Impact on farnesyltransferase inhibition of 4-chlorophenyl moiety replacement in the Zarnestra® series

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Abstract

Based on the structure of R115777 (tipifarnib, Zarnestra®), a series of farnesyltransferase inhibitors have been synthesized by modification of the 2-quinolinone motif and transposition of the 4-chlorophenyl ring to the imidazole or its replacement by 5-membered rings. This has yielded a novel series of potent farnesyltransferase inhibitors.

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1. Introduction

Ras gene mutations have been identified in approximately 30% of human cancers [1–4], evidence has prompted considerable efforts to elucidate the pathways of Ras transformations. The discovery that farnesylation is a key step for Ras transforming activity has generated considerable interest in the development of farnesyltransferase inhibitors (FTIs) as potential therapeutic agents [4–8]. And indeed, many FTIs have demonstrated excellent anti-tumoral efficacy in preclinical human xenograft models [9,10]. While some of these compounds are undergoing phase II/III clinical trials, it has become clear that the anti-tumoral activity of this class is quite complex involving other farnesylated proteins such as RhoB, centromer associated proteins or modulation of transcription events [11–22]. R115777 1 (tipifarnib, Zarnestra®) is a 4-phenylquinolinone that is currently undergoing phase II/III clinical trials for the treatment of haematological and solid tumors [23–29]. Recently, we reported the synthesis and activity of highly potent analogues of 1, namely tetrazoloquinoline 2 and tetrazolo[1,5-a]quinazoline 3 (Fig. 1) [30].

In this paper we report our further efforts to design novel inhibitors of FTase using 2 and 3 as templates. To help in this task, we initiated a molecular modeling study aiming at better understanding of the binding mode of R115777 and related analogs. Compound 1 was first submitted to a conformational analysis then manually docked in the FTase catalytic site by using the structural information available in the literature (Fig. 2). The docking model was checked against the
structure—activity relationships (SAR) of the tipifarnib series and, combined with in-house knowledge and literature data [25–32], served as a basis for rational chemical modifications. We hypothesized that transposition of the 4-chlorophenyl substituent to the methyl group of the imidazole ring should be allowed without drastic effect on binding. This was confirmed by a separate docking study of compound 4a. We also report on the direct replacement of this substituent with 5-membered heterocycles and emphasize on the related SAR.

2. Chemistry

Bromine—lithium exchange in 2-chloroquinoline 8 [34] and addition of aldehyde 7 [35] onto the resulting 6-lithio-quinoline provided the alcohol 9 in low yield (Scheme 1). Tetrazoloquinoline backbone was then obtained by reacting the 2-chloroquinoline with sodium azide to provide 4a. The 4-cyanobenzyl derivative 4b was obtained following the same sequence.

Bromine—lithium exchange in 10 [34] gave in situ 5-lithio-3-(3-chlorophenyl)benzo[c]isoxazole which was reacted with commercially available 5-chlorothiophen-2-carboxaldehyde...
to provide alcohol 11 (Scheme 2). After oxidation of the hydroxyl group into a ketone moiety, the benzisoxazole ring was reduced to \textit{ortho}-aminobenzophenone 13 using titanium trichloride Lewis acid. Acylation of 13 with trichloroacetyl chloride provided the corresponding amide 14 and in situ cyclisation by heating 14 with ammonium acetate in DMSO gave the quinazolinone 15 in good yield. Upon refluxing in POCl₃ 15 was converted to 2-chloroquinazoline 16. Then 1-methylimidazole was first deprotonated at C-2 by action of \textit{n}-butyllithium and the resulting carbanion was silylated. In the same pot, further deprotonation at C-5 by \textit{n}-butyllithium and condensation of ketone 16 at low temperature provided the alcohol 17 in a yield of 24%. Compound 17 was then condensed with sodium azide to provide the tetrazolo[1,5-a]quinazoline 18, which was subsequently reacted with thionyl chloride and ammoniac to provide 5a.

In our hands, bromine—lithium exchange on quinazoline 20 [36] and subsequent reaction with DMF was unsuccessful. Therefore we choose to convert 20 into Weinreb amide 21. 2-Methoxyquinazoline 21 was transformed into 2-chloroquinazoline 22 by reaction with POCl₃. Addition of 1-methyl-2-triethylsilyl-imidazol-5-yl moiety, prepared as described in Scheme 2, gave ketone 23 which was reduced to alcohol 24 using diisobutylaluminium hydride. After formation of the tetrazole ring, as already described, conversion of the hydroxyl group to chloride 26 and substitution of the chlorine atom by various imidazoles (27–30) provided final compounds 5b–e in low yields (Scheme 3).

3. Results and discussion

All compounds were evaluated for in vitro inhibition of FTase [22] using the Amersham scintillation proximity assay and the laminB peptide substrate (Biotin-YRASNRSCAIM) and compared to 1, 2 and 3. The structure—activity relationships are presented in Tables 1–2 (Fig. 3).

Compound 4a proved to be at least 28-times less potent towards enzyme inhibition than the corresponding tetrazoloquinoline 2 and 100-times less potent than R115777. Docking of 1 and 4a into the FTase catalytic site led to overall similar binding modes and energetically stable structures (Figs. 2 and 4). As expected the major differences are due to the impact of the 4-chlorophenyl transposition. For molecule 1 the 4-chlorophenyl ring sits at equal distances from the 3-chlorophenyl ring on one hand and the HFP hydrophobic tail on the other hand. It adopts a parallel orientation that enables ideal face-to-face pi-stacking and alkyl-pi interactions, respectively. In addition, the primary amino group performs a water-mediated hydrogen bond with an hydroxyl group of the HFP head. For molecule 4a, the transposition of the 4-chlorophenyl substituent on the imidazole ring resulted in a less favorable tilted orientation of the phenyl ring as compared to 1 and in a docked position very close to the HFP tail. Moreover, in contrast to compounds 1, 2 and 3, this new structure lacks the quaternary carbon as a result of the aryl group removal. It follows that the remaining substituents are less tightly packed allowing more rotational flexibility around this carbon. Although the spatial orientation of the substituents bearded by this carbon has been preserved during docking, the water-mediated hydrogen bond between the hydroxyl moiety and the HFP head got lost upon minimization due to excessive rotation. These observations may account for the activity drop of 4a as compared to 1 and 2. Most of the potency was recovered when replacing the 4-Cl by a 4-cyano group in 4b. This can be explained by a strong electrostatic interaction between the electron-rich cyano group and
the positively charged side chain of Arg 202. However, 4b was still 10-fold less active than R155777.

We turned our attention rather to 4-chlorophenyl replacement starting from 2-chloro-5-thiophenyl 5a as thiophenyl can be considered as a good surrogate for a phenyl ring (Table 2, Fig. 5).

Although less potent, thiophenyl compounds 18 and 5a were still in the high nanomolar range of activity towards FTase inhibition thereby proving that 4-chlorophenyl can be replaced by 5-membered rings. We therefore continued our efforts and tested compounds 5b–e where the 4-chlorophenyl ring has been replaced by substituted imidazoles. When the
imidazole is substituted by an alkyl group as in 5b, activity is comparable to 3, the tetrazoloquinazoline analogue of R115777. When the substituent is a phenyl group, then activity is improved to that of R115777. A few examples of compounds with substitution on the phenyl ring retained overall activity. This opens an avenue for further developments.

4. Conclusion

Starting from the structure of R115777 1 (tipifarnib, Zarnestra\(^a\)), a novel series of inhibitors of FTase have been

**Table 1**

Comparison of FPT inhibition for tetrazoloquinolines 4a, 4b, 1 and 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>FTase (enz) IC(_{50}) (nM)(^a)</th>
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<tr>
<td>1</td>
<td>−</td>
<td>0.9</td>
</tr>
<tr>
<td>2</td>
<td>−</td>
<td>3.5</td>
</tr>
<tr>
<td>4a</td>
<td>Cl</td>
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<tr>
<td>4b</td>
<td>CN</td>
<td>11</td>
</tr>
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</table>

\(^a\) The concentration required for a 50\% reduction of the FPT-catalyzed incorporation of \[^{3}H\]-farnesyl pyrophosphate into a biotinylated laminB peptide [23].

\(^b\) Inhibition (32\%) at 100 nM.
5. Experimental protocols

5.1. Chemistry

Proton NMR spectra were recorded at 400 MHz or at 300 MHz on a Bruker Avance 400, or 300 on a Bruker spectrometer, with Me$_4$Si as internal standard. Chemical shifts (δ) are reported in parts per million (ppm) and signals are reported as s (singlet), d (doublet), t (triplet), m (multiplet). Coupling constant are given in hertz (Hz). Electrospray mass spectra were recorded on a Waters/Micromass LCT spectrometer. Melting points were determined on a Mettler Toledo FP62 apparatus and are uncorrected. All reactions were routinely checked by TLC on silica gel Merck 60 F254. Column chromatography was carried out on Millipore silica gel (25–45 μm).

5.1.1. 2-Chloro-4-(3-chlorophenyl)-α-[1-{(4-chlorophenyl) methyl}-1H-imidazol-5-yl]-tetrazolo[1,5-a]quinoline-7-methanol 9

A mixture of 9 (0.0002 mol) and NaN$_3$ (0.0005 mol) in DMF (10 ml) was stirred at 140 °C overnight and H$_2$O was added. The mixture was extracted with CH$_2$Cl$_2$. The organic layer was washed several times with H$_2$O, dried (MgSO$_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: CH$_2$Cl$_2$/CH$_3$OH/CH$_3$CO$_2$H 97/3/0.2 to 95/5/0.1). The pure fractions were collected and the solvent was evaporated to afford 16% of 9 (0.76 g). 1H NMR (400 MHz, DMSO, 27 °C): δ = 5.13–5.22 (m, 2H, CH$_2$-Im), 5.82 (d, J = 5.0 Hz, 1H, CH=OH), 6.21 (d, J = 5.0 Hz, 1H, OH), 6.59 (s, 1H, H$_6$-imidazole), 6.86 (d, J = 8.5 Hz, 2H, 2H$_4$-chlorophenyl), 7.17 (d, J = 8.5 Hz, 2H, 2H$_4$-chlorophenyl), 7.57 (s, 1H, H$_3$-quinoline), 7.58–7.69 (m, 6H, 4H$_3$-chlorophenyl), 7.71 (s, 1H, H$_6$-imidazole), 7.92 (d, J = 7.5 Hz, 1H, H$_2$-quinoline) ppm.

5.1.2. 5-(3-Chlorophenyl)-α-[1-{(4-chlorophenyl)methyl}-1H-imidazol-5-yl]-tetrazolo[1,5-a]quinoline-7-methanol 4a

n-BuLi 1.6 M in hexane (0.0054 mol) was added at –70 °C to a solution of 6-bromo-2-chloro-4-(3-chlorophenyl)-quinoline 8 [34] (0.0048 mol) in THF (20 ml) under N$_2$ flow. The mixture was stirred at –70 °C for 1 h. A solution of 1-{(4-chlorophenyl)-methyl}-1H-imidazol-5-carboxaldehyde 7 [35] (0.0052 mol) in THF (14 ml) was added at –70 °C. The mixture was stirred at –70 °C for 1 h, then at room temperature overnight, poured into ice water and extracted with EtOAc. The organic layer was washed with H$_2$O, dried (MgSO$_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: CH$_2$Cl$_2$/CH$_3$OH/CH$_3$CO$_2$H 97/3/0.2 to 95/5/0.1). The pure fractions were collected and the solvent was evaporated to afford 16% of 9 (0.76 g). 1H NMR (400 MHz, DMSO, 27 °C): δ = 5.13–5.22 (m, 2H, CH$_2$-Im), 5.82 (d, J = 5.0 Hz, 1H, CH=OH), 6.21 (d, J = 5.0 Hz, 1H, OH), 6.59 (s, 1H, H$_6$-imidazole), 6.86 (d, J = 8.5 Hz, 2H, 2H$_4$-chlorophenyl), 7.17 (d, J = 8.5 Hz, 2H, 2H$_4$-chlorophenyl), 7.57 (s, 1H, H$_3$-quinoline), 7.58–7.69 (m, 6H, 4H$_3$-chlorophenyl), 7.71 (s, 1H, H$_6$-imidazole), 7.92 (d, J = 7.5 Hz, 1H, H$_2$-quinoline) ppm.

Table 2

<table>
<thead>
<tr>
<th>Compound</th>
<th>R</th>
<th>X</th>
<th>FTase (enz) IC$_{50}$ (nM)</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>-</td>
<td>NH$_3$</td>
<td>0.9</td>
</tr>
<tr>
<td>3</td>
<td>-</td>
<td>NH$_3$</td>
<td>3.5</td>
</tr>
<tr>
<td>18</td>
<td>2-Chloro-5-thiophenyl</td>
<td>OH</td>
<td>16</td>
</tr>
<tr>
<td>5a</td>
<td>2-Chloro-5-thiophenyl</td>
<td>NH$_3$</td>
<td>&lt;100b</td>
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<tr>
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<td>H</td>
<td>5</td>
</tr>
<tr>
<td>5c</td>
<td>2-Ph-1-imidazolyl</td>
<td>H</td>
<td>1</td>
</tr>
<tr>
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<td>2-(4-CN-Ph)-1-imidazolyl</td>
<td>H</td>
<td>4</td>
</tr>
<tr>
<td>5e</td>
<td>2-(3-CN-Ph)-1-imidazolyl</td>
<td>H</td>
<td>1</td>
</tr>
</tbody>
</table>

* See footnote ‘a’ in Table 1.

b Inhibition (69%) at 100 nM.
was stirred at 70 °C, H3-thiophene), 6.95 (d, 1H, C4-thiophene), 127.6 (C3-thiophene), 129.5 (C2-thiophene), 130.8 (C2 or 4 or 6-chlorophenyl), 131.3 (C6-benzisoxazole), 132.0 (C3 or 4 or 6-chlorophenyl), 134.7 (C1 or 3-chlorophenyl), 141.7 (C5-benzisoxazole), 148.8 (C2-thiophene), 157.6 (C6-benzisoxazole), 162.5 (C3-benzisoxazole) ppm. HRMS (ESI), calcd. for C18H11Cl2NO3S 375.9966, found 375.9969.

5.1.4. [3-(3-Chlorophenyl)-1,2-benzisoxazol-5-yl]-[5-chloro-2-thiophenyl]-methanol 11

n-BuLi 1.6 M in hexane (0.168 mol) was added dropwise at −70 °C to a solution of 5-bromo-3-(3-chlorophenyl)-1,2-benzisoxazole 10 [34] (0.129 mol) in THF (400 ml). The mixture was stirred at −70 °C for 15 min. A solution of 5-chlorothiophene-2-carboxaldehyde (0.155 mol) in THF (200 ml) was added dropwise. The mixture was stirred at −70 °C for 1 h, poured into ice water and extracted with AcOEt. The organic layer was separated, dried (MgSO4), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (35–70 μm, eluent: CH2Cl2 100%). The pure fractions were collected and the solvent was evaporated to afford 68% of 11 (33 g). M.p. = 120 °C, 1H NMR (400 MHz, DMSO-d6, 27 °C): δ = 6.00 (d, J = 4.0 Hz, 1H, CH—OH), 6.60 (d, J = 4.0 Hz, 1H, CH—OH), 6.80 (d, J = 4.0 Hz, 1H, H3-thiophene), 6.95 (d, J = 4.0 Hz, 1H, H4-thiophene), 7.44 (d, J = 9.5 Hz, 1H, H6-benzisoxazole), 7.72–7.76 (m, 3H, H7-benzisoxazole, 5-chlorophenyl, 6 or 4-chlorophenyl), 8.08 (d, J = 7.0 Hz, 1H, H4 or 4-chlorophenyl), 8.09 (s, 1H, H2-chlorophenyl), 8.13 (s, 1H, H4-benzisoxazole) ppm. 13C NMR (75 MHz, DMSO-d6, 27 °C): δ = 70.6 (CH—OH), 114.3 (Cbenzosoxazole), 115.8 (C7-benzisoxazole), 116.7 (C4-benzisoxazole), 124.2 (C3-thiophene), 125.4 (C4 or 6-chlorophenyl), 126.0 (C2-chlorophenyl), 126.8 (C4-thiophene), 127.6 (C5-thiophene), 129.5 (C1 or 3-chlorophenyl), 130.8 (C2 or 4 or 6-chlorophenyl), 131.3 (C6-benzisoxazole), 132.0 (C3 or 4 or 6-chlorophenyl), 134.7 (C1 or 3-chlorophenyl), 141.7 (C5-benzisoxazole), 148.8 (C2-thiophene), 157.6 (C6-benzisoxazole), 162.5 (C3-benzisoxazole) ppm. HRMS (ESI), calcd. for C18H11Cl2NO3S 375.9966, found 375.9969.

5.1.5. [3-(3-Chlorophenyl)-1,2-benzisoxazol-5-yl][5-chloro-2-thiophenyl]-methanone 12

MnO2 (35 g) was added to compound 11 (0.0877 mol) in dioxane (350 ml). The mixture was stirred and refluxed for 15 h, then cooled and filtered over Celite. The solvent was evaporated. The residue was washed with Et2O. The precipitate was filtered off and dried, yielding 21 g of 12 (64%). M.p. = 179 °C, 1H NMR (300 MHz, DMSO-d6, 27 °C): δ = 7.37 (d, J = 4.0 Hz, 1H, H4-thiophene), 7.70–7.65 (m, 2H, H5,6-benzoisoxazole), 7.74 (d, J = 9.5 Hz, 1H, H4-benzisoxazole), 7.85 (d, J = 9.5 Hz, 1H, H7-benzisoxazole), 7.87 (d, J = 4.0 Hz, 1H, H3-thiophene), 8.14–8.17 (m, 1H, H4-chlorophenyl), 8.19 (s, 1H, H2-chlorophenyl), 8.59 (s, 1H, H4-benzisoxazole) ppm. 13C NMR (75 MHz, DMSO-d6, 27 °C): δ = 113.8 (C3 or 5-benzisoxazole), 116.1 (C7-benzisoxazole), 125.8 (C4-benzisoxazole), 126.3 (C4-chlorophenyl), 126.9 (C2-chlorophenyl), 128.9 (C3-chlorophenyl), 129.6 (C4-thiophene), 131.0 (C6-benzisoxazole), 131.6 (C6-chlorophenyl), 132.0 (C5-chlorophenyl), 133.6 (C3 or 5-benzisoxazole), 134.7 (C2-chlorophenyl), 136.7 (C3-thiophene), 138.9 (C5-thiophene), 142.1 (C5-thiophene), 157.7 (C2-benzisoxazole), 166.2 (C3-benzisoxazole), 185.8 (C=O) ppm. LRMS (ESI), calcd. for C18H11Cl2NO3S 374.2, found 374.0 [MH]+.
5.1.7. 2,2,2-Trichloro-N-[2-(3-chlorobenzoyl)-4-[(5-chloro-2-thienyl)carbonyl]phenyl]-acetamide 14

Trichloroacetyl chloride (0.0416 mol) and Et3N (0.0416 mol) were added dropwise at 5 °C to a solution of 13 (0.0347 mol) in CH2Cl2 (130 ml) under N2 flow. The mixture was stirred at room temperature overnight, poured into ice water and extracted with CH2Cl2. The organic layer was separated, dried (MgSO4), filtered, and the solvent was evaporated, yielding 18.1 g of 14 (100%). M.p. = 194 °C, 1H NMR (300 MHz, DMSO-d6, 27 °C): δ = 7.43 (d, J = 4.0 Hz, 1H, H4-thiophene), 7.64 (d, J = 7.5 Hz, 1H, H5-chlorophenyl), 7.76 (m, 1H, H3 or 6-chlorophenyl), 7.82 (d, J = 4.0 Hz, 1H, H3-thiophene), 7.97 (d, J = 8.5 Hz, 1H, H6-2,2,2-trichlorophenylacetamide), 8.02 (d, J = 2.0 Hz, 1H, H2-2,2,2-trichlorophenylacetamide), 8.24 (dd, J = 8.5, 2.0 Hz, 1H, H3-thiophene), 8.28 (t, J = 7.5 Hz, 1H, H2-thiophene), 8.34 (dd, J = 8.0 Hz, 1H, H6 or 6-chlorophenyl), 8.72 (d, J = 3.5 Hz, 1H, H3-thiophene), 8.82 (d, J = 7.5 Hz, 1H, H4 or 6-chlorophenyl), 8.02 (s, 1H, H2-chlorophenyl), 8.22 (d, J = 8.5 Hz, 1H, H3-quinazoline), 8.43 (d, J = 8.5 Hz, 1H, H7-quinazoline), 8.48 (s, 1H, H8-quinazoline) ppm. 13C NMR (75 MHz, DMSO-d6, 27 °C): δ = 185.06 (COCCl3), 133.2 (C3-thiophene), 133.4 (C2-2,2,2-trichlorophenylacetamide), 136.4 (C3-thiophene), 139.8 (C1, C3, C5-quinazoline), 138.9 (3C, C1 or 3-chlorophenyl, C2-thiophene, C4-quinazolinone), 141.7 (C5-thiophene), 141.6 (C5-thiophene), 153.4 (quinazoline), 158.0 (C2-quinazoline), 171.5 (C4-quinazoline), 185.4 (C=O) ppm. HRMS (ESI), calcd. for C19H12Cl2N2O5 found: 418.9598.

5.1.10. 2-Chloro-4-(3-chlorophenyl)-α-(5-chloro-2-thienyl)-α-(1-methyl-1H-imidazol-5-yl)-quinazoline-6-methanol 17

n-BuLi 1.6 M in hexane (0.0404 mol) was added dropwise at −70 °C to a solution of 1-methyl-1H-imidazole (0.0404 mol) in THF (40 ml) under N2 flow. The mixture was stirred for 15 min. Chlorotriethylsilane (0.0414 mol) was added dropwise and the mixture stirred at −70 °C for 15 min. A solution of 16 (0.023 mol) in THF (100 ml) was added at −70 °C. The mixture was stirred at −70 °C for 1 h, poured into ice water, extracted with CH2Cl2 and washed with H2O. The organic layer was separated, dried (MgSO4), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15−40 μm, eluent: CH2Cl2/CH3OH/NH4OH 96/4/0.2). The pure fractions were collected and the solvent evaporated, yielding 2.75 g of 17 (24%). 1H NMR (400 MHz, DMSO-d6, 27 °C): δ = 3.38 (s, 3H, CH3(1-methylimidazole)), 6.35 (s, 1H, H4(1-methylimidazole)), 6.60 (d, J = 4.0 Hz, 1H, H4-thiophene), 6.97 (d, J = 4.0 Hz, 1H, H3-thiophene), 7.58 (s, 1H, H3(thiophene)), 7.61−7.78 (m, 5H, 4H3-chlorophenyl, H2(1-methylimidazole)), 7.97 (d, J = 1.5 Hz, 1H, H5-quinazoline), 8.08 (dd, J = 8.5, 1.5 Hz, 1H, H7-quinazoline) ppm.

5.1.11. 5-(3-Chlorophenyl)-α-(5-chloro-2-thienyl)-α-(1-methyl-1H-imidazol-5-yl)-tetrazolo[1,5-a]quinazoline-7-methanol 18

A mixture of 17 (0.0040 mol) and NaN3 (0.0119 mol) in DMF (40 ml) was stirred at 90 °C for 3 h, cooled and poured into ice water. The precipitate was filtered. The filtrate was extracted with CH2Cl2. The organic layer was brought together with the precipitate dissolved in CH2Cl2 and dried (MgSO4), filtered, and the solvent was evaporated. The residue was crystallized from CH3CN/DIPE. The precipitate was filtered off and dried, yielding: 1.33 g of 18 (66%). M.p. = 202 °C, 1H NMR (300 MHz, DMSO-d6, 27 °C): δ = 3.38 (s, 3H, NCH3 imidazole).
6.34 (s, 1H, H4-(1-methylimidazole)), 6.60 (d, J = 4.0 Hz, 1H, H3-thiophene), 6.99 (d, J = 4.0 Hz, 1H, H4-thiophene), 7.64–7.78 (m, 6H, OH, H2-(1-methylimidazole), H2,4,5-chlorophenyl), 8.08 (d, J = 1.5 Hz, 1H, H6-quinoline), 8.26 (dd, J = 8.5, 1.5 Hz, 1H, H8-quinoline), 8.74 (d, J = 8.5 Hz, 1H, H9-quinoline) ppm. 13C NMR (75 MHz, DMSO-d6, 27°C): δ = 116.7 (C9-quinazoline), 118.2 (C10-quinazoline), 125.7 (C13-thiophene), 126.6 (C6-quinazoline), 127.0 (C4-C14-thiophene), 128.7 (C5-thiophene), 128.8 (C8-chlorophenyl), 129.8 (C-chlorophenyl), 130.4 (C4-(1-methylimidazole)), 131.0 (2C, C-chlorophenyl), 133.8 (C1-quinazoline), 133.9 (C1 or 3-chlorophenyl), 134.3 (C5-(1-methylimidazole)), 134.8 (C8-quinazoline), 138.1 (C1 or 3-chlorophenyl), 141.6 (C2-(1-methylimidazole)), 145.4 (C7-quinazoline), 149.2 (C2-thiophene), 152.8 (C2-thiophene), 167.9 (C5-quinazoline) ppm. HRMS (ESI), calcld. for C23H15Cl2N7OS 507.0674, found 507.0669.

5.1.14. 4-(3-Chlorophenyl)-N,2-dimethoxy-N-methyl-6-quinazolincarboxamide 21

A mixture of 6-bromo-2-methoxy-4-(3-chlorophenyl)-quinazoline 20 (0.03146 mol), Pd(PPh3)4 (0.003146 mol) and N,N-dimethyldihydroxylamine hydrochloride (0.06923 mol) in Et3N (22 ml) and dioxane (90 ml) was stirred at 100°C for 18 h under a 5 bar pressure of CO2, then cooled, poured into ice water, extracted with CH2Cl2 and filtered over Celite. The organic layer was separated, dried (MgSO4), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–35 μm, eluent: CH3Cl/CH2Cl2/NH4OH 85/15) then CH2Cl2/CH3OH/NH4OH 98/2/0.4. The pure fractions were collected and the solvent was evaporated, yielding 3 g of 21 (27%). M.p. = 118°C. 1H NMR (400 MHz, DMSO, 27°C): δ = 3.33 (s, 3H, NCH3), 3.55 (s, 3H, NOCH3), 4.10 (s, 3H, OCH3(2-quinazoline)), 7.64–7.84 (m, 4H, 4H2-chlorophenyl), 7.93 (d, J = 8.5 Hz, 1H, H8-quinoline), 8.16 (dd, J = 8.5, 1.5 Hz, 1H, H7-quinoline), 8.21 (d, J = 1.5 Hz, 1H, H8-quinoline) ppm. 13C NMR (75 MHz, DMSO-d6, 27°C): δ = 33.1 (NCH3), 54.9 (NOCH3), 60.9 (OCH3), 118.5 (C(9-quinazoline), 126.5 (C9-chlorophenyl), 127.4 (C6-quinazoline), 128.5 (C3-chlorophenyl), 129.3 (C2-(3-chlorophenyl)), 130.3 (C1-quinazoline), 130.4 (C3-chlorophenyl), 130.6 (C3-chlorophenyl), 133.4 (C3-chlorophenyl), 134.0 (C8-quinazoline), 137.9 (C3-chlorophenyl), 153.1 (C=O), 162.5 (C3-quinazoline), 167.3 (C-quinazoline), 170.8 (C5-quinazoline) ppm. Anal. (C18H16ClN3O3, 0.05 CH2Cl2) calcld. C 59.88, H 4.48, N 11.61, found C 59.97, H 4.48, N 11.54.

5.1.15. 2-Chloro-4-(3-chlorophenyl)-N-methoxy-N-methyl-6-quinazolincarboxamide 22

POCl3 (0.084 mol) was added dropwise at room temperature to a solution of 21 (0.042 mol) in DMF (110 ml). The mixture was stirred at 80°C for 4 h, cooled, poured into ice water, extracted with EtOAc and basified with K2CO3 solid. The organic layer was separated, dried (MgSO4), filtered, and the solvent was evaporated. The residue was purified from column chromatography on silica gel (15–35 μm, eluent: CH3Cl/CH2Cl2/CH3OH/NH4OH 85/15) then CH2Cl2/CH3OH/NH4OH 98/2/0.4. The pure fractions were collected and the solvent was evaporated, yielding 59 g of 22 (59%). M.p. = 110°C. 1H NMR (400 MHz, DMSO, 27°C): δ = 3.32 (s, 3H, NCH3), 3.55 (s, 3H, NOCH3), 7.68–7.80 (m, 3H, 3H, H8-quinoline), 7.88 (s, 1H, H2-chlorophenyl), 8.12 (d, J = 9.0 Hz, 1H, H8-quinoline), 8.28 (m, 2H, H6,7-quinoline) ppm. 13C NMR (75 MHz, DMSO-d6, 27°C): δ = 34.4 (NCH3), 62.5 (NOCH3), 122.1 (C-quinazoline), 128.5 (C5 or 7-quinazoline), 128.8 (C6-quinazoline), 130.2 (C4 or 6-(3-chlorophenyl)), 131.0 (C2-(3-chlorophenyl)), 132.1 (2C, C5, C6 or 6-(3-chlorophenyl)), 134.9 (C1 or 3-(3-chlorophenyl)), 135.4 (C-quinazoline), 136.2 (C5 or 7-quinazoline), 138.4 (C1 or 3-(3-chlorophenyl)), 154.4 (C6-quinazoline), 158.3 (C2-quinazoline), 168.2 (C=O), 172.1 (C4-quinazoline) ppm. Anal. (C18H16ClN3O2) calcld. C 56.37, H 3.62, N 11.60, found C 56.49, H 3.54, N 11.42.
5.1.16. [2-Chloro-4-(3-chlorophenyl)-6-quinazolinyl]-
(1-methyl-1H-imidazol-5-yl)-methanone 23

n-ButLi 1.6 M in hexane (0.042 mol, 26.2 ml) was added
dropwise at −70 °C to a mixture of 1-methyl-1H-imidazole
(0.042 mol) in THF (80 ml) under N2 flow. The mixture
was stirred for 15 min. Chlorotriethylsilane (0.043 mol) was
added. The mixture was stirred for 15 min. n-ButLi 1.6 M in hexane
(0.037 mol, 23.2 ml) was added at −70 °C, and the mixture
was stirred for 15 min. A solution of 22 (0.024 mol) in THF
(80 ml) was added at −70 °C, and the mixture was stirred at
−70 °C for 30 min, poured into H2O and extracted with EtOAc.
The organic layer was separated, dried (MgSO4), filtered, and the solvent was evaporated,
yielding 4 g of 4-(1-methyl-1H-imidazol-5-yl)-methanone 5.1.16. 
[2-Chloro-4-(3-chlorophenyl)-6-quinazolinyl]-
(tetrazolo[1,5-a]quinazoline-7-methanol 5.1.17. 2-Chloro-4-(3-chlorophenyl)-α-(1-methyl-1H-
imidazol-5-yl)-6-quinolinemethanol 24

DIBAL-H in toluene (10 ml) was added dropwise at−70 °C to compound 23 (0.012 mol) in THF (150 ml) under
N2 flow. The mixture was stirred at −70 °C for 30 min. DIBAL-
H in toluene (50 ml) was added. The mixture was stirred at
−70 °C for 3 h, poured into ice water, extracted with CH2Cl2
and filtered over Celite. The organic layer was separated,
dried (MgSO4), filtered, and the solvent was evaporated,
yielding 2.46 g of 23 (27%). M.p. = 190 °C, 1H NMR (400 MHz, DMSO,
27 °C): δ = 3.92 (s, 3H, CH3(1-methylimidazolododecyl)), 7.60–7.75 (m, 2H, 2H3-chlorophenyl), 7.77 (s, 1H, H4-(1-methylimidazolodidecyl)), 7.83–7.89 (m, 1H,
H3-chlorophenyl), 7.97–7.99 (m, 1H, H3-chlorophenyl), 8.08 (s, 1H, H2-(1-methylimidazolodidecyl)), 8.21 (d, J = 8.5 Hz, 1H,
H8-quinazoline), 8.41 (dd, J = 8.5, 2.0 Hz, 1H, H7-quinazoline), 8.44 (d, J = 2.0 Hz, 1H, H5-quinazoline) ppm.

5.1.18. 5-(3-Chlorophenyl)-α-(1-methyl-1H-imidazol-5-yl)-
tetrazolo[1,5-a]quinazoline-7-methanol 25

NaBH3CN (0.031 mol) was added at room temperature to
compound 24 (0.0103 mol) in DMF (40 ml). The mixture was
stirred at 90 °C for 4 h, then cooled, poured into ice water
and stirred at room temperature for 1 h. The precipitate
was filtered off and dried at 80 °C under vacuo, yielding
3.4 g of 25 (84%). M.p. = 190 °C, 1H NMR (400 MHz, DMSO,
27 °C): δ = 3.60 (s, 3H, CH3(1-methylimidazolodidecyl)), 6.60 (d,
J = 5.5 Hz, 1H, CH−OH), 6.33 (d, J = 5.5 Hz, 1H, CH−OH), 6.36 (s, 1H, H4-(1-methylimidazolodidecyl)), 7.58 (s, 1H,
H2-(1-methylimidazolodidecyl)), 7.68–7.86 (m, 4H, 4H3-chlorophenyl), 8.21 (m, 2H, H6,8-quinazoline), 8.71 (d, J = 7.5 Hz, 1H,
H9-quinazoline) ppm.

5.1.19. 7-(Chloro-1-methyl-1H-imidazol-5-yl)methyl-5-(3-
chlorophenyl)-tetrazolo[1,5-a]quinazoline
monohydrochloride 26

Compound 25 (0.0025 mol) in SOCl2 (10 ml) was stirred at
65 °C for 4 h, then cooled and the solvent was evaporated till
dryness. The residue was taken up twice in CH2Cl2. The
solvent was evaporated till dryness, yielding 26. This product
was used directly in the next reaction step.

5.1.20. 5-(3-Chlorophenyl)-7-[(2-ethyl-1H-imidazol-1-yl)(1-
 methyl-1H-imidazol-5-yl)methyl]-tetrazolo-
[1,5-a]quinazoline 5b

A mixture of 26 (0.0010 mol) and 2-ethyl-1H-imidazole
(0.0015 mol) in CH2CN (5 ml) was stirred and refluxed
for 2 h, cooled, poured into ice water, extracted with
CH2Cl2 and washed with K2CO3 10%. The organic layer
was separated, dried (MgSO4), filtered, and the solvent was
evaporated. The residue was purified by column chromatography over silica gel
(10 μm, eluent: CH2Cl2/EtOAc 50/50 then CH2Cl2/CH3OH
1:1). The pure fractions were collected and the solvent was evaporated, yielding 0.084 g
of 5b (17.5%). M.p. = 120 °C, 1H NMR (400 MHz, DMSO,
27 °C): δ = 1.09 (t, J = 7.5 Hz, 3H, CH3(2-ethylimidazolodidecyl)), 2.45–2.53 (m, 1H, CH2(2-ethylimidazolodidecyl)), 2.70–2.80 (m, 1H, CH2(2-ethylimidazolodidecyl)), 3.39 (s, 3H,
CH3(1-methylimidazolodidecyl)), 6.32 (s, 1H, H4(1-methylimidazolodidecyl)), 6.65 (s, 1H, H5-(2-ethylimidazolodidecyl)), 6.85 (s, 1H, H4-(2-ethylimidazolodidecyl)), 7.24 (s, 1H, CH), 7.65–7.67 (m, 2H, 2H3-chlorophenyl), 7.74–7.76 (m, 3H, 2H3-chlorophenyl), 7.81 (s, 1H, H6-quinazoline), 8.11 (d, J = 8.5 Hz, 1H,
H9-quinazoline), 8.79 (d, J = 8.5 Hz, 1H, H8-quinazoline) ppm.
13C NMR (75 MHz, DMSO-d6, 27 °C): δ = 12.8 (CH3(2-ethylimidazolodidecyl)), 20.4 (CH2(2-ethylimidazolodidecyl)), 31.8 (CH3(1-methylimidazolodidecyl)), 53.5 (CH, 117.8 (C5-(3-chlorophenyl)), 117.2 (C5-(2-ethylimidazolodidecyl)), 119.3 (Quinazoline), 128.1 (C6-quinazoline), 128.3 (C4-(2-ethylimidazolodidecyl)), 129.3 (C3-chlorophenyl), 130.3 (C4-(1-methylimidazolodidecyl)), 130.5 (C5-(1-methylimidazolodidecyl)), 130.8 (C4-(1-methylimidazolodidecyl)), 131.5 (C3-chlorophenyl), 131.6 (C3-chlorophenyl), 133.7 (Quinazoline), 134.3 (C1 or 3-(3-chlorophenyl) ), 135.6 (C8-quinazoline), 138.6 (C1 or 3-(3-chlorophenyl) ), 139.8 (C7-quinazoline), 141.1 (C2-(1-methylimidazolodidecyl)), 149.6 (C2-(2-ethylimidazolodidecyl)), 153.3 (Quinazoline), 168.3 (C5-quinazoline) ppm. HRMS (ESI), calcd. for C24H20ClIN9O4 740.1608, found 740.1604.
5.1.21. 5-(3-Chlorophenyl)-7-{[2-phenyl-1H-imidazol-1-yl]-[1-methyl-1H-imidazol-5-yl]methyl}-tetrazolo[1,5-a]quinazoline 5c

2-Phenyl-1H-imidazole (0.0038 mol) was added at room temperature to compound 26 (0.0025 mol) in CH₂CN (10 ml). The mixture was stirred and refluxed for 2 h, poured into ice water and extracted with CH₂Cl₂/CH₃OH. The organic layer was washed with K₂CO₃, separated, dried (MgSO₄), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: CH₂Cl₂/CH₃OH/CH₃CN 96/4/0.2). The pure fractions were collected and the solvent was evaporated, yielding 0.17 g of 5c (13%). M.p. = 150 °C. ¹H NMR (400 MHz, DMSO, 27 °C): δ = 3.31 (s, 3H, CH₃(1-methylimidazole)), 6.30 (s, 1H, H₄-(1-methylimidazole)), 6.93 (s, 1H, Hphenylimidazole), 7.16 (s, 1H, CH), 7.37–7.46 (m, 5H, Hphenyl), 7.61–7.68 (m, 2H, H₆-quinazoline), 7.71 (s, 1H, H₃-(1-methylimidazole)), 7.76 (m, 2H, H₂-4-chlorophenyle), 7.83 (s, 1H, H₅-quinazoline), 8.14 (d, J = 8.5 Hz, 1H, H₂-phenylimidazole), 8.78 (d, J = 8.5 Hz, H₆-quinazoline) ppm. ¹³C NMR (75 MHz, DMSO-d₆, 27 °C): δ = 31.2 (CH₃(1-methylimidazole)), 55.0 (CH), 116.5 (2C, C₂-(3-chlorophenyle), 130.2 (C 3-chlorophenyl), 130.7 (C 2-(1-methylimidazole)), 131.0 (C 4-(1-methylimidazole)), 131.5 (C 3-chlorophenyl), 131.6 (C 5-chlorophenyl), 131.8 (2C, JC–F = 9.2 Hz, C₂,6-(4-fluorophenyl)), 133.7 (Cphenazine), 134.4 (C 2-chlorophenyl), 135.5 (C₈-quinazoline), 138.6 (C 3-chlorophenyl), 139.8 (C₇-quinazoline), 141.1 (C 2-(1-methylimidazole)), 147.3 (C 2-(4-fluorophenylimidazole)), 153.3 (Cphenazine), 157.7 (C 4-(4-fluorophenyl)), 168.3 (C₅-quinazoline) ppm. Anal. for C₂₈H₁₃ClN₉ (518.1608): calcd. C 60.21, H 3.04, N 18.44; found C 59.63, H 3.12, N 18.28.

5.1.22. 5-(3-Chlorophenyl)-7-{[2-(4-fluorophenyl)-1H-imidazol-1-yl]-[1-methyl-1H-imidazol-5-yl]methyl}-tetrazolo[1,5-a]quinazoline 5d

(2-Fluorophenyl)quinazolone (0.0026 mol) and K₂CO₃ (0.0053 mol) was added at room temperature to compound 26 (0.0017 mol) in CH₂CN (10 ml). The mixture was stirred and refluxed for 2 h, poured into ice water and extracted with CH₂Cl₂/CH₃OH. The organic layer was separated, dried (MgSO₄), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: CH₂Cl₂/CH₃OH/CH₃CN 95/5/0.2) and then again over silica gel (10 μm eluent: CH₂Cl₂ 100%). The pure fractions were collected and the solvent was evaporated, yielding: 0.048 g of 5d (5%). ¹H NMR (300 MHz, DMSO, 27 °C): δ = 3.35 (s, 3H, CH₃(1-methylimidazole)), 6.27 (s, 1H, H₄-(1-methylimidazole)), 6.98 (d, J = 2.0 Hz, 1H, H₅-(phenylimidazole)), 7.16 (d, J = 2.0 Hz, 1H, H₆-(phenylimidazole)), 7.29 (s, 1H, CH), 7.57–7.69 (m, 5H, 3H₃-chlorophenyl, 2H₄ and 5-benzonitrile), 7.75–7.77 (m, 1H, H₂-4-chlorophenyle), 7.78 (s, 1H, H₅-quinazoline), 7.85 (s, 1H, H₂-benzonitrile), 7.90 (d, J = 2.0 Hz, 1H, H₆-quinazoline), 8.08 (dd, J = 8.5, 2.0 Hz, 1H, H₅-quinazoline), 8.78 (d, J = 8.5 Hz, 1H, H₆-quinazoline) ppm. ¹³C NMR (75 MHz, DMSO-d₆, 27 °C): δ = 31.2 (CH₃(1-methylimidazole)), 54.3 (CH), 112.1 (C₁ or 3,3-benzodioxole), 117.3 (Cphenazine), 118.5 (C₇-quinazoline), 118.6 (Cquinazoline), 120.7 (C₅-(phenylimidazole)), 127.5 (C₆-quinazoline), 128.7 (Cphenazine), 129.6 (C₄-(phenylimidazole)), 129.8 (C₅-(1-methylimidazole)), 130.2 (Cphenyl), 130.5 (C₄-(1-methylimidazole)), 130.8 (Cphenyl), 130.9 (C₂-phenyl), 131.5 (Cphenyl), 132.1 (C₂-benzonitrile), 132.9 (Cphenazine), 133.1 (C₆-benzonitrile), 133.5 (Cphenyl), 134.8 (C₈-quinazoline), 137.8 (Cphenyl), 138.8 (C₇-quinazoline), 140.6 (C₂-(1-methylimidazole)), 145.5 (C₂-(4-fluorophenylimidazole)), 152.7 (Cquinazoline), 167.3 (C₅-quinazoline) ppm. HRMS (ESI), calcd. for C₂₈H₁₉ClF₂N₁₀ 543.1561, found 543.1557.

5.2. Molecular modeling

5.2.1. Conformational analysis

The conformational analysis of 1 has been achieved by using the Random Search tool available in the Sybyl 6.8
modeling software [37]. The conformational space was sampled by randomly perturbing the torsion angle of all rotatable bonds including those of the two methyl substituents. At each step, the random geometry was relaxed by a full energy minimization using the MMFF94s force field [38]. The process was performed for 1000 cycles. Search parameters were set to default value except for the energy cutoff set to 8 kcal/mol. Fifty-eight distinct conformers were generated among which the one with the lowest energy was retained. This conformer was superimposed with the X-ray structure of isolated compound 1 and was found to match tightly (Fig. 6). The major difference lies in the opposite symmetrical orientation of the 3-chlorophenyl substituent relative to the plane defined by the quinolinone scaffold. However both conformations can switch from one to another in vacuo as indicated by identical internal energy.

5.2.2. Docking

Molecule 1 was docked manually within the FTase catalytic site. The initial FTase coordinates were retrieved from the Protein Data Bank (PDB code: 1QBQ [39]) and processed to yield a model appropriate for molecular docking. In particular, the CVIM peptide was removed whereas the alpha-hydroxyfarne-syl-phosphonic acid (HFP), an analogue of the farnesyl pyrophosphate needed for farnesylation, was conserved. The lowest energy conformer identified during the conformational analysis of 1 was used as starting point. Based on internal knowledge, two anchor points were chosen to orient the ligand within the binding site: the imidazole basic nitrogen was positioned such as to coordinate the zinc atom while the quinolinone carbonyl group was moved close to a structured water molecule known to form an hydrogen bond with other FTase inhibitors [40]. Molecule 1 was then rotated around this N–C=O axis in order to orient the two chlorophenyl substituents towards the hydrophobic rear of the catalytic cavity (Fig. 2). The FTase/molecule 1/HFP ternary complex was subsequently minimized using the Tripos force field and the kollman All-Atom charge set. During the minimization, the ligands along with all residues included in a 10 Å sphere around the molecule 1 were allowed to move, while the remaining part of the protein structure, including the zinc atom, was kept rigid. Molecule 4a was constructed by structural modification of 1 which was used as a template. The molecule was then submitted to a conformational analysis and docked manually using the procedure described above (Fig. 4).

References


[37] SYBYL® release 6.8, Tripos Inc., 1699 South Hanley Rd., St Louis, Missouri, 63144, USA.


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