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Short communication

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.

Keywords: Farnesyltransferase inhibition; Tipifarnib; Zarnestra; R115777; Anticancer agents

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

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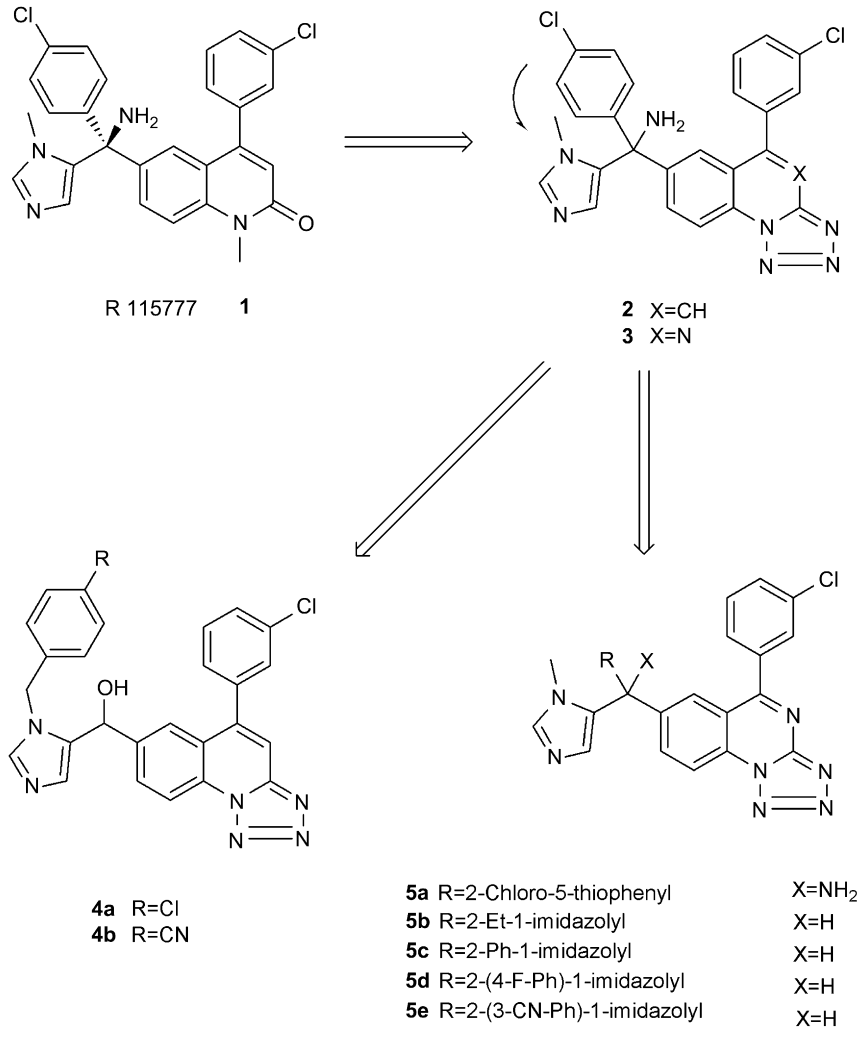


Fig. 1. Modifications of R115777 **1** and tetrazolo heterocycles **2**, **3** led to novel inhibitors of FTase **4**–**5**.

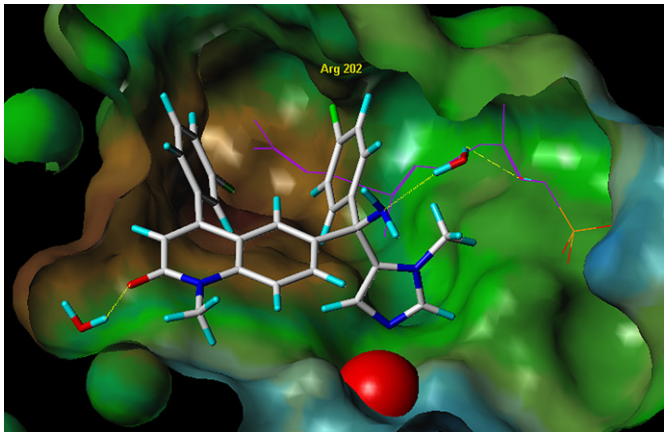


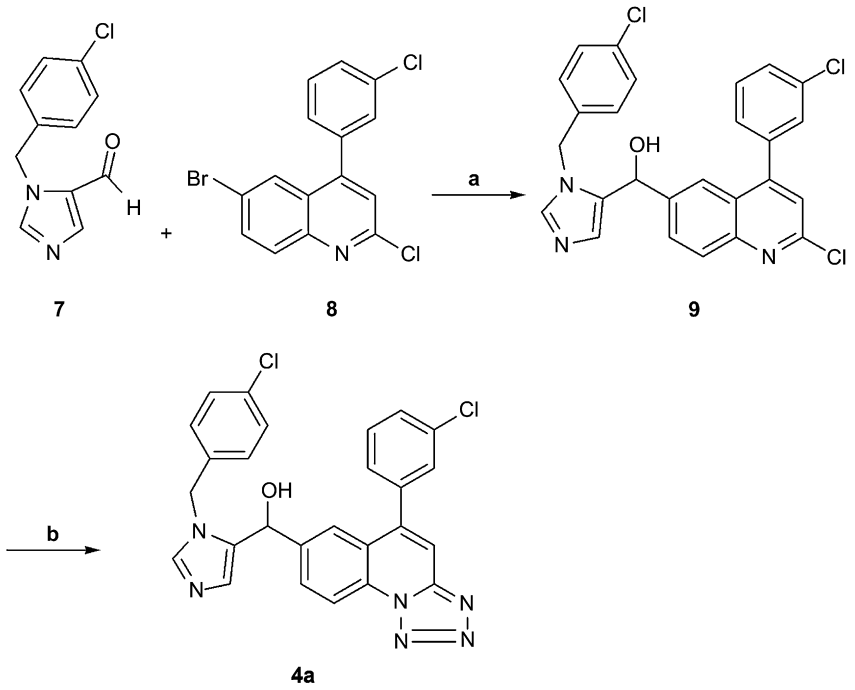
Fig. 2. Docking of molecule **1** (capped sticks) within the FTase catalytic site. Alpha-hydroxyfarnesyl-phosphonic acid (HFP) (violet lines) is sitting along the "right" wall of the catalytic site. The two water-mediated hydrogen bonds between **1** and HFP are shown as yellow dashed lines. This binding mode has been confirmed later by the elucidation of the X-ray structure of the FTase/tipifarnib/farnesyl pyrophosphate (FPP) complex [33]. (For interpretation of the references to colors in the figure legends, the reader is referred to the web version of this article.)

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



Scheme 1. (a) *n*-BuLi, THF, −70 °C, 1 h then addition of **7**, −70 °C, 1 h to RT, 16 h (16%); (b) NaN_3, DMF, 140 °C, 16 h (25%).

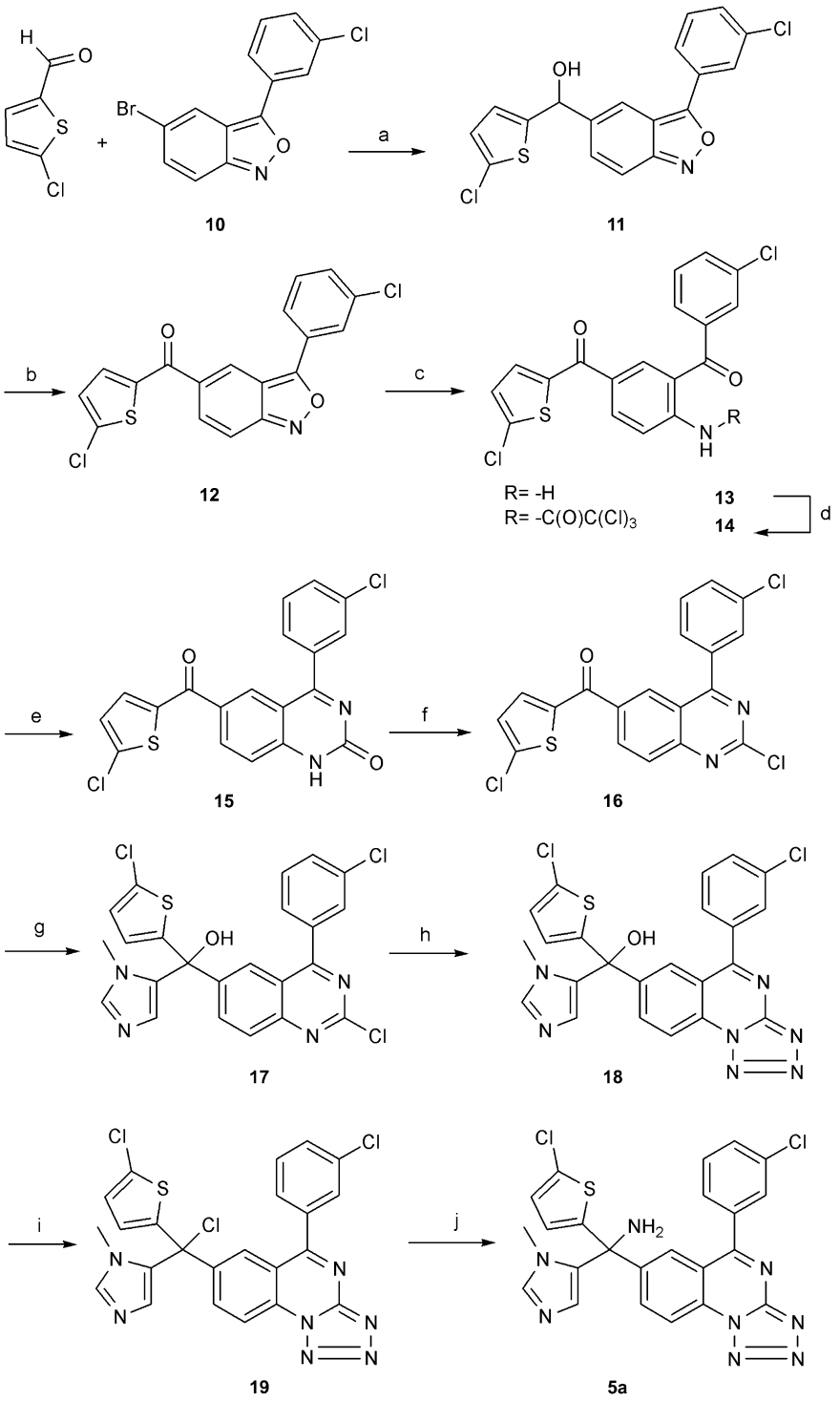
to provide alcohol **11** (Scheme 2). After oxidation of the hydroxyl group into a ketone moiety, the benzisoxazole ring was reduced to *ortho*-aminobenzophenone **13** using titanium trichloride Lewis acid. Acylation of **13** with trichloroacetyl chloride provided the corresponding amide **14** and in situ cyclisation by heating **14** with ammonium acetate in DMSO gave the quinazolinone **15** in good yield. Upon refluxing in $POCl_3$ **15** was converted to 2-chloroquinazoline **16**. Then 1-methylimidazole was first deprotonated at C-2 by action of *n*-butyllithium and the resulting carbanion was silylated. In the same pot, further deprotonation at C-5 by *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 $POCl_3$. 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

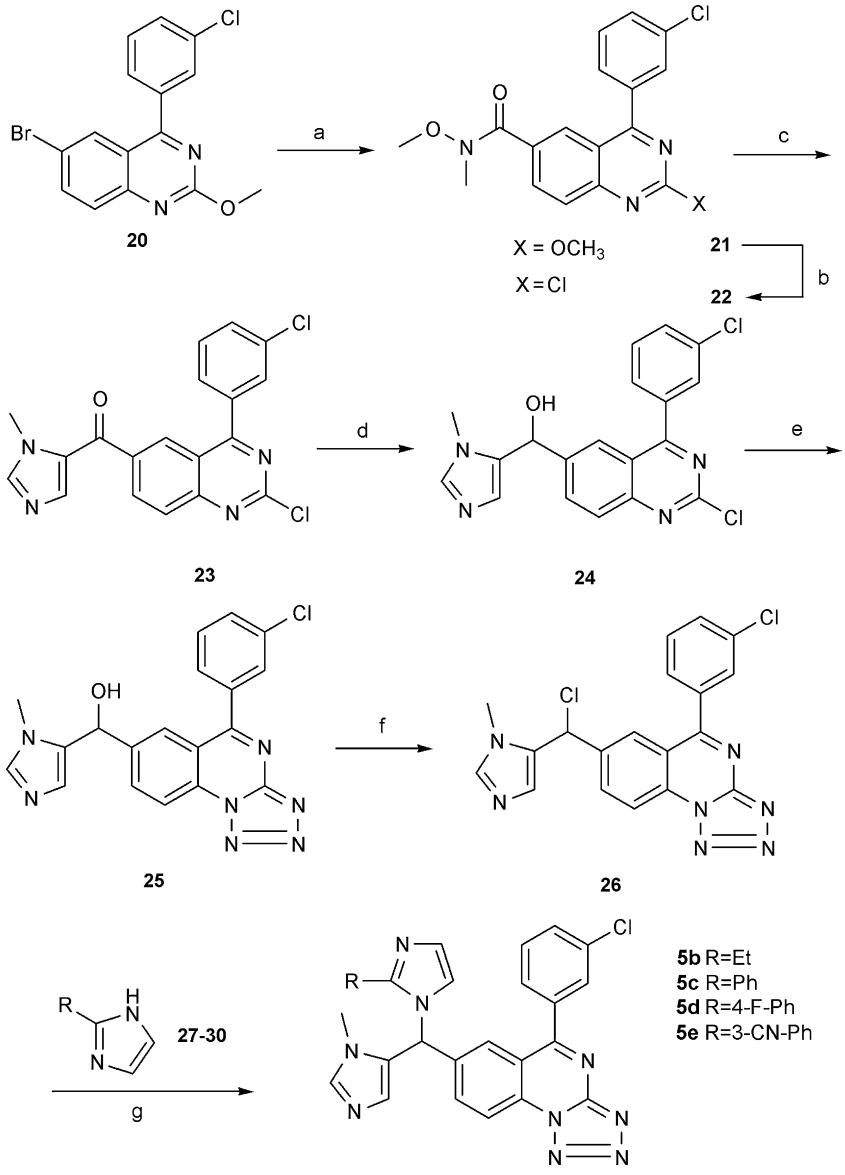


Scheme 2. (a) *n*-BuLi, THF, −70 °C, 1 h (68%); (b) MnO_2, dioxane, reflux, 15 h (64%); (c) $TiCl_3$, H_2O/THF, RT, 3 h (100%); (d) trichloroacetyl chloride, NEt_3, CH_2Cl_2, RT, overnight (100%); (e) ammonium acetate, DMSO, 60 °C, 4 h (83%); (f) $POCl_3$, 100 °C, 2 h (88%); (g) (1) 1-methylimidazole, *n*-BuLi, THF, $ClSiEt_3$, −70 °C; (2) *n*-BuLi, THF, −70 °C, 1 h (24%); (h) NaN_3, DMF, 90 °C, 3 h (66%); (i) $SOCl_2$, 60 °C, 3 h (30%); (j) NH_3/*i*PrOH, THF, RT, 15 h (18%).

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



Scheme 3. (a) CH_3NHOCH_3, CO 5 bar, $Pd(PPh_3)_4$, Et_3N, dioxane, 100 °C, 18 h (27%); (b) $POCl_3$, DMF, 80 °C, 4 h (59%); (c) 1-methylimidazole, *n*-BuLi, $ClSiEt_3$, THF, −70 °C (27%); (d) DIBAL in toluene, THF, −70 °C, 4 h (86%); (e) NaN_3, DMF, 90 °C, 4 h (84%); (f) $SOCl_2$, 65 °C, 4 h; (g) 2-substitued-1*H*-imidazole, (K_2CO_3), CH_3CN, reflux, 2 h (5–20%).

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.

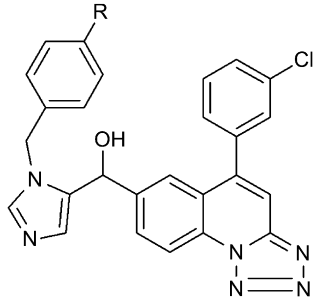


Fig. 3.

4. Conclusion

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

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

Compound	R	FTase (enz) IC_{50} (nM)[a]
1	–	0.9
2	–	3.5
4a	Cl	>100[b]
4b	CN	11

[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.

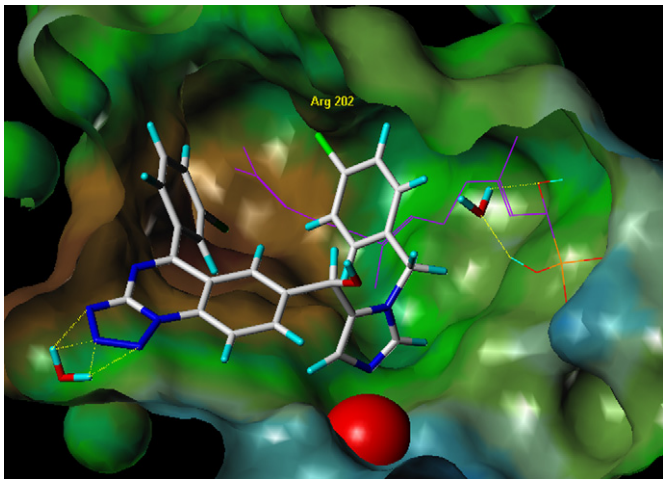


Fig. 4. Docking of molecule **4a** (capped sticks) within the FTase catalytic site. HFP (violet lines) is sitting along the "right" wall of the catalytic site. As compared to **1**, the 4-chlorophenyl ring adopts a tilted orientation less favorable for stacking interactions and only one water-mediated hydrogen bond remains after minimization. (For interpretation of the references to colors in the figure legends, the reader is referred to the web version of this article.)

synthesized by modification of the 2-quinolinone motif and replacement or displacement of the 4-chlorophenyl moiety. We have identified compounds with in vitro potency in the same range as that of R115777. These encouraging results warrant further efforts to optimize the synthesis and pharmacokinetic properties of these highly potent FTIs.

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_4Si 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 F_{254}. Column chromatography was carried out on Millipore silica gel (25–45 μM).

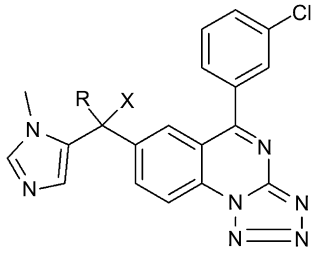


Fig. 5.

Table 2
FTase inhibition for tetrazoloquinazolines **5b–e**

Compound	R	X	FTase (enz) IC_{50} (nM)[a]
1	-	NH_2	0.9
3	-	NH_2	3.5
18	2-Chloro-5-thiophenyl	OH	16
5a	2-Chloro-5-thiophenyl	NH_2	<100[b]
5b	2-Et-1-imidazolyl	H	5
5c	2-Ph-1-imidazolyl	H	1
5d	2-(4-F–Ph)-1-imidazolyl	H	4
5e	2-(3-CN–Ph)-1-imidazolyl	H	1

[a] See footnote 'a' in Table 1.
[b] Inhibition (69%) at 100 nM.

5.1.1. 2-Chloro-4-(3-chlorophenyl)-α-[1-[(4-chlorophenyl)-methyl]-1H-imidazol-5-yl]-quinoline-6-methanol **9**

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)]-1*H*-imidazole-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_2O, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 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): $\delta = 5.13–5.22$ (m, 2H, CH_2-Im), 5.82 (d, $J = 5.0$ Hz, 1H, C*H*–OH), 6.21 (d, $J = 5.0$ Hz, 1H, OH), 6.59 (s, 1H, $H_{4\text{-imidazole}}$), 6.86 (d, $J = 8.5$ Hz, 2H, $2H_{4\text{-chlorophenyl}}$), 7.17 (d, $J = 8.5$ Hz, 2H, $2H_{4\text{-chlorophenyl}}$), 7.57 (s, 1H, $H_{3\text{-quinoline}}$), 7.58–7.69 (m, 6H, $4H_{3\text{-chlorophenyl}}$, $H_{5,7\text{-quinoline}}$), 7.71 (s, 1H, $H_{2\text{-imidazole}}$), 7.92 (d, $J = 7.5$ Hz, 1H, $H_{8\text{-quinoline}}$) ppm.

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

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_2O was added. The mixture was extracted with CH_2Cl_2. The organic layer was washed several times with H_2O, dried (MgSO4), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (10 μm, eluent: CH_2Cl_2/CH_3OH 98/2 to 95/5). The pure fractions were collected and the solvent was evaporated; the compound was washed with diethyl ether and dried, yielding 0.041 g of **4a** (25%). M.p. = 140 °C, ^1H NMR (400 MHz, DMSO-d_6, 27 °C): $\delta = 5.17$ (m, $J = 16.0$ Hz, 2H, CH_2-Im), 5.91 (d, $J = 5.0$ Hz, 1H, C*H*–OH), 6.32 (d, $J = 5.0$ Hz, 1H, OH), 6.66 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 6.79 (d, $J = 8.5$ Hz, 2H, $2H_{4\text{-chlorophenyl}}$), 7.09 (d, $J = 8.5$ Hz, 2H, $2H_{4\text{-chlorophenyl}}$), 7.51 (dd, $J = 1.5$, 7.0 Hz, 1H, $H_{3\text{-chlorophenyl}}$), 7.61–7.67 (m, 3H, $3H_{3\text{-chlorophenyl}}$), 7.69 (s, 1H, $H_{2\text{-(1-methylimidazole)}}$), 7.73 (s, 1H, $H_{6\text{-quinoline}}$), 7.80 (dd, $J = 1.5$, 8.5 Hz, 1H, $H_{8\text{-quinoline}}$), 8.11 (s, 1H, $H_{4\text{-quinoline}}$), 8.58 (d, $J = 8.5$ Hz, 1H, $H_{9\text{-quinoline}}$)

ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 47.4$ (CH_2), 65.9 (CH), 113.0 ($C_{4\text{-quinoline}}$), 117.1 ($C_{9\text{-quinoline}}$), 122.9 ($C_{\text{quinoline}}$), 124.9 ($C_{6\text{-quinoline}}$), 128.4–128.7 (7C, $2C_{3\text{-chlorophenyl}}$, $4C_{4\text{-chlorophenyl}}$, $C_{4\text{-limidazole}}$), 129.5 ($C_{\text{quinoline}}$), 129.6 ($C_{3\text{-chlorophenyl}}$), 130.6 ($C_{8\text{-quinoline}}$), 131.3 ($C_{3\text{-chlorophenyl}}$), 131.8 ($C_{4\text{-chlorophenyl}}$), 133.5 ($C_{3\text{-chlorophenyl}}$), 133.6 ($C_{5\text{-imidazole}}$), 136.3 ($C_{4\text{-chlorophenyl}}$), 138.4 ($C_{5\text{-quinoline}}$), 139.4 ($C_{2\text{-imidazole}}$), 142.3 ($C_{7\text{-quinoline}}$), 143.9 ($C_{1\text{-(3-chlorophenyl)}}$), 146.4 ($C_{\text{quinoline}}$) ppm. HRMS (ESI), calcd. for $C_{26}H_{18}Cl_2N_6O$ 501.0997, found 501.0995.

5.1.3. 4-[[5-[[5-(3-Chlorophenyl)tetrazolo[1,5-a]quinolin-7-yl]hydroxymethyl]-1H-imidazol-1-yl]methyl]-benzonitrile **4b**

Compound **4b** was synthesized using the same procedure as above (yield = 36%). M.p. = 158 °C, ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): $\delta = 5.20$ (s, 2H, CH_2-Im), 5.91 (d, $J = 5.0$ Hz, 1H, C*H*–OH), 6.31 (d, $J = 5.0$ Hz, 1H, OH), 6.67 (s, 1H, $H_{4\text{-imidazole}}$), 6.95 (d, $J = 8.5$ Hz, 2H, $2H_{2,6\text{-benzonitrile}}$), 7.51–7.55 (m, 3H, $H_{3\text{-chlorophenyl}}$, $2H_{3,5\text{-benzonitrile}}$), 7.62–7.68 (m, 3H, $3H_{3\text{-chlorophenyl}}$), 7.73 (s, 1H, $H_{2\text{-imidazole}}$), 7.75 (s, 1H, $H_{6\text{-quinoline}}$), 7.79 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinoline}}$), 8.11 (s, 1H, $H_{4\text{-quinoline}}$), 8.57 (d, $J = 8.5$ Hz, 1H, $H_{9\text{-quinoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 47.3$ (CH_2), 65.3 (CH), 109.5 (CN), 112.6 ($C_{4\text{-quinoline}}$), 116.6 ($C_{9\text{-quinoline}}$), 118.1 ($C_{1\text{-benzonitrile}}$), 122.4 ($C_{\text{quinoline}}$), 124.4 ($C_{6\text{-quinoline}}$), 127.1 (2C, $C_{3,5\text{-benzonitrile}}$), 128.1 ($C_{3\text{-chlorophenyl}}$), 128.2 ($C_{4\text{-imidazole}}$), 129.0–129.2 (3C, $C_{\text{quinoline}}$, $2C_{3\text{-chlorophenyl}}$), 130.2 ($C_{8\text{-quinoline}}$), 130.6 ($C_{3\text{-chlorophenyl}}$), 131.9 (2C, $C_{2,6\text{-benzonitrile}}$), 133.5 ($C_{5\text{-imidazole}}$), 133.6 ($C_{3\text{-(3-chlorophenyl)}}$), 138.4 ($C_{5\text{-quinoline}}$), 139.6 ($C_{2\text{-imidazole}}$), 142.5 ($C_{7\text{-quinoline}}$), 143.1 ($C_{4\text{-benzonitrile}}$), 143.8 ($C_{1\text{-(3-chlorophenyl)}}$), 146.5 ($C_{\text{quinoline}}$) ppm. HRMS (ESI), calcd. for $C_{27}H_{18}ClN_7O$ 492.1340, found 492.1335.

5.1.4. [3-(3-Chlorophenyl)-1,2-benzisoxazol-5-yl]-(5-chloro-2-thienyl)-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-carbaldehyde (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 ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (35–70 μm, eluent: CH_2Cl_2 100%). The pure fractions were collected and the solvent was evaporated to afford 68% of **11** (33 g). M.p. = 120 °C, ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): $\delta = 6.00$ (d, $J = 4.0$ Hz, 1H, C*H*–OH), 6.60 (d, $J = 4.0$ Hz, 1H, OH), 6.80 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 6.95 (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.44 (d, $J = 9.5$ Hz, 1H, $H_{6\text{-benzoisoxazole}}$), 7.72–7.66 (m, 3H, $H_{7\text{-benzoisoxazole, 5-chlorophenyl, 6 or 4-chlorophenyl}}$), 8.08 (d, $J = 7.0$ Hz, 1H, $H_{6\text{ or }4\text{-chlorophenyl}}$), 8.09 (s, 1H, $H_{2\text{-chlorophenyl}}$), 8.13 (s, 1H, $H_{4\text{-benzoisoxazole}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 70.6$ (*C*H–OH), 114.3 ($C_{\text{benzoisoxazole}}$), 115.8 ($C_{7\text{-benzoisoxazole}}$), 116.7 ($C_{4\text{-benzoisoxazole}}$), 124.2 ($C_{3\text{-thiophene}}$), 125.4 ($C_{4\text{ or }6\text{-chlorophenyl}}$), 126.0 ($C_{2\text{-chlorophenyl}}$), 126.8 ($C_{4\text{-thiophene}}$), 127.6 ($C_{5\text{-thiophene}}$), 129.5 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 130.8 ($C_{3\text{ or }4\text{ or }6\text{-chlorophenyl}}$), 131.3 ($C_{6\text{-benzoisoxazole}}$), 132.0 ($C_{3\text{ or }4\text{ or }6\text{-chlorophenyl}}$), 134.7 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 141.7 ($C_{5\text{-benzoisoxazole}}$), 148.8 ($C_{2\text{-thiophene}}$), 157.6 ($C_{\text{benzoisoxazole}}$), 162.5 ($C_{3\text{-benzoisoxazole}}$) ppm. HRMS (ESI), calcd. for $C_{18}H_{11}Cl_2NO_2S$ 375.9966, found 375.9969.

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

MnO_2 (33 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 Et_2O. The precipitate was filtered off and dried, yielding 21 g of **12** (64%). M.p. = 179 °C, ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C): $\delta = 7.37$ (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.70–7.65 (m, 2H, $H_{5,6\text{-chlorophenyl}}$), 7.74 (d, $J = 9.5$ Hz, 1H, $H_{6\text{-benzoisoxazole}}$), 7.85 (d, $J = 9.5$ Hz, 1H, $H_{7\text{-benzoisoxazole}}$), 7.87 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 8.14–8.17 (m, 1H, $H_{4\text{-chlorophenyl}}$), 8.19 (s, 1H, $H_{2\text{-chlorophenyl}}$), 8.59 (s, 1H, $H_{4\text{-benzoisoxazole}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 113.8$ ($C_{3\text{ or }5\text{-benzoisoxazole}}$), 116.1 ($C_{7\text{-benzoisoxazole}}$), 125.8 ($C_{4\text{-benzoisoxazole}}$), 126.3 ($C_{4\text{-chlorophenyl}}$), 126.9 ($C_{2\text{-chlorophenyl}}$), 128.9 ($C_{3\text{-chlorophenyl}}$), 129.6 ($C_{4\text{-thiophene}}$), 131.0 ($C_{6\text{-benzoisoxazole}}$), 131.6 ($C_{6\text{-chlorophenyl}}$), 132.0 ($C_{5\text{-chlorophenyl}}$), 133.6 ($C_{3\text{ or }5\text{-benzoisoxazole}}$), 134.7 ($C_{2\text{-chlorophenyl}}$), 136.7 ($C_{3\text{-thiophene}}$), 138.9 ($C_{2\text{-thiophene}}$), 142.1 ($C_{5\text{-thiophene}}$), 157.7 ($C_{\text{benzoisoxazole}}$), 166.2 ($C_{3\text{-benzoisoxazole}}$), 185.8 (C=O) ppm. LRMS (ESI), calcd. for $C_{18}H_9Cl_2NO_2S$ 374.2, found 374.0 $[MH]^+$.

5.1.6. [4-Amino-3-(3-chlorobenzoyl)phenyl]-(5-chloro-2-thienyl)-methanone **13**

$TiCl_3$ 15% in H_2O (130 ml) was added dropwise to a solution of **12** (0.0561 mol) in THF (200 ml). The mixture was stirred 3 h at room temperature. The mixture was poured into ice water, extracted with CH_2Cl_2, basified with K_2CO_3 10%, extracted and washed with H_2O. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 21.2 g of **13** (100%). M.p. = 132 °C, ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C): $\delta = 7.00$ (d, $J = 9.0$ Hz, 1H, $H_{5\text{-aminophenyl}}$), 7.25 (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.56 (t, $J = 7.5$ Hz, 1H, $H_{5\text{-chlorophenyl}}$), 7.60 (d, $J = 7.5$ Hz, 1H, $H_{6\text{-chlorophenyl}}$), 7.62 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 7.67 (d, $J = 7.5$ Hz, 1H, $H_{4\text{-chlorophenyl}}$), 7.74 (s, 1H, $H_{2\text{-chlorophenyl}}$), 7.84 (dd, $J = 9.0$, 2.0 Hz, 1H, $H_{6\text{-aminophenyl}}$), 7.88 (d, $J = 2.0$ Hz, 1H, $H_{2\text{-aminophenyl}}$), 8.00 (s, 2H, NH_2) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 114.9$ ($C_{3\text{-aminophenyl}}$), 117.7 ($C_{5\text{-aminophenyl}}$), 122.5 ($C_{1\text{-aminophenyl}}$), 127.6 ($C_{6\text{-chlorophenyl}}$), 128.6 (2C, $C_{2\text{-chlorophenyl}}$, $C_{4\text{-thiophene}}$), 130.7 ($C_{5\text{-chlorophenyl}}$), 131.4 ($C_{4\text{-chlorophenyl}}$), 133.5 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 133.9 ($C_{3\text{-thiophene}}$), 135.1 ($C_{6\text{-aminophenyl}}$), 136.8 ($C_{2\text{-thiophene}}$), 138.1 ($C_{2\text{-aminophenyl}}$), 141.5 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 142.4 ($C_{5\text{-thiophene}}$), 155.9 ($C_{4\text{-aminophenyl}}$), 183.6 ($C{=}O_{\text{thiophene}}$), 196.2 ($C{=}O_{\text{chlorophenyl}}$) ppm. HRMS (ESI), calcd. for $C_{18}H_{11}Cl_2NO_2S$ 373.9966, found 373.9963.

*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 Et_3N (0.0416 mol) were added dropwise at 5 °C to a solution of **13** (0.0347 mol) in CH_2Cl_2 (130 ml) under N_2 flow. The mixture was stirred at room temperature overnight, poured into ice water and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 18.1 g of **14** (100%). M.p. = 194 °C, ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C): $\delta = 7.43$ (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.64 (d, $J = 7.5$ Hz, 1H, $H_{5\text{-chlorophenyl}}$), 7.76 (m, 1H, $H_{3\text{ or }6\text{-chlorophenyl}}$), 7.76–7.79 (m, 2H, $H_{2,3\text{ or }6\text{-chlorophenyl}}$), 7.82 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 7.97 (d, $J = 8.5$ Hz, 1H, $H_{6\text{-2,2,2 trichlorophenylacetamide}}$), 8.02 (d, $J = 2.0$ Hz, 1H, $H_{3\text{-2,2,2 trichlorophenylacetamide}}$), 8.24 (dd, $J = 8.5$, 2.0 Hz, 1H, $H_{5\text{-2,2,2 trichlorophenylacetamide}}$), 11.6 (br s, 1H, NH) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 80$ (CCl_3), 125.28 ($C_{6\text{-2,2,2 trichlorophenylacetamide}}$), 128.7 ($C_{3\text{ or }6\text{-chlorophenyl}}$), 129.5 (2C, $C_{4\text{-thiophene}}$, $C_{2\text{-chlorophenyl}}$), 130.85 (2C, $C_{2,4\text{-2,2,2-trichlorophenylacetamide}}$), 132.0 ($C_{3\text{-2,2,2-trichlorophenylacetamide}}$), 133.2 ($C_{3\text{ or }6\text{-chlorophenyl}}$), 133.4 ($C_{5\text{-2,2,2-trichlorophenylacetamide}}$), 133.7 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 133.9 ($C_{4\text{-2,2,2-trichlorophenylacetamide}}$), 136.4 ($C_{3\text{-thiophene}}$), 138.9 (3C, $C_{1\text{ or }3\text{-chlorophenyl}}$, $C_{2\text{-thiophene}}$, $C_{1\text{-2,2,2-trichlorophenylacetamide}}$), 141.7 ($C_{5\text{-thiophene}}$), 160.0 ($C{=}O_{CCl_3}$), 185.06 ($C{=}O_{\text{thiophene}}$), 193.8 ($C{=}O_{3\text{-chlorophenyl}}$) ppm.

*5.1.8. 4-(3-Chlorophenyl)-6-[(5-chloro-2-thienyl)carbony]-2(1H)quinazolinone **15***

Ammonium acetate (0.0694 mol) was added to a solution of **14** (0.0347 mol) in DMSO (180 ml). The mixture was stirred at 60 °C for 4 h, then cooled and poured into ice water. The precipitate was filtered, taken up in CH_3CN (warm), filtered, washed with CH_3CN and diethyl ether and dried under vacuum, yielding 11.7 g of **15** (83%). M.p. > 260 °C, ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C): $\delta = 7.31$ (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.46 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazolinone}}$), 7.58–7.66 (m, 2H, $H_{4,5\text{-chlorophenyl}}$), 7.69 (dd, $J = 7.5$, 1.5 Hz, 1H, $H_{6\text{-chlorophenyl}}$), 7.71 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 7.84 (t, $J = 1.5$ Hz, 1H, $H_{2\text{-chlorophenyl}}$), 8.02 (d, $J = 1.5$ Hz, 1H, $H_{5\text{-quinazolinone}}$), 8.14 (dd, $J = 8.5$, 2.0 Hz, 1H, $H_{7\text{-quinazolinone}}$), 11.3 (br s, 1H, $NH_{\text{quinazolinone}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 113.7$ ($C_{\text{quinazolinone}}$), 116.78 ($C_{8\text{-quinazolinone}}$), 127.63 ($C_{6\text{-chlorophenyl}}$), 129.3 (2C, $C_{2\text{-chlorophenyl}}$, $C_{4\text{-thiophene}}$), 131.0 (3C, $C_{5\text{-quinazolinone}}$, $C_{5,4\text{-chlorophenyl}}$), 133.67 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 135.4 ($C_{7\text{-quinazolinone}}$), 135.7 ($C_{3\text{-thiophene}}$), 138.2 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 138.5 ($C_{2\text{-thiophene}}$), 141.84 ($C_{5\text{-thiophene}}$), 146.9 ($C_{\text{quinazolinone}}$), 155.2 ($C_{2\text{-quinazolinone}}$), 174.2 ($C_{4\text{-quinazolinone}}$), 184.7 (C=O) ppm. HRMS (ESI), calcd. for $C_{19}H_{10}Cl_2N_2O_2S$ 400.9918, found 400.9925.

*5.1.9. [2-Chloro-4-(3-chlorophenyl)-6-quinazolinyl](5-chloro-2-thienyl)-methanone **16***

Compound **15** (0.0279 mol) in $POCl_3$ (70 ml) was stirred at 100 °C for 2 h and cooled. $POCl_3$ was evaporated. The residue was taken up in CH_2Cl_2. The solvent was evaporated and the residue was taken up in CH_2Cl_2, poured into ice water, basified with K_2CO_3, extracted with CH_2Cl_2 and washed with H_2O. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was crystallized from CH_3CN. The precipitate was filtered off and dried, yielding: 10.35 g of **16** (88%). M.p. = 186 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 7.40$ (d, $J = 3.5$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.69 (t, $J = 7.5$ Hz, 1H, $H_{5\text{-chlorophenyl}}$), 7.76 (d, $J = 8.0$ Hz, 1H, $H_{4\text{ or }6\text{-chlorophenyl}}$), 7.82 (d, $J = 3.5$ Hz, 1H, $H_{3\text{-thiophene}}$), 7.88 (d, $J = 7.5$ Hz, 1H, $H_{4\text{ or }6\text{-chlorophenyl}}$), 8.02 (s, 1H, $H_{2\text{-chlorophenyl}}$), 8.22 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.43 (d, $J = 8.5$ Hz, 1H, $H_{7\text{-quinazoline}}$), 8.48 (s, 1H, $H_{5\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 121.0$ ($C_{\text{quinazoline}}$), 128.7 ($C_{8\text{-quinazoline}}$), 129.3 ($C_{6\text{-chlorophenyl}}$), 129.5 ($C_{4\text{-thiophene}}$), 130.0 ($C_{5\text{-quinazoline}}$), 130.2 ($C_{2\text{-chlorophenyl}}$), 130.8 ($C_{5\text{-chlorophenyl}}$), 131.3 ($C_{4\text{-chlorophenyl}}$), 133.9 ($C_{3\text{-chlorophenyl}}$), 134.9 ($C_{7\text{-quinazoline}}$), 136.2 ($C_{\text{quinazoline}}$), 136.7 ($C_{3\text{-thiophene}}$), 137.2 ($C_{1\text{-chlorophenyl}}$), 139.4 ($C_{2\text{-thiophene}}$), 141.6 ($C_{5\text{-thiophene}}$), 154.3 ($C_{\text{quinazoline}}$), 158.0 ($C_{2\text{-quinazoline}}$), 171.5 ($C_{4\text{-quinazoline}}$), 185.4 (C=O) ppm. HRMS (ESI), calcd. for $C_{19}H_9Cl_3N_2OS$ 418.9579, 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-1*H*-imidazole (0.0404 mol) in THF (40 ml) under N_2 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. *n*-BuLi 1.6 M in hexane (0.0356 mol) was added dropwise, the mixture was stirred 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 CH_2Cl_2 and washed with H_2O. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 96/4/0.2). The pure fractions were collected and the solvent was evaporated, yielding 2.75 g of **17** (24%). ^{1}H NMR (400 MHz, DMSO-d_6, 27 °C): $\delta = 3.38$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.35 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 6.60 (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 6.97 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 7.58 (s, 1H, H_{OH}), 7.61–7.78 (m, 5H, $4H_{3\text{-chlorophenyl}}$, $H_{2\text{-(1-methylimidazole)}}$), 7.97 (d, $J = 1.5$ Hz, 1H, $H_{5\text{-quinazoline}}$), 8.08 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.12 (dd, $J = 8.5$, 1.5 Hz, 1H, $H_{7\text{-quinazoline}}$) 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 NaN_3 (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 CH_2Cl_2. The organic layer was brought together with the precipitate dissolved in CH_2Cl_2 and dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was crystallized from CH_3CN/DIPE. The precipitate was filtered off and dried, yielding 1.33 g of **18** (66%). M.p. = 202 °C, ^{1}H NMR (300 MHz, DMSO-d_6, 27 °C): $\delta = 3.38$ (s, 3H, $NCH_{3\text{ imidazole}}$),

6.34 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 6.60 (d, $J = 4.0$ Hz, 1H, $H_{3\text{-thiophene}}$), 6.99 (d, $J = 4.0$ Hz, 1H, $H_{4\text{-thiophene}}$), 7.64–7.78 (m, 6H, OH, $H_{2\text{-(1-methylimidazole)}}$, $H_{2,4,5,6\text{-chlorophenyl}}$), 8.08 (d, $J = 1.5$ Hz, 1H, $H_{6\text{-quinoline}}$), 8.26 (dd, $J = 8.5$, 1.5 Hz, 1H, $H_{8\text{-quinazoline}}$), 8.74 (d, $J = 8.5$ Hz, 1H, $H_{9\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 116.7$ ($C_{9\text{-quinazoline}}$), 118.2 ($C_{\text{quinazoline}}$), 125.7 ($C_{3\text{-thiophene}}$), 126.6 ($C_{6\text{-quinazoline}}$), 127.0 ($C_{4\text{-thiophene}}$), 128.7 ($C_{5\text{-thiophene}}$), 128.8 ($C_{\text{chlorophenyl}}$), 129.8 ($C_{\text{chlorophenyl}}$), 130.4 ($C_{4\text{-(1-methylimidazole)}}$), 131.0 (2C, $C_{\text{chlorophenyl}}$), 133.8 ($C_{\text{quinazoline}}$), 133.9 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 134.3 ($C_{5\text{-(1-methylimidazole)}}$), 134.8 ($C_{8\text{-quinazoline}}$), 138.1 ($C_{1\text{ or }3\text{-chlorophenyl}}$), 141.6 ($C_{2\text{-(1-methylimidazole)}}$), 145.4 ($C_{7\text{-quinazoline}}$), 149.2 ($C_{2\text{-thiophene}}$), 152.8 ($C_{\text{quinazoline}}$), 167.9 ($C_{5\text{-quinazoline}}$) ppm. HRMS (ESI), calcd. for $C_{23}H_{15}Cl_2N_7OS$ 508.0514, found 508.0522.

5.1.12. 7-[Chloro-(5-chloro-2-thienyl)(1-methyl-1H-imidazol-5-yl]-5-(3-chlorophenyl)-tetrazolo-[1,5-a]quinazoline **19**

Compound **18** (0.0019 mol) in $SOCl_2$ (20 ml) was stirred at 60 °C for 3 h and 30 min, then cooled and the solvent was evaporated. The residue was taken up twice in CH_2Cl_2 and the solvent was evaporated, yielding **19**, which was used without further purification.

5.1.13. 5-(3-Chlorophenyl)-α-(5-chloro-2-thienyl)-α-(1-methyl-1H-imidazol-5-yl)-tetrazolo[1,5-a]quinazoline-7-methanamine **5a**

NH_3/*i*PrOH (20 ml) was added dropwise at 5 °C compound of **19** (0.0019 mol) in THF (20 ml) under N_2 flow. The mixture was stirred at 5 °C for 45 min, then at room temperature overnight, poured into ice water and extracted with CH_2Cl_2. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: CH_2Cl_2/CH_3OH/NH_4OH 97/3/0.1) and again over silica gel (10 μm, eluent: CH_3CN/H_2O 40/60). The pure fractions were collected and the solvent was evaporated. This fraction was crystallized from CH_3CN. The precipitate was filtered off and dried, yielding 0.018 g of **5a** (18%). M.p. = 220 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.42$ (s, 3H, $CH_{3\text{(1-methylimidazole)}}$), 3.55 (s, 2H, NH_2), 6.22 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 6.65 (d, $J = 3.5$ Hz, 1H, $H_{4\text{-thiophene}}$), 6.98 (d, $J = 3.5$ Hz, 1H, $H_{3\text{-thiophene}}$), 7.61–7.77 (m, 5H, $4H_{3\text{-chlorophenyl}}$, $H_{2\text{-(1-methylimidazole)}}$), 7.91 (d, $J = 2.0$ Hz, 1H, $H_{6\text{-quinazoline}}$), 8.39 (dd, $J = 8.5$, 2.0 Hz, 1H, $H_{8\text{-quinazoline}}$), 8.57 (d, $J = 8.5$ Hz, 1H, $H_{9\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 33.1$ ($CH_{3\text{(1-methylimidazole)}}$), 59.5 (CH), 116.2 ($C_{9\text{-quinazoline}}$), 117.6 ($C_{\text{quinazoline}}$), 125.6 ($C_{4\text{-thiophene}}$), 126.5 ($C_{3\text{-thiophene}}$), 126.7 ($C_{6\text{-quinazoline}}$), 127.9 ($C_{3\text{-chlorophenyl}}$), 128.4 ($C_{3\text{-chlorophenyl}}$), 129.4 ($C_{4\text{-(1-methylimidazole)}}$), 130.5 ($C_{3\text{-chlorophenyl}}$), 130.6 ($C_{3\text{-chlorophenyl}}$), 132.3 ($C_{\text{quinazoline}}$), 133.4 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 135.1 ($C_{8\text{-quinazoline}}$), 135.7 ($C_{5\text{-(1-methylimidazole)}}$), 137.7 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 140.8 ($C_{2\text{-(1-methylimidazole)}}$), 146.4 ($C_{7\text{-quinazoline}}$), 150.9 ($C_{2\text{-thiophene}}$, $C_{5\text{-thiophene}}$), 152.5 ($C_{\text{quinazoline}}$), 167.5 ($C_{\text{quinazoline}}$). HRMS (ESI), calcd. for $C_{23}H_{16}Cl_2N_8S$ 507.0674, found 507.0669.

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

A mixture of 6-bromo-2-methoxy-4-(3-chlorophenyl)-quinazoline **20** (0.03146 mol), $Pd(PPh_3)_4$ (0.003146 mol) and *N*,*O*-dimethylhydroxylamine hydrochloride (0.06923 mol) in Et_3N (22 ml) and dioxane (90 ml) was stirred at 100 °C for 18 h under a 5 bar pressure of CO, then cooled, poured into ice water, extracted with CH_2Cl_2 and filtered over Celite. The organic layer was separated, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–35 μm, eluent: CH_2Cl_2/EtOAc 85/15 then CH_2Cl_2/CH_3OH/NH_4OH 98/2/0.4). The pure fractions were collected and the solvent was evaporated, yielding 3 g of **21** (27%). M.p. = 118 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.33$ (s, 3H, NCH_3), 3.55 (s, 3H, $NOCH_3$), 4.10 (s, 3H, $OCH_{3\text{(2-quinazoline)}}$), 7.64–7.84 (m, 4H, $4H_{3\text{-chlorophenyl}}$), 7.93 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.16 (dd, $J = 8.5$, 1.5 Hz, 1H, $H_{7\text{-quinazoline}}$), 8.21 (d, $J = 1.5$ Hz, 1H, $H_{8\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 33.1$ (NCH_3), 54.9 ($NOCH_3$), 60.9 (OCH_3), 118.5 ($C_{\text{quinazoline}}$), 126.5 ($C_{9\text{-quinazoline}}$), 127.4 ($C_{6\text{-quinazoline}}$), 128.5 ($C_{3\text{-chlorophenyl}}$), 129.3 ($C_{2\text{-(3-chlorophenyl)}}$), 130.3 ($C_{7\text{-quinazoline}}$), 130.4 ($C_{3\text{-chlorophenyl}}$), 130.6 ($C_{3\text{-chlorophenyl}}$), 133.4 ($C_{3\text{-chlorophenyl}}$), 134.0 ($C_{8\text{-quinazoline}}$), 137.9 ($C_{3\text{-chlorophenyl}}$), 153.1 (C=O), 162.5 ($C_{3\text{-quinazoline}}$), 167.3 ($C_{\text{quinazoline}}$), 170.8 ($C_{5\text{-quinazoline}}$) ppm. Anal. ($C_{18}H_{16}ClN_3O_3$, 0.05 CH_2Cl_2) calcd. 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-quinazolinecarboxamide **22**

$POCl_3$ (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 K_2CO_3 solid. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was crystallized from DMF. The precipitate was filtered off and dried, yielding 9 g of **22** (59%). M.p. = 110 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.32$ (s, 3H, NCH_3), 3.55 (s, 3H, $NOCH_3$), 7.68–7.80 (m, 3H, $H_{4,5,6\text{-chlorophenyl}}$), 7.88 (s, 1H, $H_{2\text{-chlorophenyl}}$), 8.12 (d, $J = 9.0$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.28 (m, 2H, $H_{6,7\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 34.4$ (NCH_3), 62.5 ($NOCH_3$), 122.1 ($C_{\text{quinazoline}}$), 128.5 ($C_{5\text{ or }7\text{-quinazoline}}$), 128.8 ($C_{8\text{-quinazoline}}$), 130.2 ($C_{4\text{ or }6\text{-(3-chlorophenyl)}}$), 131.0 ($C_{2\text{-(3-chlorophenyl)}}$), 132.1 (2C, $C_{5,4\text{ or }6\text{-(3-chlorophenyl)}}$), 134.9 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 135.4 ($C_{\text{quinazoline}}$), 136.2 ($C_{5\text{ or }7\text{-quinazoline}}$), 138.4 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 154.4 ($C_{6\text{-quinazoline}}$), 158.3 ($C_{2\text{-quinazoline}}$), 168.2 (C=O), 172.1 ($C_{4\text{-quinazoline}}$) ppm. Anal. ($C_{17}H_{13}Cl_2N_3O_2$) calcd. 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-BuLi 1.6 M in hexane (0.042 mol, 26.2 ml) was added dropwise at −70 °C to a mixture of 1-methyl-1*H*-imidazole (0.042 mol) in THF (80 ml) under N_2 flow. The mixture was stirred for 15 min. Chlorotriethylsilane (0.043 mol) was added. The mixture was stirred for 15 min. *n*-BuLi 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 H_2O and extracted with EtOAc. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified twice by column chromatography over silica gel (15–35 μm, eluent: CH_2Cl_2/CH_3OH 97/3) followed by (15–40 μm, eluent: CH_2Cl_2/EtOAc 50/50 then CH_2Cl_2/CH_3OH 97/3). The pure fractions were collected and the solvent was evaporated, yielding 2.46 g of **23** (27%). M.p. = 190 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.92$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 7.60–7.75 (m, 2H, $2H_{3\text{-chlorophenyl}}$), 7.77 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 7.83–7.89 (m, 1H, $H_{3\text{-chlorophenyl}}$), 7.97–7.99 (m, 1H, $H_{3\text{-chlorophenyl}}$), 8.08 (s, 1H, $H_{2\text{-(1-methylimidazole)}}$), 8.21 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.41 (dd, $J = 8.5$, 2.0 Hz, 1H, $H_{7\text{-quinazoline}}$), 8.44 (d, $J = 2.0$ Hz, 1H, $H_{5\text{-quinazoline}}$) ppm.

*5.1.17. 2-Chloro-4-(3-chlorophenyl)-α-(1-methyl-1H-imidazol-5-yl)-6-quinazolinemethanol **24***

DIBAL-H in toluene (10 ml) was added dropwise at −70 °C to compound **23** (0.012 mol) in THF (150 ml) under N_2 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 CH_2Cl_2 and filtered over Celite. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated, yielding 4 g of **24** (86%). M.p. = 140 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.59$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.03 (d, $J = 5.5$ Hz, 1H, C*H*–OH), 6.24 (d, $J = 5.5$ Hz, 1H, OH), 6.38 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 7.55 (s, 1H, $H_{2\text{-(1-methylimidazole)}}$), 7.64–7.78 (m, 3H, $3H_{3\text{-chlorophenyl}}$), 7.84–7.86 (m, 1H, $H_{3\text{-chlorophenyl}}$), 8.01–8.08 (m, $J = 8.5$, 1.5 Hz, 2H, $H_{7,8\text{-quinazoline}}$), 8.15 (s, 1H, $H_{5\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 31.5$ (NCH_3), 65.1 (CH), 120.0 ($C_{\text{quinazoline}}$), 123.3 ($C_{5\text{-quinazoline}}$), 127.2 ($C_{7\text{-quinazoline}}$), 127.7 ($C_{4\text{-(1-methylimidazole)}}$), 128.7 ($C_{3\text{-chlorophenyl}}$), 129.5 ($C_{2\text{-(3-chlorophenyl)}}$), 130.5 ($C_{3\text{-chlorophenyl}}$), 130.6 ($C_{3\text{-chlorophenyl}}$), 133.4 ($C_{3\text{-chlorophenyl}}$), 134.8 ($C_{8\text{-quinazoline}}$), 137.5 ($C_{3\text{-chlorophenyl}}$), 139.2 ($C_{2\text{-(1-methylimidazole)}}$), 143.6 ($C_{6\text{-quinazoline}}$), 152.0 ($C_{\text{quinazoline}}$), 155.6 ($C_{2\text{-quinazoline}}$), 169.7 ($C_{4\text{-quinazoline}}$) ppm. HRMS (ESI), calcd. for $C_{19}H_{14}Cl_2N_4O$ 385.0623, found 385.0618.

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

NaN_3 (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, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.60$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.60 (d, $J = 5.5$ Hz, 1H, OH), 6.33 (d, $J = 5.5$ Hz, 1H, C*H*–OH), 6.36 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 7.58 (s, 1H, $H_{2\text{-(1-methylimidazole)}}$), 7.68–7.86 (m, 4H, $4H_{3\text{-chlorophenyl}}$), 8.21 (m, 2H, $H_{6,8\text{-quinazoline}}$), 8.71 (d, J = 7.5 Hz, 1H, $H_{9\text{-quinazoline}}$) 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 $SOCl_2$ (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 CH_2Cl_2. 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-1*H*-imidazole (0.0015 mol) in CH_3CN (5 ml) was stirred and refluxed for 2 h, cooled, poured into ice water, extracted with CH_2Cl_2 and washed with K_2CO_3 10%. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (10 μm, eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95/5/0.2). The pure fractions were collected and the solvent was evaporated, yielding 0.084 g of **5b** (17.5%). M.p. = 120 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 1.09$ (t, $J = 7.5$ Hz, 3H, $CH_{3(2\text{-ethylimidazole})}$), 2.45–2.53 (m, 1H, $CH_{2(2\text{-ethylimidazole})}$), 2.70–2.80 (m, 1H, $CH_{2(2\text{-ethylimidazole})}$), 3.39 (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.32 (s, 1H, $H_{4\text{-(1-methylimidazole)}}$), 6.65 (s, 1H, $H_{5\text{-(2-ethylimidazole)}}$), 6.85 (s, 1H, $H_{4\text{-(2-ethylimidazole)}}$), 7.24 (s, 1H, CH), 7.65–7.67 (m, 2H, $2H_{3\text{-chlorophenyl}}$), 7.74–7.76 (m, 3H, $2H_{3\text{-chlorophenyl}}$, $H_{5\text{-(1-methylimidazole)}}$), 7.81 (s, 1H, $H_{6\text{-quinazoline}}$), 8.11 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.79 (d, $J = 8.5$ Hz, 1H, $H_{9\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 12.8$ ($CH_{3(2\text{-ethylimidazole})}$), 20.4 ($CH_{2(2\text{-ethylimidazole})}$), 31.8 ($CH_{3(1\text{-methylimidazole})}$), 53.5 (CH), 117.8 ($C_{9\text{-quinazoline}}$), 117.2 ($C_{5\text{-(2-ethylimidazole)}}$), 119.3 ($C_{\text{quinazoline}}$), 128.1 ($C_{6\text{-quinazoline}}$), 128.3 ($C_{4\text{-(2-ethylimidazole)}}$), 129.3 ($C_{3\text{-chlorophenyl}}$), 130.3 ($C_{3\text{-chlorophenyl}}$), 130.5 ($C_{5\text{-(1-methylimidazole)}}$), 130.8 ($C_{4\text{-(1-methylimidazole)}}$), 131.5 ($C_{3\text{-chlorophenyl}}$), 131.6 ($C_{3\text{-chlorophenyl}}$), 133.7 ($C_{\text{quinazoline}}$), 134.3 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 135.6 ($C_{8\text{-quinazoline}}$), 138.6 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 139.8 ($C_{7\text{-quinazoline}}$), 141.1 ($C_{2\text{-(1-methylimidazole)}}$), 149.6 ($C_{2\text{-(2-ethylimidazole)}}$), 153.3 ($C_{\text{quinazoline}}$), 168.3 ($C_{5\text{-quinazoline}}$) ppm. HRMS (ESI), calcd. for $C_{24}H_{20}ClN_9$ 470.1608, found 470.1604.

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-1*H*-imidazole (0.0038 mol) was added at room temperature to compound **26** (0.0025 mol) in CH_3CN (10 ml). The mixture was stirred and refluxed for 2 h, poured into ice water and extracted with CH_2Cl_2/CH_3OH. The organic layer was washed with K_2CO_3, separated, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 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, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.31$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.30 (s, 1H, $H_{4\text{-}(1\text{-methylimidazole})}$), 6.93 (s, 1H, $H_{phenylimidazole}$), 7.10 (s, 1H, $H_{phenylimidazole}$), 7.16 (s, 1H, CH), 7.37–7.46 (m, 5H, H_{phenyl}), 7.61–7.68 (m, 2H, $H_{5,6\text{-chlorophenyl}}$), 7.71 (s, 1H, $H_{2\text{-}(1\text{-methylimidazole})}$), 7.76 (m, 2H, $H_{2,4\text{-chlorophenyl}}$), 7.83 (s, 1H, $H_{6\text{-quinazoline}}$), 8.14 (d, $J = 8.5$ Hz, 1H, $H_{8\text{-quinazoline}}$), 8.78 (d, $J = 8.5$ Hz, $H_{9\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 31.2$ ($CH_{3(1\text{-methylimidazole})}$), 54.5 (CH), 117.3 ($C_{9\text{-quinazoline}}$), 118.7 ($C_{quinazoline}$), 119.9 ($C_{3\text{ or }4\text{-(2-phenylimidazole)}}$), 127.7 ($C_{6\text{-quinazoline}}$), 128.8 ($C_{6\text{-(3-chlorophenyl)}}$), 128.9 (2C, C_{phenyl}), 129.0 (2C, C_{phenyl}), 129.3 (C_{phenyl}), 129.4 ($C_{3\text{ or }4\text{-(2-phenylimidazole)}}$), 129.7 ($C_{2\text{-(3-chlorophenyl)}}$), 130.3 ($C_{5\text{-(1-methylimidazole)}}$), 130.4 ($C_{1\text{-phenyl}}$), 130.6 ($C_{4\text{-(1-methylimidazole)}}$), 131.0 ($C_{5\text{-(3-chlorophenyl)}}$), 131.1 ($C_{4\text{-(3-chlorophenyl)}}$), 133.2 ($C_{quinazoline}$), 133.9 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 134.0 ($C_{8\text{-quinazoline}}$), 138.0 ($C_{1\text{ or }3\text{-(3-chlorophenyl)}}$), 139.4 ($C_{7\text{-quinazoline}}$), 140.6 ($C_{2\text{-(1-methylimidazole)}}$), 147.7 ($C_{2\text{-(2-phenylimidazole)}}$), 152.8 ($C_{quinazoline}$), 167.8 ($C_{5\text{-quinazoline}}$) ppm. HRMS (ESI), calcd. for $C_{28}H_{20}ClN_9$ 518.1608, found 518.1614.

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-(4-Fluorophenyl)-1*H*-imidazole (0.0015 mol) was added at room temperature to compound **26** (0.001 mol) in CH_3CN (5 ml). The mixture was stirred and refluxed for 2 h, then cooled, poured into ice water and extracted with CH_2Cl_2. The organic layer was washed with K_2CO_3 10%, separated, dried ($MgSO_4$), filtered and the solvent was evaporated. The residue was purified by column chromatography over silica gel (10 μm, eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95/5/0.2). The pure fractions were collected and the solvent was evaporated, yielding 0.11 g of **5d** (20%). M.p. = 154 °C, ^{1}H NMR (400 MHz, DMSO, 27 °C): $\delta = 3.32$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.29 (s, 1H, $H_{4\text{-}(1\text{-methylimidazole})}$), 6.93 (s, 1H, $H_{5\text{-}(4\text{-fluorophenylimidazole})}$), 7.10 (s, 1H, $H_{4\text{-}(4\text{-fluorophenylimidazole})}$),7.12 (s, 1H, CH), 7.25 (t, $J = 9.0$ Hz, 2H, $H_{3,5\text{-}(4\text{-fluorophenyl})}$), 7.38–7.42 (m, 2H, $H_{2,6\text{-}(4\text{-fluorophenyl})}$), 7.62–7.68 (m, 2H, $H_{3\text{-chlorophenyl}}$), 7.72 (s, 1H, $H_{2\text{-}(1\text{-methylimidazole})}$), 7.76–7.77 (m, 2H, $H_{3\text{-chlorophenyl}}$), 7.83 (d, $J = 2.0$ Hz, 1H, $H_{6\text{-quinazoline}}$), 8.13 (dd, $J = 8.5$, 2.0 Hz, 1H, $H_{8\text{-quinazoline}}$), 8.79 (d, $J = 8.5$ Hz, $H_{9\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 31.7$ ($CH_{3(1\text{-methylimidazole})}$), 55.0 (CH), 116.5 (2C, $J_{C-F} = 22$ Hz, $C_{3,5\text{-}(4\text{-fluoropheny})}$), 117.9 ($C_{9\text{-quinazoline}}$), 119.2 ($C_{quinazoline}$), 120.5 ($C_{5\text{-}(4\text{-fluorophenylimidazole})}$), 127.6 ($C_{1\text{-}(4\text{-fluorophenyl})}$), 128.3 ($C_{6\text{-quinazoline}}$), 129.3 ($C_{3\text{-chlorophenyl}}$), 129.8 ($C_{4\text{-}(4\text{-fluorophenylimidazole})}$), 130.2 ($C_{3\text{-chlorophenyl}}$), 130.7 ($C_{5\text{-}(1\text{-methylimidazole})}$), 131.0 ($C_{4\text{-}(1\text{-methylimidazole})}$), 131.5 ($C_{3\text{-chlorophenyl}}$), 131.6 ($C_{3\text{-chlorophenyl}}$), 131.8 (2C, $J_{C-F} = 9.2$ Hz, $C_{2,6\text{-}(4\text{-fluorophenyl})}$), 133.7 ($C_{quinazoline}$), 134.4 ($C_{3\text{-chlorophenyl}}$), 135.5 ($C_{8\text{-quinazoline}}$), 138.6 ($C_{3\text{-chlorophenyl}}$), 139.8 ($C_{7\text{-quinazoline}}$), 141.1 ($C_{2\text{-}(1\text{-methylimidazole})}$), 147.3 ($C_{2\text{-}(4\text{-fluorophenylimidazole})}$), 153.3 ($C_{quinazoline}$), 157.7 ($C_{4\text{-}(4\text{-fluorophenyl})}$), 168.3 ($C_{5\text{-quinazoline}}$) ppm. Anal. ($C_{28}H_{19}ClFN_9$, 0.35 H_2O, 0.25 CH_2Cl_2) calcd. C 60.21, H 3.61, N 22.37, found C 60.00, H 3.45, N 22.04.

5.1.23. 3-[1-[[5-(3-Chlorophenyl)tetrazolo[1,5-a]quinazolin-7-yl](1-methyl-1H-imidazol-5-yl)methyl]-1H-imidazol-2-yl]-benzonitrile ***5e***

A solution of 3-(1*H*-imidazol-2-yl)-benzonitrile (0.0026 mol) and K_2CO_3 (0.0053 mol) was added at room temperature to compound **26** (0.0017 mol) in CH_3CN (10 ml). The mixture was stirred and refluxed for 2 h, poured into ice water and extracted with CH_2Cl_2/CH_3OH. The organic layer was separated, dried ($MgSO_4$), filtered, and the solvent was evaporated. The residue was purified by column chromatography over silica gel (15–40 μm, eluent: $CH_2Cl_2/CH_3OH/NH_4OH$ 95/5/0.2) and then again over silica gel (10 μm eluent: CH_2Cl_2 100%). The pure fractions were collected and the solvent was evaporated, yielding: 0.048 g of **5e** (5%). ^{1}H NMR (300 MHz, DMSO, 27 °C): $\delta = 3.35$ (s, 3H, $CH_{3(1\text{-methylimidazole})}$), 6.27 (s, 1H, $H_{4\text{-}(1\text{-methylimidazole})}$), 6.98 (d, $J = 2.0$ Hz, 1H, $H_{5\text{-}(phenylimidazole)}$), 7.16 (d, $J = 2.0$ Hz, 1H, $H_{4\text{-phenylimidazole}}$), 7.29 (s, 1H, CH), 7.57–7.69 (m, 5H, $3H_{3\text{-chlorophenyl}}$, $2H_{4\text{ and }5\text{-benzonitrile}}$), 7.75–7.77 (m, 1H, $H_{3\text{-chlorophenyl}}$), 7.73 (s, 1H, $H_{2\text{-}(1\text{-methylimidazole})}$), 7.78 (d, $J = 2.0$ Hz, 1H, $H_{6\text{-quinazoline}}$), 7.85 (s, 1H, $H_{2\text{-benzonitrile}}$), 7.90 (d, 1H, $H_{6\text{-benzonitrile}}$), 8.08 (dd, $J = 8.5$, 2.0 Hz, 1H, $H_{8\text{-quinazoline}}$), 8.78 (d, $J = 8.5$ Hz, 1H, $H_{9\text{-quinazoline}}$) ppm. ^{13}C NMR (75 MHz, DMSO-d_6, 27 °C): $\delta = 31.2$ ($CH_{3(1\text{-methylimidazole})}$), 54.3 (CH), 112.1 ($C_{1\text{ or }3\text{-benzonitrile}}$), 117.3 ($C_{9\text{-quinazoline}}$), 118.5 (CN), 118.6 ($C_{quinazoline}$), 120.7 ($C_{5\text{-(phenylimidazole)}}$), 127.5 ($C_{6\text{-quinazoline}}$), 128.7 ($C_{phenyl}$), 129.6 ($C_{4\text{-(phenylimidazole)}}$), 129.8 ($C_{5\text{-}(1\text{-methylimidazole})}$), 130.2 ($C_{phenyl}$), 130.5 ($C_{4\text{-}(1\text{-methylimidazole})}$), 130.8 ($C_{phenyl}$), 130.9 (2C, C_{phenyl}), 131.5 (C_{phenyl}), 132.1 ($C_{2\text{-benzonitrile}}$), 132.9 ($C_{quinazoline}$), 133.1 ($C_{6\text{-benzonitrile}}$), 133.5 ($C_{phenyl}$), 133.8 ($C_{phenyl}$), 134.8 ($C_{8\text{-quinazoline}}$), 137.8 ($C_{phenyl}$), 138.8 ($C_{7\text{-quinazoline}}$), 140.6 ($C_{2\text{-}(1\text{-methylimidazole})}$), 145.5 ($C_{2\text{-(phenylimidazole)}}$), 152.7 ($C_{quinazoline}$), 167.3 ($C_{5\text{-quinazoline}}$) ppm. HRMS (ESI), calcd. for $C_{29}H_{19}ClN_{10}$ 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

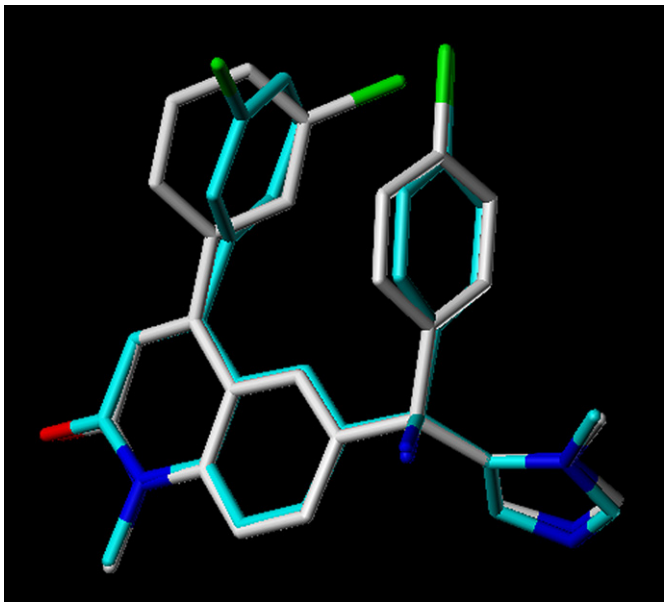


Fig. 6. Least square alignment between the lowest energy conformer (colored by atom type) and the X-ray structure (cyan) of isolated compound **1**. Only the heavy atoms have been considered for the alignment. The RMS fit of corresponding atom pairs is 1.11 but drops to 0.23 when the 3-chlorophenyl ring is oriented in the crystallographic conformation. (For interpretation of the references to colors in the figure legends, the reader is referred to the web version of this article.)

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-hydroxyfarnesyl-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).

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