27. Found: 58.86; 6.28; 13.11.

Preparation of (*S*) 4-{5-[(1-(2-phenylethyl)-2-oxo-azepan-3-ylamino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (30a). Compound **30a** was prepared as the dihydrochloride (0.215 g, 22%) by the same procedure used for **28** with compound **13a** (0.5 g, 2.15 mmol), **17** (0.45 g, 2.15 mmol) and $NaBH(OAc)_3$ (0.64 g, 3 mmol). Mp: 167 °C; RMN; IR similar to **30**. Anal. ($C_{25}H_{27}N_5O$) 2HCl, 2H_2O: C, H, N calcd: 58.14; 5.96; 13.04. Found: 57.76; 6.33; 12.76. The enantiomeric excess was determined by HPLC using a Chiralcel OD column (eluant: heptane/isopropanol/DEA; 500:500:0.5) and a UV detector at 210 nm. (ee 85%).

Preparation of (*R*,*S*) 4-{5-[1-(3-phenylpropyl)-2-oxo-azepan-3-ylamino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (31). Compound **31** was prepared as the fumarate (0.12 g, 19%) by the same procedure used for **28** with compound **14** (0.3 g, 1.2 mmol), **17** (0.26 g, 1.2 mmol) and $NaBH(OAc)_3$ (0.52 g, 2.45 mmol). Mp: 180 °C; ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.75–7.1 (m, 10H, N*CH*N *PhPh*), 6.8 (s, 1H, N*CH*), 6.6 (s, 2H, *CH=CH*), 5.45 (s, 2H, PhCH_2NH), 3.5 (AB, 2H, ImCH_2N), 3.1–2.55 (m, 7H, *CH*NH, 2 CH_2N, CH_2Ph), 1.8–1.1 (m, 8H, (CH_2), $(CH_2)_3$). IR: V_{max} 2800–1800, 2229, 1655, 1577 cm^{-1}. Anal. ($C_{27}H_{31}N_5O$) $C_4H_4O_4$: C, H, N calcd: 66.77; 6.33; 12.56. Found: 66.67; 6.38; 12.54. M^-H^+ (440)

Preparation of (*R*,*S*) 4-{5-[(1-(benzenesulfonyl)-2-oxo-azepan-3-ylamino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (32). Compound **32** was prepared as the free base (0.193 g, 37%) by the same procedure used for **28** with compound **15** (0.3 g, 1.11 mmol), **17** (0.236 g, 1.11 mmol) and $NaBH(OAc)_3$ (0.33 g, 1.56 mmol). ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.95–7.25 (m, 10H, N*CH*N *PhPh*), 6.75 (s, 1H, N*CH*=), 6.5 (s, 2H, *CH=CH*), 5.35 (s, 2H, PhCH_2N), 4.35 (m, 1H, *CH*NH), 3.8–3.6 (2dd, 2H, CH_2NH), 3.4 (m, 2H, CH_2NSO_2), 2.0–1.1 (m, 6H, $(CH_2)_3$). IR: V_{max} 3308, 2227, 1693, 1348, 1164 cm^{-1}. Anal. ($C_{24}H_{25}N_5O_3S$) $C_4H_4O_4$: C, H, N calcd: 62.19; 5.44; 15.11. Found: 62.15; 5.44; 14.80. MH^+ (464).

Preparation of (*R*,*S*) 4-{5-[(1-(benzenesulfonylmethyl)-2-oxo-azepan-3-ylamino)-methyl]-imidazol-1-ylmethyl}-benzonitrile (33). Compound **33** was prepared as the dihydrochloride (0.156 g, 27%) by the same procedure used for **28** with compound **16** (0.27 g, 1.04 mmol), **17** (0.2 g, 1.04 mmol) and $NaBH(OAc)_3$ (0.44 g, 2.1 mmol). Mp: decomposed. ^1H NMR (200 MHz, DMSO-d_6): δ (ppm) 9.2 (s, 1H, N*CH*N), 7.95–7.4 (m, 9H, *PhPh*), 7.75 (s, 1H, N*CH*=), 5.75 (AB, 2H, PhCH_2N), 5.05 (AB, 2H, SO_2CH_2N), 4.35 (AB, 2H, CH_2NH), 3.8–3.6 (2dd, 2H, CH_2NH), 3.4 (m, 2H, CH_2NSO_2), 2.35–1.3 (m, 6H, $(CH_2)_3$). IR: V_{max} 3407, 2800–2300, 2231, 1643, 1321, 1141 cm^{-1}. Anal. ($C_{25}H_{27}N_5O_3S$) 2HCl, H_2O: C, H, N, S calcd: 52.77; 5.45; 12.31; 5.62. Found: 52.72; 5.55; 11.75; 5.45. MH^+ (478).

Preparation of compounds 34a–53a: general procedure

To a solution of compound **4a** (0.23 g; 1 mmol) in THF (5 mL) was added NaH 80% in oil (32 mg) at 0 °C. After 5 min agitation, nBu_4NI (37 mg; 1% mol) and the corresponding benzyl bromide (1.05 mmol) were added. After 1 h agitation at room temperature the reaction was quenched by adding of saturated NH_4Cl solution (1 mL) and H_2O (4 mL) then extracted twice with AcOEt (5 mL). The organic layer was dried over $MgSO_4$ filtrated and evaporated to dryness. The crude compound was treated with HCl (4 M) solution in dioxane (10 mL) and agitated at room temperature until complete Boc deprotection. The solvent was removed and the residue was dissolved in CH_2Cl_2 (10 mL) and washed with saturated $NaHCO_3$ (5 mL) dried over $MgSO_4$ filtrated and evaporated to dryness. The residue was dissolved in CH_2Cl_2 (5 mL) and aldehyde **17** (1 equiv) and $NaBH(OAc)_3$ (1.5 equiv) were added successively. After one night at room temperature was added saturated $NaHCO_3$ (5 mL) and the compound was extracted twice with CH_2Cl_2 (5 mL). The organic layer was dried over $MgSO_4$ filtrated and evaporated to dryness. The final compound was purified by reversed phase SPE affording the pure corresponding compound as a TFA salt. This salt was dissolved in CH_2Cl_2 (10 mL) and washed with saturated $NaHCO_3$ solution (5 mL). The organic layer was dried over $MgSO_4$ filtrated and evaporated to dryness to afford the free base of compounds **34a** to **53a** which was transformed into hydrochloride or fumarate salt to form an amorphous solid (%, Table 7).

Preparation of compounds 54a–63a

Compounds **54a**–**63a** were prepared as dihydrochloride or fumarate by the same procedure used for **29a** by reductive amination of the various aldehydes **18**–**27** with the intermediate amine of **39a** and $NaBH(OAc)_3$ (%, Table 8).

Preparation of (*S*) 1-benzyl-3-[1-trityl-imidazol-4-yl-methyl)amino]-azepan-2-one (64a). To a mixture of compounds **12a** (7 g, 24.2 mmol) and 1-trityl-4-carboxaldehyde imidazol (8.2 g, 24.2 mmol) in DCE (85 mL), was added $NaBH(OAc)_3$ (10.2 g, 48.5 mmol) at 0 °C. Then this mixture was stirred overnight at room temperature and concentrated in vacuo. The residue was diluted with CH_2Cl_2 washed with saturated $NaHCO_3$, brine, dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography (silica gel, CH_2Cl_2/MeOH; 95:5)

gave compound **64a** (10.5 g, 81%) as a foam. ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.45–7.1 (m, 20H, 4 *Ph*), 7.35 (s, 1H, NC*H*N), 6.75 (s, 1H, NC*H*=), 4.55 (AB, 2H, CH_2Ph), 3.8–3.5 (m, 3H, C*H*NH, CH_2N), 1.9–1.1 (m, 6H, (CH_2)$_3$). IR: V_{max} 3300, 1644 cm^{-1}.

Preparation of (*S*) 1-benzyl-3-[3H-imidazol-4-ylmethyl)-amino]-azepan-2-one(65a). A solution of **64a** (0.5 g, 0.925 mol) in HCO_2H (5 mL) was stirred 3 h at room temperature and then concentrated in vacuo. The residue was diluted in ether and extracted with 1 N aqueous HCl. The aqueous phase was treated with 10 N aqueous NaOH (to pH > 10) and extracted with CH_2Cl_2. The organic phase was dried over Na_2SO_4 and concentrated in vacuo to give **65a** which was crystallised as the fumarate (0.26 g, 67%). Mp: 60 °C decomposed; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.65 (s, 1H, NC*H*N), 7.3 (m, 5H, *Ph*), 7.0 (s, 1H, NC*H*=), 6.5 (s, 2H, *CH*=*CH*), 4.55 (AB, 2H, CH_2NH), 3.85 (AB, 2H, CH_2NH), 3.55–3.2 (m, 2H, CH_2N), 1.95–1.3 (m, 6H, (CH_2)$_3$). IR: V_{max} 3600, 2200, 1705–1659, 1620, 1567 cm^{-1}. Anal. ($C_{17}H_{22}N_4O$) 1.25 $C_4H_4O_4$, 1H_2O: C, H, N calcd: 57.28; 6.72; 12.14; 5.62. Found: 57.56; 6.70; 11.90.

Preparation of (*S*) 4-{[3-(4-cyanobenzyl-3H-imidazol-4-ylmethyl)-(1-benzyl-2-oxo-azepan-3-yl)-amino]-methyl}-benzonitrile (66a). Preparation of (*S*) 4-{[1-trityl-1H-imidazol-4-ylmethyl)-(1-benzyl-2-oxo-azepan-3-yl)-amino]-methyl}-benzonitrile. To a suspension of NaH (60% in oil, 0.11 g, 2.75 mmol) in DMF (6 mL) was added a solution of compound **64a** (1 g, 1.85 mmol) in DMF (5 mL) at 0 °C. Then this mixture was stirred 1 h and a solution of *p*-cyanobenzyl bromide (0.725 g, 2.7 mmol) and NBu_4I (0.07 g, 0.2 mmol) in DMF (2 mL) was added. After an additional stirring of 2 h, the reaction mixture was cooled down to 0 °C, treated with a saturated solution of NH_4Cl and extracted with AcOEt. The organic phase was dried over Na_2SO_4, filtrated and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, heptane/AcOEt; 50:50) to give the title compound as a yellow foam (0.585 g, 48%) which was used directly for the next step.

Preparation of (66a). To a solution of the compound obtained in step a (0.25 g, 0.38 mmol) in AcOEt (1 mL) was added a solution of *p*-cyanobenzyl bromide (0.075 g, 0.38 mmol) in AcOEt (1 mL) at room temperature. Then the reaction mixture was heated at 60 °C overnight and concentrated in vacuo. The residue was treated with MeOH (2 mL), heated at 60 °C for 2 h and concentrated in vacuo. The residue was purified by flash chromatography (silica gel, AcOEt/EtOH, 90:10) to give **66a** which was crystallized as a fumarate (0.036 g, 18%). Mp: > 100 °C decomposed; ^{1}H NMR (200 MHz, DMSO-d_6): δ (ppm) 7.8–7.0 (m, 15H, NC*H*=, NC*H*N, 3*Ph*), 7.75 (s, 1H, NC*H*=), 5.21 (AB, 2H, PhCH_2N), 4.55 (AB, 2H, PhCH_2N), 4.2–3.5 (2AB, 4H, CH_2NCH_2), 3.6 (m, 1H, C*H*N), 3.15 (m, 2H, CH_2N), 2.35–1.3 (m, 6H, (CH_2)$_3$). IR: V_{max} 3300–2300, 2228, 1700, 1645 cm^{-1}. Anal. ($C_{33}H_{32}N_6O$) 1$C_4H_4O_4$, 0.5H_2O: C, H, N calcd: 67.89; 5.66; 12.84. Found: 67.87; 5.91; 12.32.

Preparation of compounds 67a–96a. Preparation of compounds 67a–82a. General procedure. To a solution of compound **64a** (200 mg, 0.37 mmol) in CH_2Cl_2 (5 mL) is added the corresponding aldehyde (0.37 mmol) at room temperature followed by $NaBH(OAc)_3$ (118 mg, 0.55 mmol). After one night at room temperature is added saturated $NaHCO_3$ (5 mL). After 10 min the organic layer was recovered and the aqueous solution was extracted twice with CH_2Cl_2 (5 mL). The organic layers were pooled and dried over magnesium sulphate, filtrated and evaporated to dryness. The recovered compound was dissolved in CH_2Cl_2 (2 mL), treated by Et_3SiH (0.1 mL) and TFA (2 mL). After overnight agitation at room temperature, the solvent was removed under vacuum and the final compound was purified by reversed-phase chromatography affording the pure corresponding compound as a TFA salt (%, Table 9).

Preparation of compounds 83a–96a. General procedure. To a solution of compound **10** (170 mg, 0.31 mmol) in THF (5 mL) is added polyvinyl pyridine (500 mg) and the corresponding acylchloride or chloroformiate (0.4 mmol) at room temperature. After overnight agitation, the reaction mixture is filtrated and the resin is washed twice with ethyl acetate (5 mL). The organic layer was concentrated and the compound purified by quick filtration on normal phase SPE (AcOEt/CH_2Cl_2). The recovered compound was dissolved in CH_2Cl_2 (2 mL), treated by Et_3SiH (0.1 mL) and TFA (2 mL). After overnight agitation at room temperature, the solvent was removed under vacuum and the final compound was purified by reversed phase chromatography affording the pure corresponding compound as a TFA salt (%, Table 9).

Enzyme assays

FTase and GGTase-1 were purified from rat brains and enzymatic activities were evaluated using the Amersham scintillation proximity assay with the C-terminal sequence of lamin B as a substrate for FTase (biotin-YRASNRSCAIM) and the biotin-TKCVIL sequence for GGTase; [^{3}H]-farnesyl- and [^{3}H]-geranylgeranyl-pyrophosphate were used as donors. IC_{50} of each product on each enzyme was calculated by EXCEL software using three points in the central linear range of fluorescence inhibition.

Cellular assays

The rat cell line Ras#1 obtained after transfection of fibroblastic RAT2 cells with *v-H-Ras*[17] reverts to the fibroblastic phenotype after treatment with FTase inhibitors. This effect is accompanied by a reduction in growth kinetics and cell viability. When quantifying cell number as a function of dose, this translates in the formation of a plateau at doses effective to inhibit FTase eventually followed by a cytotoxic decrease at higher doses. A serum batch was chosen to ensure that this plateau occurred at less than 50% of the cell number so that IC_{50s} indicated in the tables refer to cell inhibition of FTase when appropriate and not to unrelated cytotoxicity of

the compounds. Cells were treated for four days in 96-well tissue culture plates and cell number estimated indirectly through a tetrazolium viability assay.

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