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4-Methyl-1,2,4-triazol-3-yl Heterocycle as an Alternative to the 1-Methylimidazol-5-yl Moiety in the Farnesyltransferase Inhibitor ZARNESTRA™

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Abstract—Replacement of the 1-methylimidazol-5-yl moiety in the farnesyltransferase inhibitor ZARNESTRA™ series by a 4-methyl-1,2,4-triazol-3-yl group gave us compounds with similar structure–activity relationship profiles showing that this triazole is potentially a good surrogate to imidazole for farnesyltransferase inhibition.

The past decade has seen a growing interest in Farnesyl Protein Transferase (FPT), an enzyme which catalyses a key step in the anchoring of Ras protein to the cell membrane. Once attached to the cell membrane Ras is acting as a signal transducer from external growth factors to the cell nucleus. Mutated forms of Ras are found in approximately 30% of human cancers[1,2] providing a continuous signal for the transformation and uncontrolled growth of malignant tumor cells.[3–11] Therefore it was hypothesized that inhibitors of farnesyl protein transferase (FTI's) would be important and novel cancer therapeutic drugs. Several FTI's are currently undergoing clinical trials. Data from these trials as well as from recent biological research has shown that the antitumor activity of the class is not only related to Ras functioning. Rather it also involves other farnesylated proteins such as RhoB, and centromere associated proteins or modulation of transcription events.[11–21]

One of the first FTI's to enter clinical trials, R115777[22,23] **1** (ZARNESTRA™) is a 4-phenylquinolinone bearing an asymmetric carbon atom substituted by the 1-methylimidazol-5-yl moiety (Fig. 1). Earlier studies have shown that this imidazole is uniquely active conferring FPT inhibitory activity to analogues of R115777.[24–26] Furthermore, the substitutions introduced on the imidazole heterocycle either in position-2 or -4 led to a dramatic decrease in potency.[25,26]

Herein, we describe the synthesis and inhibitory profile of a series of 6-[amino(4-chlorophenyl)(heterocyclyl)methyl]-4-(3-chlorophenyl)-1-methyl-2(1*H*)-quinolinones, evaluating the replacement of either the C-2 or/and the C-4 imidazole carbon atoms by nitrogen in order to identify imidazole surrogates.[27]

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Taking the hydroxy analogue **2** of R115777 as a reference compound[24] (Fig. 1) we first replaced the C-4 by a nitrogen (Scheme 1).

Nitrile **4** was obtained by reacting the ketone **3**[24] with tosylmethylisocyanide and was then hydrolyzed into the acid **5**. Coupling **5** with 4-methylthiosemicarbazide gave **6** which was cyclized under basic conditions into **7**. Subsequent alkylation of the sulfur gave **8**. Finally, reductive removal of the methylsulfide provided us with our first analogue **9**. Deprotonation and oxidation of **9** enabled us to introduce the hydroxy moiety which was further transformed into the amino **12** after chlorination followed by substitution of the chlorine by ammonia.

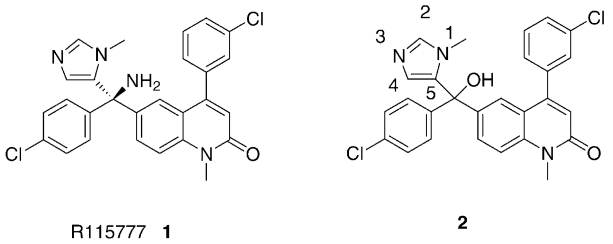
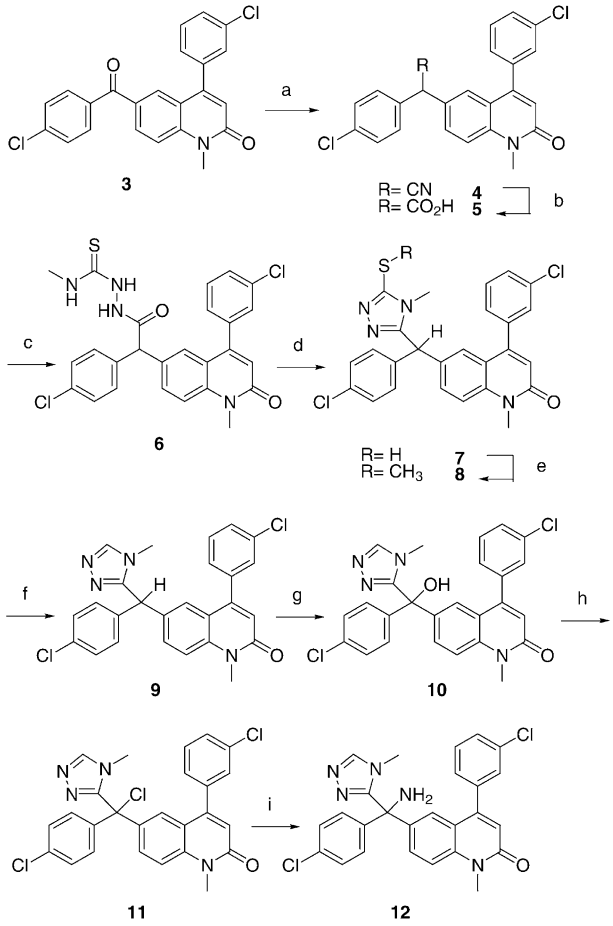


Figure 1. Structure of R115777 **1** and its hydroxy analogue **2** (racemic form).



Scheme 1. Reagents and conditions: (a) $TsCH_2NC$, *t*-BuOK, DME, DMF, *t*-BuOH, rt 3 h, 74%; (b) AcOH, H_2SO_4, 110 °C, 3 h, 92%; (c) $MeNHC(S)NHNH_2$, DIEA, HOBT, THF, rt, 18 h, 50%; (d) MeONa, MeOH, 60 °C, 3 h; then (e) CH_3I, rt, 0.5 h, 84%; (f) Ni–Raney, EtOH, 80 °C, 18 h, 66%; (g) *t*-BuOK, DME, air bubbling, rt, 1 h, 77%; (h) $SOCl_2$, rt, 4 h; (i) NH_3/*i*-PrOH, THF, 5 °C–rt, 1 h, 57%.

Further introduction of a nitrogen on this five membered ring led us to prepare 1-methyltetrazol-5-yl derivatives such as **15** (Scheme 2). Conversion of the nitrile **4** to the tetrazolyl derivative **13** was achieved by reacting **4** with sodium azide. Alkylation of **13** by methyliodide gave **14** and the hydroxy substituent was introduced by oxidation to provide **15**.

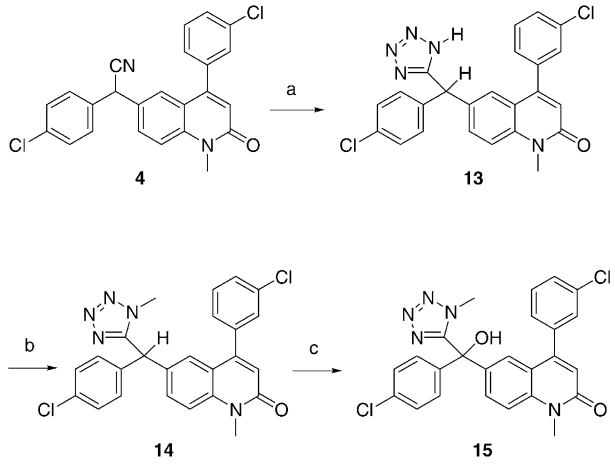
These compounds were evaluated for inhibition of FPT in vitro and compared to **1** and **2** (Table 1).

Triazoles **10** and **12** showed the same potency range for enzymatic inhibition as the imidazole compounds **1** and **2** whereas the tetrazole **15** proved to be inactive at a 0.1 μM concentration. For the latter, modification of the heterocycle basicity probably explains the drastic loss in potency concomitant to a loss of zinc binding properties. Therefore the tetrazoles were not further studied.

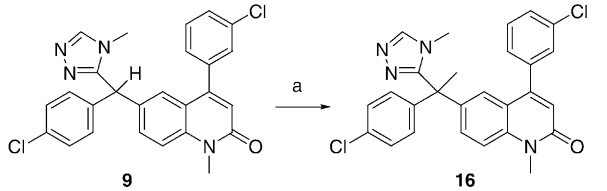
Two other triazole examples **16** and **21** were prepared and compared to the equivalent imidazoles **23** and **24**[24] (Table 1).

Deprotonation of **9** (Scheme 3) and subsequent alkylation of the generated anion gave **16** with a moderate yield (Scheme 3).

The *N*-demethylated quinolinone analogue of **10** was obtained as described in Scheme 4 starting from the 2-chloroquinoline **17**.[29] The chlorine moiety was sub-

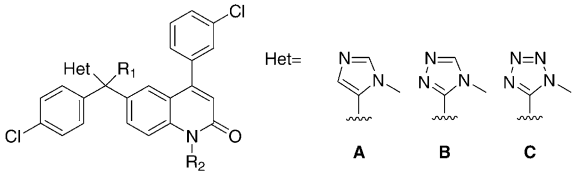


Scheme 2. Reagents and conditions: (a) NaN_3, NH_4Cl, DMF, 120 °C, 24 h, 88%; (b) CH_3I, K_2CO_3, CH_3CN, 80 °C, 2 h, 25%; (c) *t*-BuOK, DME, air bubbling, 5 °C–rt, 6 h, 24%.



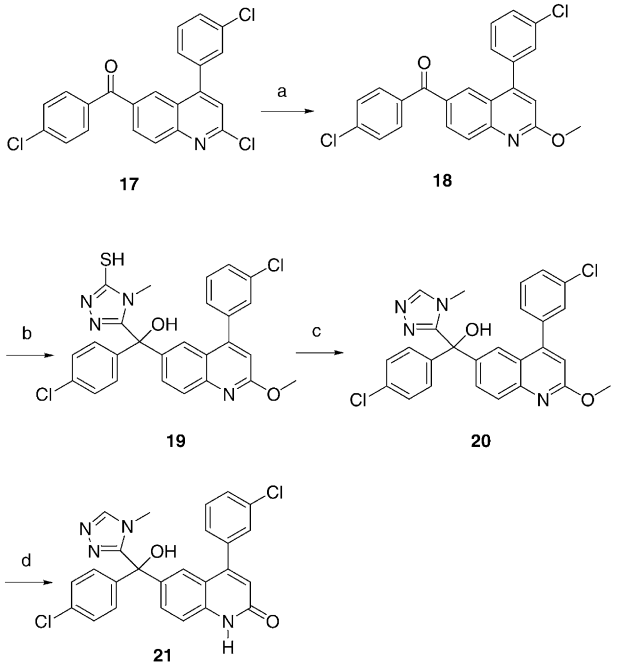
Scheme 3. Reagents and conditions: (a) CH_3I, *t*-BuOK, DME, rt, 0.5 h, 54%.

Table 1. FPT inhibition comparison between the tetrazole, triazole and the imidazole series



Compd	Heterocycle	R_1	R_2	FPT (enz) IC_{50}, nM[a]	Cell proliferation IC_{50}, nM[b]
2	A	OH	CH_3	1.7	12
1	A	NH_2	CH_3	0.9	1.7
10	B	OH	CH_3	6	151
12	B	NH_2	CH_3	1.3	24
15	C	OH	CH_3	0% inh. @ 0.1μM	nt
22[c]	A	H	CH_3	3	174
9	B	H	CH_3	1	80
23[c]	A	CH_3	CH_3	4	58
16	B	CH_3	CH_3	5	nt
24[c]	A	OH	H	4	400
21	B	OH	H	26	nt

nt, not tested.
[a]The concentration required for a 50% reduction of the FPT-catalyzed incorporation of [3*H*]-farnesylpyrophosphate into a biotinylated lamin B peptide.
[b]See ref 28.
[c]Synthesis described in ref 24.



Scheme 4. Reagents and conditions: (a) MeONa, MeOH, 80 °C, 16 h, 62% two steps; (b) 4-methyl-1,2,4-triazole-3-thione, *n*-BuLi, THF, −70 to 0 °C, 2.5 h, 35%; (c) $NaNO_2$, HNO_3, THF, H_2O, rt, 0.25 h, 61%; (d) HCl 6 N, 100 °C, 48 h, 73%.

stituted by a methoxy group easier to remove afterwards. The triazole heterocycle was then introduced directly by addition of the 5-lithio-4-methyl-1,2,4-triazole-3-thione onto ketone **18** followed by removal of the thiol (Scheme 4). This procedure is more straightforward than the triazole ring construction depicted in Scheme 1. Then the 2-methoxy group was cleaved in acidic media to provide **21**.

Table 2. Comparison of in vitro and in vivo results for compounds R115777 and **9**

Compd	Metabolization[a] (%)	In vivo[b] % inhib
R115777	66	37
9	77	45

[a]Percentage of parent drug remaining after 120 min incubation with human liver microsomes.[28]
[b]In vivo screening at 25 mg/kg in mice inoculated with T24H-ras NIH 3T3 cells, percentage of tumor weight inhibition.[28] Compounds were administered orally once daily starting 2 days after tumor inoculation and tumor growth inhibition was measured 17 days after administration.

With the exception of the last entry (compound **21**) where a slight decrease of activity was observed the triazolyl quinolinones showed the same enzymatic activity as the corresponding imidazoles, but changing imidazole to triazole induced a decrease in antiproliferative activity in cell based assays (**10**, **12** vs **2**, **1**, respectively). Compound **9**, being one of the most chemically accessible member of this series, it was chosen as a first example to draw a preliminary pharmacological profile of triazole series (Table 2).

Although both changes (imidazole to triazole and NH_2 into H) induced a decreased activity in cell based assays, and being a racemic mixture, triazole **9** proved to have similar in vivo potency as R115777 in this preliminary experiment.

In vitro results have shown that 4-methyl-1,2,4-triazol-3-yl moiety can potentially replace 1-methylimidazol-5-yl heterocycle while designing FTI's. Preliminary in vivo results have encouraged us to further compare solubility, metabolism, pharmacokinetics and in vivo profiles of such triazolyl inhibitors with ZARNESTRA™.

Acknowledgements

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