Controlled Exploration of Structural Databases: The Case of Farnesyl Transferase Inhibitors

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Abstract: Among the newer and promising weapons against cancer are Farnesyl Transferase Inhibitors (FTI). Indeed it is known that the enzyme Farnesyl Transferase (FT), catalyses the prenylation of cysteine residues of several proteins associated with cancer progression, including oncogenic forms of Ras.FTI could alter tumour progression.

Exploration of our corporate structural database, based on concepts of diversity and similarity, brought forward a quinazoline-2,4-dione possessing weak farnesyl transferase inhibitory properties. A systematic modulation of structural parameters allowed the elaboration of a series of analogs out of which the most potent compound (**21b**) exhibited an IC_{50} of 19 nM on FT, an excellent cellular activity on the oncogenic H-Ras-transfected cell line Ras #1, as well as selectivity (ratio of IC_{50} on parental RAT2 cells/ IC_{50} on Ras#1 cells > 2000). Moreover this compound also showed encouraging *"in vivo"* activity. The synthesis of these new chemical entities as well as the structure activity relationships found following pharmacological testing, is described.

Key Words: Database mining, farnesyl transferase, farnesyl transferase inhibitors, quinazoline-2,4-diones, structure activity.

This paper is dedicated to the memory of Dr. Gilbert Regnier.

[#]**Author Profile:** Dr. Solo Goldstein received his PhD in Organic Chemistry from the Université Catholique de Louvain, Louvain-La-Neuve, Belgium, in 1983. He started his career in Medicinal Chemistry at the Pharmaceutical Sector of Union Chimique in Belgium where he held various positions culminating with Head of Structure Activity and Molecular Modelling Department. He was Visiting Research Professor at the Western Psychiatric Institute and Clinic, Pittsburgh, USA (1985-1986) and Lecturer on Molecular Orbitals in Organic and Medicinal Chemistry at the Sherbrooke University, Québec, Canada (1990-1993). In 1997 he joined the Servier Research Institute in France, as Director of Chemistry Research Division A. Dr Solo Goldstein is the main inventor of more than 10 international patents and author of more than 50 publications in scientific international journals and conference proceedings.

INTRODUCTION

The last decade has witnessed a number of critical advances achieved in molecular biology, pharmacological screening and synthesis that induced new paradigms in the process of drug design and discovery; strategies used to detect, pharmacologically evaluate, and optimize new chemical entities were modified in order to take advantage of the new knowledge [1].

One of these new paradigms involves exploration of structural databases (DB Mining); indeed the progress made in molecular biology coupled with advances in High Throughput Screening (HTS), brought forward the possibility of screening thousands of compounds on existing or new (sometimes orphan) targets [2]. Therefore proprietary, commercially available, or non-profit structural databases became quickly an important source from which stem new medicinal chemistry series that were investigated in various therapeutic areas. A number of different approaches have been used to start medicinal chemistry programs by such explorations of structural databases, the initial tendency being massive screening supported by the newly developed HTS technique [3]. However it was soon realized that other, more controlled methods, based on diversity and/or similarity [4], virtual screening [5], or pharmacophore search [6] allowed to select upfront subsets representing either the whole database or possessing the required characteristics recognized by a given target. It was anticipated that such selections will allow easier result analysis and conclusion making about the next steps to be taken [1].

Farnesyl Transferase (FT) and Farnesyl Transferase Inhibitors (FTI) have been investigated in great depth in order to find new promising weapons against cancer [7]. Indeed it is known that there is a high frequency of Ras protein mutations in cancer and that prenylation (addition of farnesyl or geranyl geranyl isoprenoid, to cystein residues), catalyzed by farnesyl and geranyl geranyl transferases is required for the transforming activity of Ras. Therefore farnesyl transferase inhibitors were investigated as a means to block tumour progression [8].

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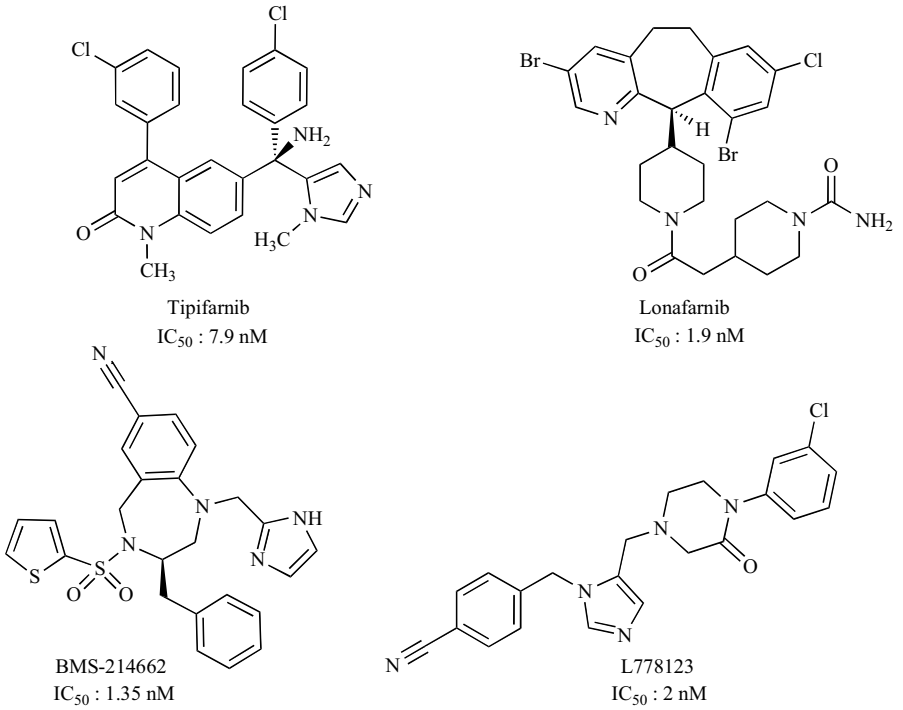


Fig. (1). FT inhibitors that reached clinical stages.

Prenylation is shared by many proteins and the effective targets of FT turned out to be more elusive than Ras [9]. FTI were found to be effective against the growth of certain tumor cells both *in vitro* and *in vivo*. Several FTI reached clinical stages (Fig. **1**) but none showed the expected efficacy.

Our own work in this area capitalized on a series of new quinazolines-2,4-diones that were elaborated starting from a weak hit. The outcome of this research is presented and analysed in this paper.

RESULTS AND DISCUSSION

The quest for new FT inhibitors started with the investigation of our corporate structural database. Thus based on principles of structural diversity and similarity (using similarity function within Isis Base) a set of molecules were chosen and tested on farnesyl transferase isolated from rat brain (see experimental). Investigation of the compounds at different concentrations in DMSO singled out the quinazolinedione **1** (Fig. (**2**)) as a weak hit.

In order to fully explore the potential of such structure types we decided to further elaborate this initial hit. Thus a series of related compounds found in the database by a substructure search or synthetized as described (*vide infra*) were investigated. Indeed as can be seen from Fig. (**2**) the FT inhibition level is increased when both nitrogens of the quinazoline-2,4-dione moiety are substituted, especially when substitution is an aromatic ring (**1b**, **1d**, Fig. (**2**)). Also it appears that inhibition levels depend on the regiochemistry of substitution, the imidazolyl alkyl chain on the *1* nitrogen providing more efficiency on inhibition (compare **1b** and **1c**, **1d** and **1e**, Fig. (**2**)). With these elements in hand we set up a systematic hit optimisation program starting from compound **1d**. Three structural elements (Fig. (**3**)) have been modulated in order to establish the impact on FT inhibition:

(a) position of the imidazole ring substitution;

(b) phenyl ring substitution;

(c) linker (between quinazoline-2,4-dione and imidazole) substitution;

All compounds could be synthetized following either of the two practical pathways illustrated by the retrosynthetic analysis in Scheme **1**, both being initiated from literature described or commercially available starting materials. Representative syntheses are exemplified in Scheme **2**.

Inhibition was evaluated on rat brain purified FT enzyme (see experimental) while the cellular activity of compounds was assayed on RAT2 fibroblastic cells and by measuring the phenotypic reversion of the Ras#1 cell line (RAT2 cells transfected with an oncogenic form of H-Ras known to be farnesylated) to the RAT2 phenotype (see experimental). Thus RAT2 cells allow to establish the intrinsic toxicity of the compound while transfected Ras#1 cells allow the evaluation of a specific effect on cellular FT. Therefore the ratio between these two activities gives an idea on the specificity of the compound.

Table **1** summarizes the results obtained by variation of the structural parameters mentioned before.

Thus entries **1d-9** Table **1** illustrate the influence of the imidazole ring orientation on FT inhibition and cellular activities (Ras#1, RAT2 tests).

Compared to **1d**, linking to the *5*-imidazole ring position (**8**,**9**) improved the FT inhibition but RAT2/Ras#1 selectivity, although slightly better for **1d**, remains modest.

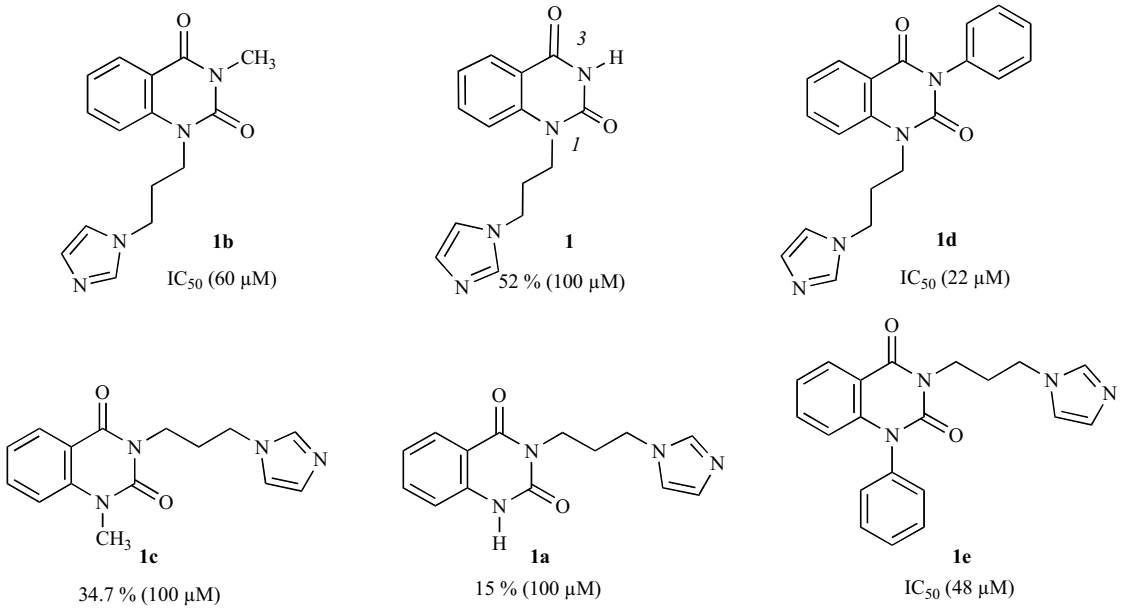


Fig. (2). Inhibition of FT: Initial hit exploration.

Linking to position *4* of the imidazole nucleus provides comparable results to those of **1d** (N-H), all the other compounds (**2**, **3**, **6**, **7**) being ineffective on FT.

Next the influence of N-Phe (quinazoline-2,4-dione) substitution was investigated. Results for salient substituents and positions are exemplified for **1d** analogs (**10-13**, Table **1**). Better FT inhibition and improved selectivities (RAT2/ Ras#1) were obtained for all four compounds (the selectivity of *m,p*-dichloro substitution being roughly the same as that of **1d**). Therefore it appears that this parameter greatly influences both the inhibition potency and the selectivity, compounds **11** and **12** (*m*-Br and *p*-NHtBoc) exhibiting the best profile obtained at this point.

As for the substitution on the linker between the imidazole nucleus and the quinazoline-2,4-dione substructure, two main aspects have to be considered namely the position of the substituent and generation of an asymmetric center, bringing along the need to consider the profiles of both enantiomers. Again, Table **1** presents analoging of **1d** in this respect, the results obtained with "naked" phenyl and benzyl substituents being analyzed (all substitutions on both phenyl or benzyl rings induced a marked drop in FT inhibitory activity).

As can be seen from the results obtained for compounds **14-18** (Table **1**), all substitutions by phenyl rings irrespective of their position on the linker diminished the RAT2/ Ras#1 selectivity; nevertheless several of these compounds exhibited FT inhibition in the lower micro molar range (**17**, **18**, *m*-Br and *p*-NHtBoc substitutions). Also, enantiomers **15a** and **15b** demonstrated clear-cut differences both in FT inhibitory power and selectivity.

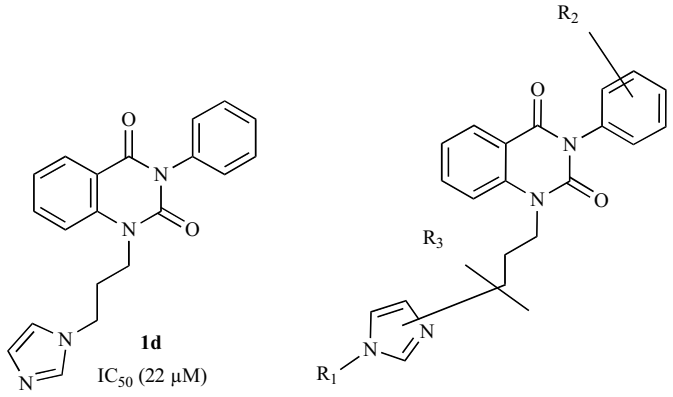
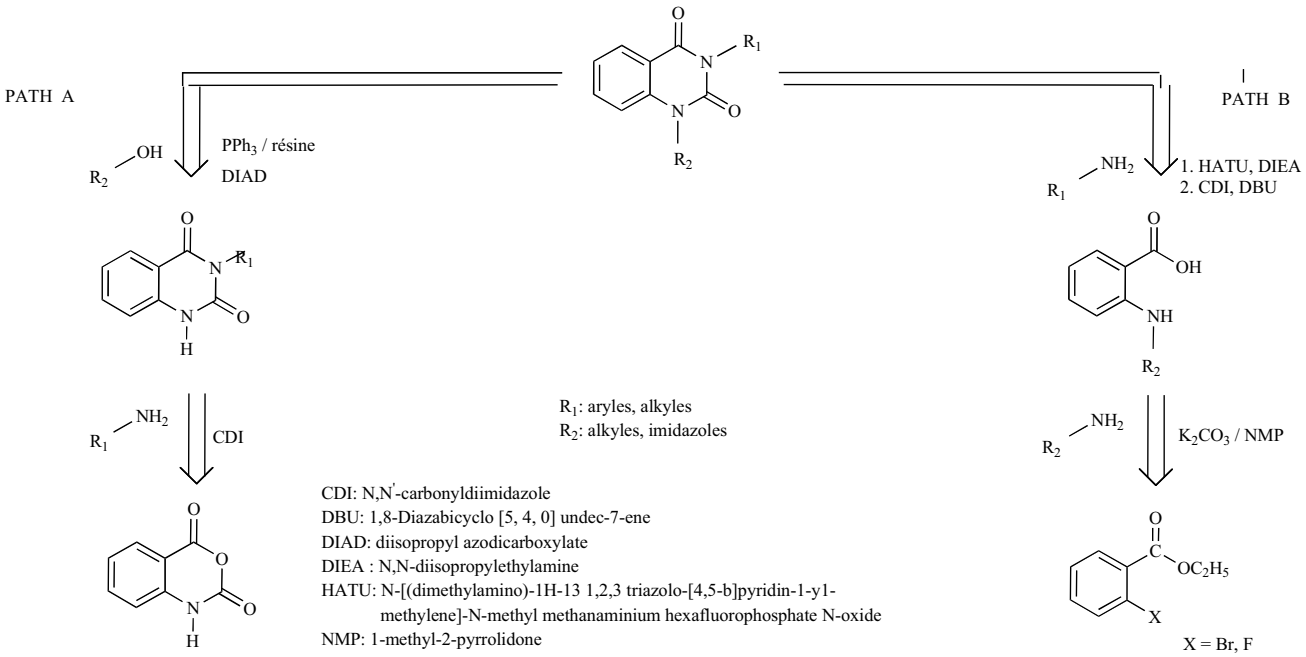


Fig. (3). Inhibition of FT: Optimisation.



Scheme 1. Synthetic approaches to quinazoline-2,4-diones.

Benzyl ring substitution on the *2* position of the linker was far more satisfactory. Thus all these analogs of **1d** (**18-21**, Table **1**) were a great deal more effective on FT inhibition and RAT2/Ras#1 selectivity. The combination of the p-NHtBoc substitution on the quinazoline-2,4-dione and 2-benzyl substitution on the linker provided powerful FT inhibition and cellular activity combined to an 800 fold RAT2/Ras#1 selectivity (**21**, Table **1**). One of the **21** enantiomers proved to be the best compound in the series (**21b**, Table **1**), strongly inhibiting the FT enzyme (IC_{50} = 19 nM) and having a remarkable cellular activity and selectivity (greater than 2000 fold RAT2/Ras#1). Inhibition of FT is much more effective than that observed for GGT (IC_{50} : 10-100 μM). For best compounds a bigger than 100 fold selectivity was observed.

Moreover **21b** evidenced an encouraging *in vivo* activity at 50 and 100 mg/kg (2 times/daily), when administered orally to Swiss nude mice which were previously transplanted (sc) with Ras#1 cells.

In conclusion starting with a weak hit detected by rational DB Mining, a family of new quinazoline-2,4-diones FT inhibitors, could be elaborated. Several of the family members, besides having potent FT inhibiting properties (in the nanomolar range for the best) also possess very good cellular activities (Ras#1) and selectivities (RAT2/Ras#1). An encouraging *in vivo* activity has also been observed.

Further results on this family of compounds will be reported in due time.

EXPERIMENTAL SECTION

Chemistry

Compounds were prepared following one or both of the two strategies outlined in Scheme **1**. Representative syntheses outlined in Scheme **2** are described in detail.

Melting points were measured on a Tottoli Büchi apparatus and are uncorrected. FT-IR spectra were measured on a Brucker IFS 28 apparatus. ^{1}H-NMR spectra were recorded on a Brucker 300 MHz apparatus. The chemical shifts are reported in ppm (δ value) downfield from tetramethylsilane (TMS) used as internal standard.

Reagents and solvents were purchased from Aldrich-Sigma, Interchim or Novabiochem and were used as such. Chromatography was performed on Kieselgel 60 (230-400 mesh) silica gel (Merck).

Microwave reactions were performed using a multimode NORMATRON 112® oven (irradiation as indicated in procedure). The elemental analyses were established using a THERMO EA 1112 apparatus. Chiral separation was performed on a Chirapack AD 600X60 column.

1-(3-Imidazol-1-yl-2-phenyl-propyl)-3-phenyl-1H-quinazoline-2,4-dione (15)

3-Imidazol-1-yl-2-phenyl Propionic Acid Ethyl Ester

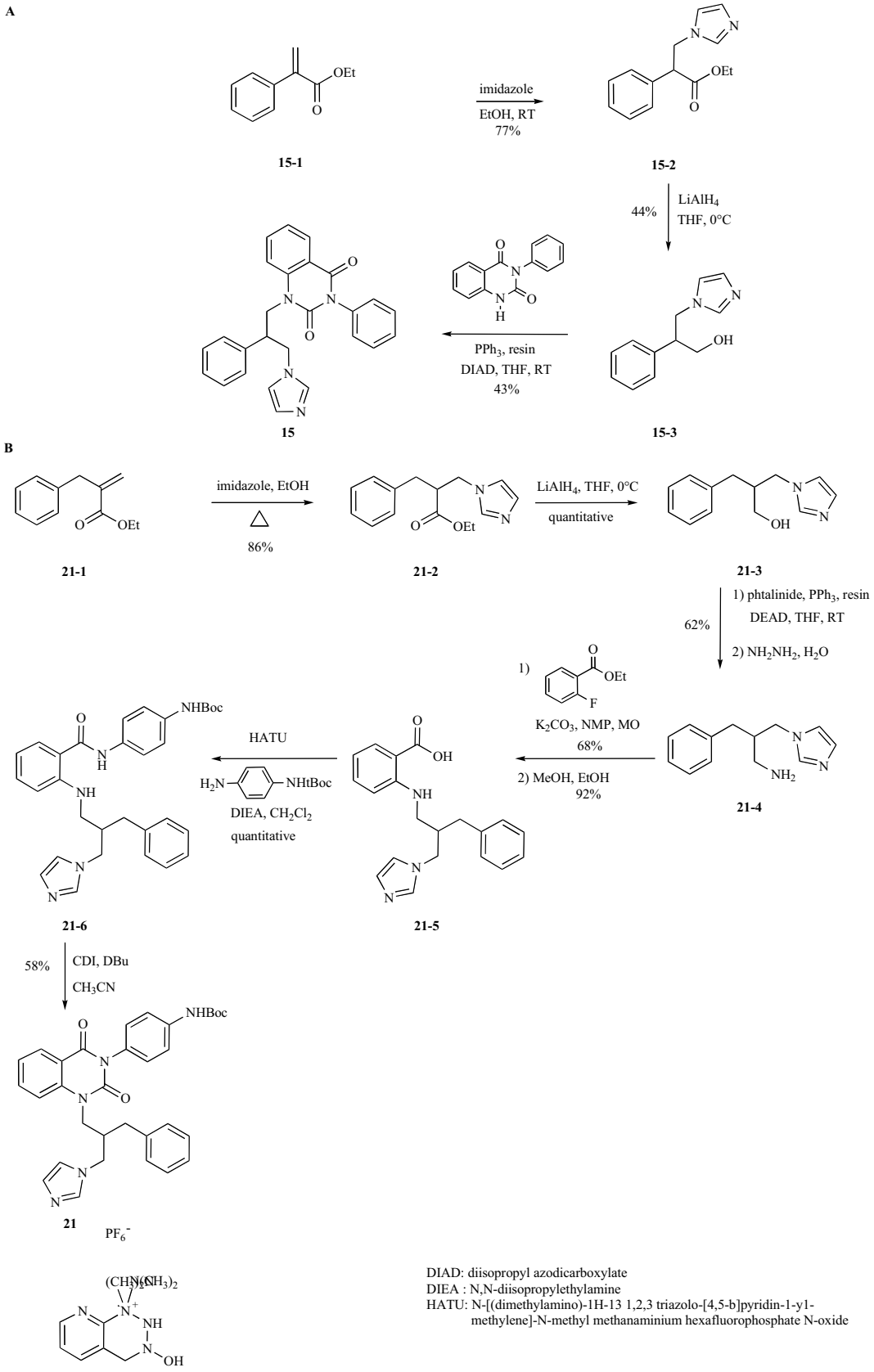
To a solution of 10 g (58 mmol), 2-phenyl-acrylic acid ethyl ester [10] in 100 mL ethanol were added 10 g (147 mmol) imidazole and the reaction mixture was stirred for 24 hours at room temperature. After removal of the solvent under reduced pressure the oily residue is purified by chromatography on silica gel using a mixture of dichloromethane/methanol 95/5 to afford 11 g (77%) of an oil.

IR (neat): 1728 cm^{-1} ($\nu_{C=O}$ ester)

^{1}H-NMR: ($CDCl_3$) δ 7.35 (m, 4H); 7.25 (m, 2H); 7.00 (s, 1H); 6.80 (s, 1H); 4.65 (m, 1H); 4.15 (m, 3H); 3.90 (m, 1H); 1.15 (t, 3H).

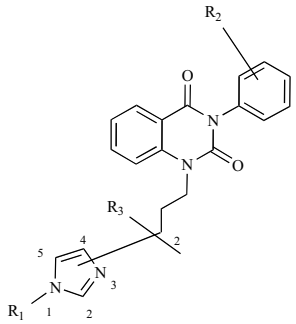
3-Imidazol-1-yl-2-phenyl-propan-1-ol

2.2 g (110 mmol) $LiAlH_4$ were added at 0°C, under an inert atmosphere, during 30 minutes, to a solution of the



Scheme 2. Synthesis of representative quinazoline-2,4-diones.

Table 1. Structure Activity Data of the Quinazoline-2,4-dione



Cmpd. No.	R1 / Imid.	R2	R3	FT inh. IC_{50} (µM) [a]	Ras#1 IC_{50} (µM) [a]	RAT2 IC_{50} (µM) [a]	IC_{50} Ratios RAT2/Ras#1
1d	-/1-Imid.	H	H	22	46	421	~ 9
2	H/2-Imid.	H	H	> 100	-	-	NT [c]
3	Me/2-Imid.	H	H	> 100	-	-	NT [c]
4	Bz/2-Imid.	H	H	> 100	-	-	NT [c]
5	H/4-Imid.	H	H	42	43	236	5.5
6	Me/4-Imid.	H	H	> 100	-	-	NT [c]
7	Bz/4-Imid.	H	H	> 100	-	-	NT [c]
8	Me/5-Imid.	H	H	8.2	17	81	~ 5
9	Bz/5-Imid.	H	H	2.4	8	26	~ 3
10	1-Imid.	OMe	H	8.1	15.5	382	24
11	1-Imid.	m-Br	H	2.7	4.8	224.3	46
12	1-Imid.	p-NHtBoc	H	1.5	2.8	79.2	28
13	1-Imid.	m,p-diCl	H	4.5	9	87	9
14	1-Imid.	H	1-Phe	4.1	10	100	10
15	1-Imid.	H	2-Phe	2.1	14.2	138.2	10
15a	1-Imid.	H	2-Phe (en 1) [b]	26	37	100	3
15b	1-Imid.	H	2-Phe (en 2) [b]	0.9	6	100	16
16	1-Imid.	H	3-Phe	30	35	96	3
17	1-Imid.	m-Br	2-Phe	1.5	2.9	33	11
18	1-Imid.	p-NHtBoc	2-Ph	1	0.9	14	15
19	1-Imid.	H	2-Bz	0.5	1.8	98.7	55
20	1-Imid.	m-Br	2-Bz	0.19	0.32	26.4	82
21	1-Imid.	p-NHtBoc	2-Bz	0.073	0.023	18.7	813
21a	1-Imid.	p-NHtBoc	2-Bz (en 1) [b]	0.258	0.258	14.5	56.2
21b	1-Imid.	p-NHtBoc	2-Bz (en 2) [b]	0.019	0.009	10.5	2166

[a]: see experimental part for description of biological assays

[b]: enantiomer

[c]: not tested

Me : methyle ; Bz : benzyle ; Phe : phenyle

The substituted or unsubstituted imidazole ring (R_1=H) is linked to the quinazoline-2,4-dione part of the molecule via the ring atoms numbered as indicated. In compound 1d, nitrogen 1 of the imidazole ring, connects via the side chain to the quinazoline-2,4-dione part.

above obtained ester (11 g, 45 mmol) in 300 mL tetrahydrofuran. After stirring at room temperature during 16 hours wet sodium sulphate was added and the mixture was filtered. The solvent was removed *in vacuo*, the residue was taken up in dichloromethane; the organic phase was washed with water then dried on $MgSO_4$. Removal of the solvent afforded 6 g (44%) of the desired alcohol used as such for the preparation of the quinazoline-dione.

IR (neat): 3200 cm^{-1} (ν_{OH}); 1511, 1496, 1453 cm^{-1} ($\nu_{C=C}$ & $\nu_{C=N}$)

^{1}H-NMR: ($CDCl_3$) δ 7.30 (m, 4H); 7.15 (m, 2H); 6.95 (s, 1H); 6.70 (s, 1H);4.40-4.20 (2dd, 2H); 3.80 (d, 2H); 3.10 (q, 1H); 2.00 (OH)

To a suspension of 640 mg (2.7 mmol) 3-phenyl-1H-quinazoline-2,4-dione (Aldrich), 600 mg (2.7 mmol) of the above alcohol and 2.5g (4.5 mmol) triphenylphosphine on resin (1.8 mmol/g) in 100 mL of tetrahydrofuran were added dropwise 950 µL (4.8 mmol) of diisopropyl azodicarboxylate (DIAD).

The reaction mixture was stirred 16 hours at room temperature, solids were removed by filtration and the solvent was removed under reduced pressure.

Chromatography on silica gel using a mixture of toluene/ethanol 95/5 afforded 200 mg (43%) of the quinazoline-dione.

m.p. (cap) 132-135°C

Anal. calcd. for $C_{26}H_{22}N_4O_2$: C, 73.92; H, 5.25; N, 13.26; Found: C, 73.66; H, 5.32; N, 12.88

IR (nujol): 1710, 1663 cm^{-1} ($\nu_{C=O}$); 1607 cm^{-1} ($\nu_{C=C}$) ^{1}H-NMR: (DMSO-d6) δ 8.05 (d, 1H); 7.80 (t, 1H); 7.50-7.10 (m, 13H); 7.00-6.75 (2s, 2H); 4.60-4.30 (m, 4H); 3.65 (m, 1H)

Separation of the enantiomers **15a**, **15b** (> 99% ee) was achieved using a chiral column and a mixture of n-heptane/ethanol/diethylamine 500/500/1 (v/v) as eluent.

{4-[1-(2-benzyl-3-imidazol-1-yl-propyl)-2,4-dioxo-1,4-dihydro-2H-quinazolin-3-yl]phenyl}-carbamic Acid *tert*-butyl Ester (21)

2-Benzyl-3-imidazol-1-yl-propionic Acid Ethyl Ester

To a solution of 20 g (0.1 mmol) 2-benzyl-acrylic acid ethyl ester [11] in 200 mL ethanol were added 20 g (0.29 mmol) of imidazole. After stirring at reflux for 48 hours the solvent was removed under vacuum and the remaining oil was purified by chromatography on silica gel using dichloromethane as eluent affording 21 g (86%) of the ester.

IR (neat): 1730 cm^{-1} (ester)

^{1}H-NMR: ($CDCl_3$) δ 7.42 (s, 1H); 7.39-7.20 (m, 3H); 7.15 (d, 2H); 7.04 (s, 1H); 6.85 (s, 1H); 4.29 (dd, 1H); 4.05 (m, 3H); 3.08 (m, 1H); 3.00 (dd, 1H); 2.79 (dd, 1H); 1.20 (t, 3H)

2-Benzyl-3-imidazol-1-yl-propan-1-ol

The reduction of the above mentioned ester was performed using a procedure identical with that described for the corresponding phenyl ester.

Yield 19.8 g (quantitative).

IR (neat): 3300 cm^{-1} large (ν_{OH})

^{1}H-NMR: ($CDCl_3$) δ 7.45 (s, 1H); 7.35-7.15 (m, 5H); 7.00 (s, 1H); 6.90 (s, 1H); 4.05 (dd, 1H); 3.95 (dd, 1H); 3.49 (d, 2H); 2.80-2.50 (m, 2H); 2.20 (m, 1H); 3.10 (large s, 1H)

2-Benzyl-3-imidazol-1-yl-propylamine

A suspension of 150 g (100 mmol) phtalimide, 19.5 g (90 mmol) of the alcohol obtained above and 150 g (180 mmol) triphenylphosphine on resin (1.2 mmol/g) in 1.5 L of tetrahydrofuran was stirred at room temperature while a solution of 40 mL (185 mmol) diisopropyl azodicarboxylate (DIAD) in 100 mL of tetrahydrofuran were added dropwise.

The stirring was continued for 16 hours at room temperature followed by filtration and removal of solvent *in vacuo* affording 75 g of an oil.

After the addition of 500 mL ethanol and 20 mL hydrazine hydrate the reaction mixture was heated with stirring at 40-50°C for 3 hours, followed by addition of 20 mL from a 6N HCl solution, and continued stirring for 1 hour at 50°C. The mixture was filtered and the residue chromatographed on silica gel using a mixture of dichloromethane/methanol 95/5 as eluent. 12.2 g (62%) of the amine were thus recovered.

IR (neat): 3350-3290 cm^{-1} (ν_{NH2}); 1656 cm^{-1} (s_{NH2})

^{1}H-NMR: ($CDCl_3$) δ 7.45 (s, 1H); 7.30 (m, 3H); 7.20 (d, 2H); 7.05 (s, 1H); 6.90 (s, 1H); 4.00 (m, 2H); 2.65 (m, 4H); 2.15 (m, 1H); 1.25 (s, 2H)

2-(2-Benzyl-3-imidazol-1-yl-propylamino)-benzoic Acid

A suspension of 12 g (56 mmol) amine obtained as above, 10.5 g (59 mmol) 2-fluoro-benzoic acid ethyl ester (Alpha Aesar), 9 g K_2CO_3 and 70 mL of *N*-methyl-pyrrolidone (NMP) was stirred at reflux in a microwave oven during 30 minutes. Solids were removed by filtration and the solvent removed *in vacuo*.

The residue was purified by chromatography on silica gel using a mixture of dichloromethane/methanol 97/3 as eluent affording 13.7 g of an oil. Hydrolysis (aqueous sodium hydroxide/ethanol, 50°C, 2 hours), afforded 10.5 g (63%) of an acidic material used as such for the next step.

IR (nujol): 3280 cm^{-1} (ν_{NH}); 1660-1620 cm^{-1} ($\nu_{C=O}$)

^{1}H-NMR: (DMSO-d6) δ 7.80 (dd, 1H); 7.50 (s, 1H); 7.20 (m, 7H); 6.90 (s, 1H); 6.50 (t, 1H); 6.35 (d, 1H); 4.05 (d, 2H); 2.90 (m, 2H); 2.60 (m, 2H); 2.50 (m, 1H)

{4-[2-(2-Benzyl-3-imidazol-1-yl-propylamino)-benzoylamino]-phenyl}-carbonic Acid tert-butyl Ester

To a solution of 90 g (27 mmol) above acid in 35 mL dichloromethane were added 10 g (27 mmol) HATU (Novabiochem), 5.5 g (27 mmol) *N*-Boc-phenylenediamine (Aldrich), and 11 mL (63 mmol) DIEA (*N,N*-diisopropyl-ethylamine).

The reaction mixture was stirred for 16 hours at room temperature more dichloromethane was added followed by washing with brine. After drying and removal of solvent the residue was chromatographed on silica gel using dichloro-

methane/methanol 95/5 as eluent to afford 18 g (quantitative) of the end product used as such in the last step.

^{1}H-NMR: (DMSO-d6) δ 10.00-9.30 (NH); 7.70 (d, 2H); 7.60 (s&d, 2H); 7.40 (d, 2H);7.30-7.20 (m, 6H); 7.15-6.90 (2s, 2H); 6.65 (t, 1H); 6.45 (d, 1H); 4.05 (d, 2H); 2.90-2.60 (2m, 4H); 2.45 (m, 1H); 1.50 (s, 9H)

7 g (13.3 mmol) of the compound obtained above, 7 g, (43 mmol) of CDI (carbonyl diimidazole) and 4.2 mL (28 mmol) DBU in 21 mL acetronitrile were stirred for 30 minutes at room temperature ; stirring was continued in a microwave oven (irradiation at 250 W) for 10 minutes. Most of the solvent was removed; the residue was taken up in dicholoromethane followed by washing with water, separation of the organic phase and drying. The solvent was removed *in vacuo*, and the residue was purified by chromatography on silica gel using a mixture of dichloromethane/methanol 90/10 as eluent, to afford 4.2 g (58%) of the desired quinazolinedione.

mp (cap) > 150°C (dec) Anal. Calcd. for $C_{32}H_{33}N_5O_4$: C, 69.67; H, 6.03; N, 12.70; Found: C, 69.16; H, 6.17; N, 12.10.

IR (nujol): 3600-3300cm^{-1} (ν_{NH}); 1712, 1664 cm^{-1} ($\nu_{C=O}$)

^{1}H-NMR: (DMSO-d6) δ 8.00 (d, 1H); 7.70 (s, 1H); 7.60 (t, 1H); 7.50 (d, 2H); 7.25 (m, 4H); 7.20-6.90 (2s, 2H); 7.15 (d, 4H); 6.95 (d, 1H); 4.20-4.00 (m, 4H); 2.80 (m, 1H); 2.60 (m, 2H); 1.50 (s, 9H)

The two enantiomers **21a** and **21b** (>99 ee) could be separated as done for compound **15**.

Biological Assays

FTase was purified from rat brain and its enzymatic activity evaluated in a scintillation proximity assay (Amersham, GE Healthcare) with streptavidin-coated beads: the biotinylated C-terminal sequence of lamin B was used as a substrate (biotin-YRASNRSCAIM) for farnesylation with [^{3}H]-farnesyl-pyrophosphate as a donor. Tested products were assayed at various concentrations directly in the assay solution and radioactivity transferred on lamin B after farnesylation was evaluated in a Topcount counter (PerkinElmer). IC_{50}s values or % inhibition at a given dose were derived from the corresponding dose-response curves.

The cellular activity of compounds was assayed by measuring the phenotypic reversion of a cell line transformed by a farnesylated oncogene. The rat cell line Ras#1 was obtained after transfection of immortalised fibroblastic RAT2 cells (obtained from the American Tissue Culture Collection) with the *v-H-ras* oncogene whose farnesylation is required for maintaining transformation. Spindle-shaped Ras#1 cells revert to the parental flat RAT2 fibroblastic morphology 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, a plateau 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_{50}s refer to cell inhibition of FTase when appropriate and not to unrelated cytotoxicity of the compounds. tetrazolium viability assay.

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ABBREVIATIONS

cap	=	Capillary
CDI	=	N,N'-carbonyldiimidazole
DB	=	Database
DBU	=	1,8-diazobicyclo[5,4,0]undec-7-ene
DIAD	=	Diisopropyl azodicarboxylate
DIEA	=	N,N-diisopropylethylamine
DMSO	=	Dimethyl sulfoxyde
FT	=	Farnesyl transferase
FTI	=	Farnesyl transferase inhibitors
HTS	=	High throughput screening
NMP	=	1-methyl-2-pyrrolidone
TMS	=	Tetramethylsilane

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