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Synthesis of *N*,*N*′-disubstituted 3-aminobenzo[*c*] and [*d*]azepin-2-ones as potent and specific farnesyl transferase inhibitors

Thierry Le Diguarher,[a] Jean-Claude Ortuno,[a] David Shanks,[a] Nicolas Guilbaud,[b,†] Alain Pierré,[b] Eric Raimbaud,[a] Jean-Luc Fauchère,[a,✠] John A. Hickman,[b] Gordon C. Tucker[b] and Patrick J. Casara[a,*]

[a]*Department of Medicinal Chemistry, Institut de Recherches Servier, 125 chemin de Ronde, 78290 Croissy sur Seine, France*
[b]*Department of Experimental Oncology, Institut de Recherches Servier, 125 chemin de Ronde, 78290 Croissy sur Seine, France*

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This paper is dedicated to the memory of Professor Jean-Luc Fauchère, who left us prematurely after a long illness, on 30 May 2003

Abstract—A structure–activity study was performed by synthesis on *N*,*N*′-disubstitution of 3-aminobenzo[*c*] and [*d*]azepin-2-one **2** and **3** to afford potent and specific farnesyl transferase inhibitors with low nM enzymatic and cellular activities.

1. 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 Ras protein.[1] This lipid side chain is critical for the cell membrane anchoring of these proteins,[2] and therefore FTase inhibition has been recognised as a valuable antitumoral therapeutic approach.[3] Recently, we described caprolactam derivatives **1** as bioisostere of the consensus terminal sequence CVIM of the Ras protein. Selected compounds were found to be very potent FTase inhibitors with low nM IC_{50}, and their activities were validated in vitro in a cellular assay, and in vivo in an antitumoral model.[4] Conformational analysis of this series of compounds is in agreement with the crystal structures of the complex FTase/CVIM in an extended conformation in the active site.[6] The presence of an hydrophobic cleft formed by aromatic residues in the active site was confirmed by the van der Walls interactions observed with the phenylalanine residue of CVFM and the peptidomimetic L-739,750.[5] These findings prompted us to investigate benzo[*c*] and [*d*]azepinones, **2** and **3** respectively, as new cores for FTase inhibitors endowed with new putative interactions by comparison with our previous caprolactam scaffold. These structures included the proximal *N*-*p*-cyanobenzyl substitution on the imidazole as a zinc-complexing function and the SAR was focused on substituent R on the lactam nitrogen atom to replace the lipophilic non-polar side chains of the isoleucine and/or methionine (Fig. 1).

2. Chemical methods

2.1. Synthesis of benzo[*c*]azepin-2-ones 2

In order to validate this hypothesis and investigate the *N*-lactam substitution, the chemical strategy was based on two selective *N*-alkylations of the benzolactam **6**.[4] Since the *S* stereospecificity of the inhibition was observed for **1**, the *S*-phenyl alanine carboxamide **4** was used as the starting material (route 1). Protection of the primary amine as a methyl carbamate, allowed the formation of the relatively stable acetoxymethylamide **5** in quantitative yield.[7] The ring closure of these compounds was achieved smoothly at room temperature by acidic catalysis to afford the desired lactam **6**.[8] In order to compare this series with the most active type **1** compounds, the various substitutions R were concentrated on aryl, benzyl and cyclohexylmethyl derivatives. The *N*-alkylation of **6** gave access to a large variety of substituted amines **7a**–**i**. The *N*-arylation was achieved by

* Corresponding author. Tel.: +33-1-5572-2361; fax: +33-1-5572-2470; e-mail: patrick.casara@fr.netgrs.com
† Present address: Onco*design*, Parc Technologique Toison d'Or, 28 rue Louis de Broglie, 21000 Dijon, France.

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treatment of **6** with various triarylbismuth compounds to give **7j**, **7m**, **7p** and **7o**.[9] However, the *N*-arylation was dependent on the formation of the triarylbismuth reagent which is not an easy process and often not compatible with all the desired substitutions. Therefore, a more general approach was developed based on selective substitutions of α,α′-dibromo-*o*-xylene (**10**, route 2). The mono alkylation of the bulky *N*-Boc-aminomalonate **9** was easily controlled to give the adduct **11** without any cyclic side product obtained with the acetamide.[10] This key intermediate allowed the introduction of all kinds of primary amines by substitution of the second benzyl bromide to afford compounds **12**. The lactam formation was achieved under basic conditions to give, after decarboxylation and deprotection, the amines **7j–o** in their racemic form. All these amines **7a–q** were converted by reductive amination with the aldehyde **8** to the desired products **2a–q** (Scheme 1, Table 1).[11]

2.2. Synthesis of benzo[*d*]azepin-2-ones 3

All attempts to obtain the corresponding benzo[*d*]azepinone **16** in a chiral form from a *S*-phenylglycine by a sequence similar to Scheme 1, failed. Therefore, the ring closure was achieved on the other side of the molecule by using the procedure described by Ben-Ishai.[12] The bis carbamate **14**, easily obtained by heating in methanol phenethylamine and the glyoxalique derivative **13**, was stirred in methane sulfonic acid to give the lactam **15** in the racemic form. By similar procedures, benzylic alkylation and arylation of the *N*-lactam afforded, after acidic deprotection, the amines **16a–j** which were converted to the corresponding products **3a–j** by reductive amination with the aldehyde **8** (Scheme 2, Table 2).[11]

3. Biological evaluation

The inhibitory activities of these compounds were examined in a binding assay against FTase and their cellular activities were determined on a cell line transfected with the *v-H-Ras* gene[13] as described.[4] The results were compared to the clinical reference **BMS-214662**[14] (Table 1).

In the benzo[*c*]azepinone series, the introduction of selected benzyl substituents R afforded very potent FTase inhibitors with almost the same potency in the cell based assay (compounds **2a–i**, Table 1). By comparison, the corresponding caprolactam series **1** showed the same range of activity and even an identical SAR with, at least an one order of magnitude lower potency for *para*-substituted derivatives (e.g., **2e** and **2f**). Aryl substitutions showed no major difference in potency with the corresponding benzyl derivatives in this series (e.g., **2a** and **2j**; **2d** and **2l**; **2g** and **2p**). Therefore, the introduction of a fused phenyl ring in the structure **1** to give benzo[*c*]azepinone **2**, did not alter, nor improve significantly the potency of these compounds.

In the benzo[*d*]azepinone series, the introduction of selected benzyl or aryl substituents R afforded less

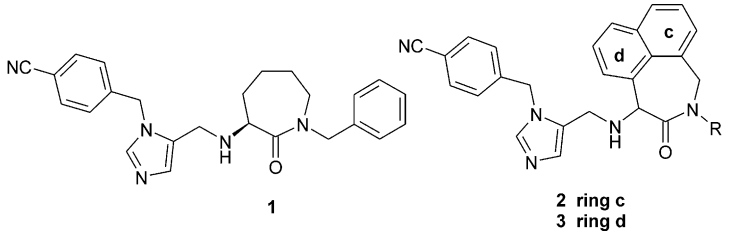
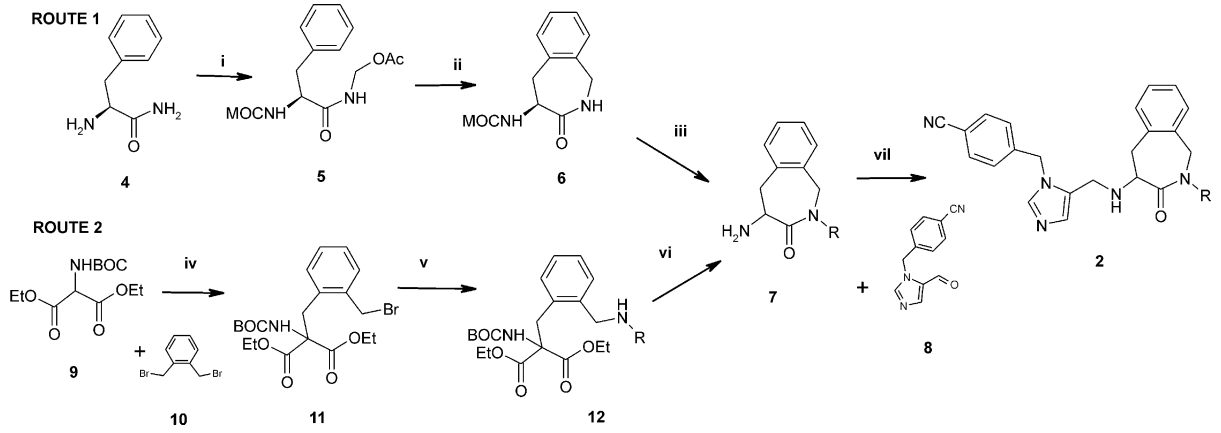
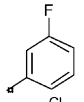
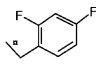
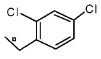
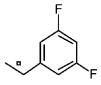
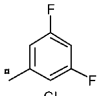
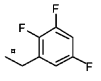
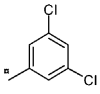


Figure 1.

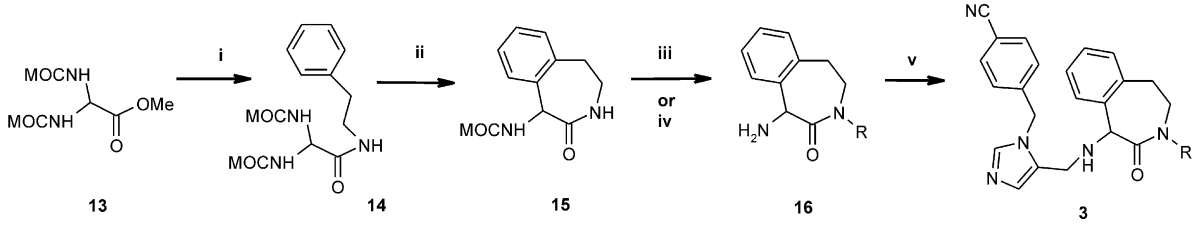


Scheme 1. Synthesis of compounds **2**. Reagents and conditions: (i) (a) $ClCO_2Me$, CH_2Cl_2 pyridine, 0 °C, rt, 24 h; (b) $(CHO)_n$, $(Ac)_2O$, AcOH, rt, 24 h (**5**, 90%); (ii) TfOH, CH_2Cl_2, rt, 24 h (**6**, 77%); (iii) (a) NaH, RBr, Bu_4NI, rt, 24 h; (b) HBr, AcOH, (*S*, **7a–i**); (iii) $BiPh_3$, pyridine, $Cu(OAc)_2$, DMF, CH_2Cl_2, rt, 24 h (*S*, **7j**, **7l**, **7p** and **7q**); (iv) EtONa, EtOH, rt, 12 h, (**10**, 88%); (v) RNH_2, THF, NEt_3, reflux; (vi) (a) KOH; (b) EDC; (c) LiI, pyridine, reflux; (d) HCl, ether, (*RS*, **7j–o**); (vii) (a) $NaBH(OAc)_3$, CH_2Cl_2, 0 °C, rt, 12 h; (b) HCl, ether, or fumaric acid, EtOH (**2a–q**).

Table 1. Structures and yields of compounds **7** and **2** and biological data of compounds **2**

R	7 (conf.) Yield (%)	2 (conf.) Yield (%)	FTase IC_{50} nM ± SEM (*n*)	Ras cell IC_{50} nM ± SEM (*n*)	R	7 (conf.) Yield (%)	2 (conf.) Yield (%)	FTase IC_{50} nM ± SEM (*n*)	Ras cell IC_{50} nM ± SEM (*n*)
		BMS-214662[3] found	1.3 16	25 29		**7j** (*RS*) 32	**2j** (*RS*) 43	26 ± 1(3)	36 ± 3(3)
		1	8 ± 6(2)	10 ± 1(15)		**7j** (*S*) 73	**2j** (*S*) 53	19 ± 4(3)	16 ± 2(3)
	**7a** (*S*) 89	**2a** (*S*) 48	17 ± 5(3)	6 ± 2(6)		**7k** (*RS*) 24	**2k** (*RS*) 16	9 ± 1(2)	39 ± 10(3)
	**7b** (*S*) 62	**2b** (*S*) 39	3 ± 1(4)	4 ± 0(2)		**7l** (*RS*) 60	**2l** (*RS*) 34	4 ± 0(2)	16 ± 4(2)
	**7c** (*S*) 66	**2c** (*S*) 68	10 ± 4(3)	6 ± 1(6)		**7l** (*S*) 38	**2l** (*S*) 41	1 ± 1(2)	7 ± 1(2)
	**7d** (*S*) 74	**2d** (*S*) 70	6 ± 3(2)	4 ± 1(6)		**7m** (*RS*) 15	**2m** (*RS*) 47	15 ± 2(2)	5 ± 1(3)
	**7e** (*S*) 60	**2e** (*S*) 39	55 ± 11(3)	115 ± 36(2)		**7n** (*RS*) 44	**2n** (*RS*) 25	6 ± 0(2)	15 ± 3(2)
	**7f** (*S*) 43	**2f** (*S*) 28	142 ± 66(9)	141 ± 30(2)		**7o** (*RS*) 8	**2o** (*RS*) 46	13 ± 2(2)	46 ± 9(2)
	**7g** (*S*) 75	**2g** (*S*) 32.5	8 ± 1(2)	16 ± 2(3)		**7p** (*S*) 68	**2p** (*S*) 24	10 ± 3(2)	40 ± 4(2)
	**7h** (*S*) 56	**2h** (*S*) 35	18 ± 5(2)	47 ± 2(2)		**7q** (*S*) 82	**2q** (*S*) 36	15 ± 4(3)	7 ± 4(2)
	**7i** (*S*) 58	**2i** (*S*) 43	47 ± 5(2)	26 ± 0(2)					

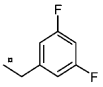
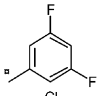
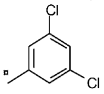
n, number of experiments.



Scheme 2. Synthesis of compounds **3a**–**j**. Reagents and conditions: (i) $Ph(CH_2)_2NH_2$, MeOH, reflux (**15**, 89%); (ii) MsOH, rt, 72 h (**16**, 78%); (iii) (a) NaH, RBr, Bu_4NI, rt, 24 h (**17a**–**f**, % Table 1); (iv) (a) $Bi(R)_3$, pyridine, $Cu(OAc)_2$, DMF, CH_2Cl_2, rt, 24 h; (b) HBr, AcOH, (**17g**–**j**,% Table 1). (v) (a) **8**, $NaBH(OAc)_3$, CH_2Cl_2, 0 °C, rt, 12 h; (b) HCl, ether, or fumaric acid, EtOH (**3a**–**j**).

potent FTase inhibitors with IC_{50} between 10 and 100 nM. A 5- to 10-fold lower potency was observed in the cell based assay (compounds **3a**–**j**, Table 2). By comparison with the corresponding benzo[*c*]azepinone series, a significant loss of potency is observed, taking into account that we are in presence of the racemate form, either for the benzyl substitution (**2b** and **3a**; **2c** and **3b**; **2d** and **3d**; **2g** and **3f**) or for the aryl substitution (**2j** and **3g**; **2l** and **3h**; **2p** and **3i**). An exception to this general observation could be made for the bis-*m*-chloroaryl substitution present in **2q** and **3j**. These two compounds are equipotent, and slightly more active in the cellular assays. No simple explanation to this phenomena could be provided, since cellular potency is dependent on many different parameters such as membrane transport, protein binding, chemical stability and so on. However, a comparative conformational analysis based on the FTase X-ray crystal structure described recently with the cyclic compound U49,[15] showed that the total complex energy is 5 kcal mol^{-1} lower for **2b** than for **3a**. These data

Table 2. Structures and yields of compounds **16** and **3** and biological data of compounds **3**

R	**16** Yield (%)	**3** Yield (%)	FTase IC_{50} nM ± SEM (*n*)	Ras cell IC_{50} nM ± SEM (*n*)	R	**16** Yield (%)	**3** Yield (%)	FTase IC_{50} nM ± SEM (*n*)	Ras cell IC_{50} nM ± SEM (*n*)
	**16a** 42	**3a** 67	54 ± 9(2)	96 ± 14(5)		**16f** 74	**3f** 26	26 ± 6(2)	379 ± 60(2)
	**16b** 38	**3b** 60	23 ± 12(2)	92 ± 31(2)		**16g** 66	**3g** 45	54 ± 9(2)	95 ± 32(3)
	**16c** 56	**3c** 29	78 ± 33(2)	67 ± 9(2)		**16h** 41	**3h** 33	19 ± 5(2)	39 ± 7(3)
	**16d** 65	**3d** 45	23 ± 1(2)	183 ± 61(2)		**16i** 34	**3i** 29	62 ± 16(3)	86 ± 41(2)
	**16e** 95	**3e** 34	22 ± 4(2)	60 ± 6(3)		**16j** 30	**3j** 49	32 ± 7(2)	19 ± 5(2)

n, number of experiments.

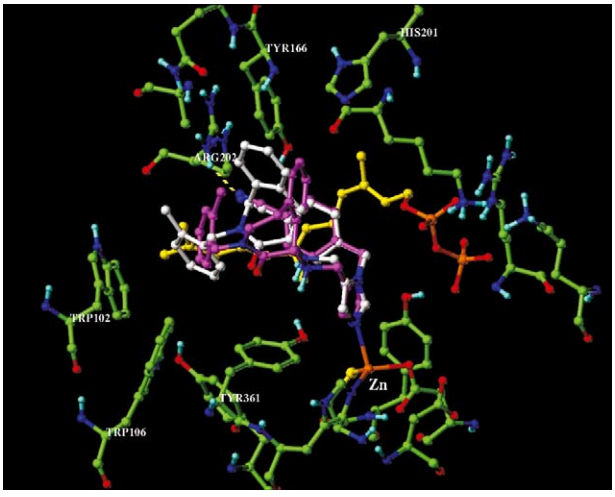


Figure 2. Conformational analysis of compounds **2b** and **3a**. Compounds **2b** (*white*) and **3a** (*magenta*) were docked manually in the binding cavity and their conformations were fully optimised with Tripos force field to obtain the best zinc chelation and protein interactions.[16]

could be explained by a π stacking between the [*c*]phenyl ring of **2b** and Tyrα166 and is consistent with the better FTase inhibitory activities for all this series (Fig. 2).[16]

4. Pharmalogical 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 rat fibroblasts were grafted sc into nude mice, and compounds were administered at 100 and 200 mg/kg p.o. for 5 days, starting from day 6 after tumour implantation.[4] Among these compounds only **2l** and **3h** showed a significant dose-dependent activity with a marked reduction of the tumour volume (65 and 57%, respectively, at 200 mg/kg) an effect less important than for the reference **BMS-214662**. These results could be explained by the relatively poor metabolic stability of the compounds **2l** and **3h** (< 10% in the presence of rat and human hepatic microsomes) despite a complete absorption (100% in the Caco2 cell permeability assay). Nevertheless, these compounds, selective for FTase versus the geranyl–geranyl transferase enzyme (GGTase), were selected for further pharmacological evaluation and the synthesis of the active enantiomer of **3h** is in progress (Table 3).

Table 3. Antitumoral evaluation and GGTase activity

#	%Control 100 mg/kg po	% Control 200 mg/kg po	GGTase IC_{50} μM (*n*)
BMS-214662	48	22	31
2b (*S*)	76	57	7.1 ± 1(2)
2l (*S*)	60	35	25 ± 2(2)
3h (*RS*)	70	43	7.7 ± 3(2)

5. Conclusion

The studies described herein the SAR of *N*,*N*′-disubstituted 3-amino benzo[*c*] and [*d*]azepinones, **2** and **3**, as new series of FTase inhibitors. The [*c*] series, almost comparable to the caprolactam derivative **1**, afforded very potent compounds with low nM IC_{50}, whereas the [*d*] series showed a significant decrease in potency by a factor 5–10. The activities of the selected compounds were validated in vitro in a cellular assay, and two compounds, **2l** and **3h**, shown significant activities in vivo in a tumour model.

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16. All structures were modelled in SYBYL® 6.9. using standard bond lengths and angles. The whole protein, including the zinc and the farnesyl di-phosphate (*yellow*), was kept fixed in aggregate, except the side chains of some of the hydrophobic amino-acid residues of the beta chain at the active site in close contact with the ligands which were allowed to change their conformations depending on the ligands docked, that is TRP102, TRP106, TYR154, TRP303, TYR361 and TYR365. All the hydrogen atoms were added on the crystal structure pdb1LD8 of the enzyme and their geometries were optimised using the Tripos force field, using Kollman charges on all atom of the residues of the protein and Gasteiger–Hückel charges on the other non-protein molecules. All the water, U49 and acetate molecules were removed before docking.