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Novel triazole based inhibitors of Ras farnesyl transferase

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Abstract—A novel series of potent inhibitors of Ras farnesyl transferase possessing a 1,2,4-triazole pharmacophore is described. These inhibitors were discovered from a parallel synthesis effort and were subsequently optimized to in vitro IC_{50} value of less than 1 nM.

The zinc-metalloenzyme farnesyl transferase (FTase) catalyzes the transfer of a farnesyl group to a cysteine thiol group contained in the C-terminal tetra peptide signal sequence of Ras, frequently referred to as a CAAX motif.[1] Farnesylation causes membrane localization of Ras which, in turn, determines the switch from an inactive to an active Ras-GTP-bound form.[2] Among the Ras isoforms H-*ras*, N-*ras*, and K-*ras*, mutations in the K-*ras* isoform are most relevant to human cancers in particular pancreatic, colon, and lung cancers, which exhibit approximately 90, 40, and 25% incidence of K-*ras* mutations, respectively. Inhibitors of FTase prevent membrane localization of the Ras oncogene and have the ability to revert the transformed phenotype, providing the rationale for the development of farnesyl transferase inhibitors (FTIs) as anticancer drugs.[3] While the exact mechanism of action of FTIs remains unclear, they appear to block the farnesylation of several additional proteins, such as RhoB, other Ras GTPases such as Rheb, and centromere-associated proteins CENP-E and CENP-F.[4] FTIs do appear to have clinical activity in leukemias and in some solid tumors, and at least two FTIs viz. Tipifarnib (Johnson & Johnson) and Sarasar (Schering-Plough) remain in advanced stages of clinical development despite early setbacks.[5]

One of these advanced inhibitors R115777 (Tipifarnib) inhibits farnesyl transferase with an IC_{50} of 0.9 nM. R115777 (Fig. 1) also proved efficacious preclinically in in vivo tumor studies and was the first FTI to enter clinical trials.[6] A key pharmacophore in R115777 is the 4-substituted imidazole moiety. The imidazole functionality has also been reported to be important in a number of other potent FTIs such as L-778123[7] (Merck) and BMS-214662[8] (Bristol Myers Squibb). In fact, imidazole is a key feature for such FTIs since it chelates with the active site Zn atom in the FT protein much in the same manner as the cysteine thiol group in the substrate CAAX-peptides.[9] During a lead exploration program in another therapeutic area, we had synthesized libraries of 4-substituted imidazole derivatives **1**–**3** (Fig. 2).[10] Screening of this collection of imidazoles, which lacked the 4-cyanobenzyl group found in the Merck inhibitors related to L-778123, did not display significant inhibition of the FTase enzyme. In yet another unrelated drug discovery program, we prepared 3-substituted 1,2,4-triazole libraries **4**. With the FTase program in mind, we considered whether preparing a small series of 1-(4-cyanobenzyl)-2,4-substituted triazoles might lead to potentially favorable FTIs. Such a triazole series (**5**), if found active, would offer novelty over the rapidly developing and increasingly competitive landscape of FTI research that still very much focused on the imidazole pharmacophore.[11] We also believed that a successful strategy would require an efficient and versatile synthetic

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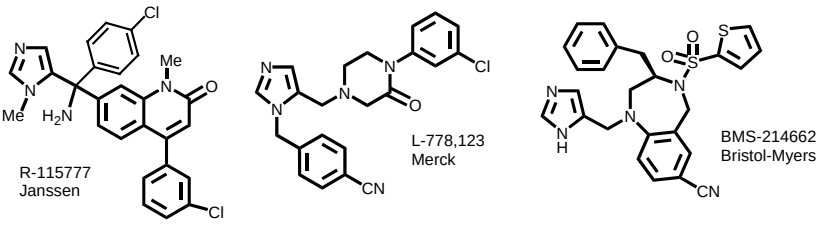


Figure 1. Imidazole based inhibitors of farnesyl transferase.

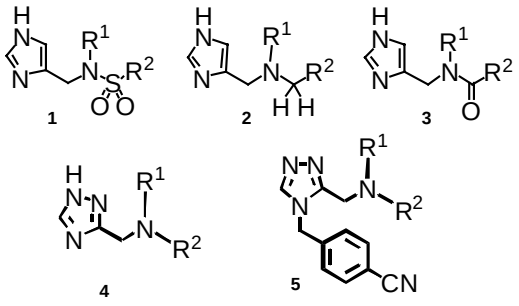
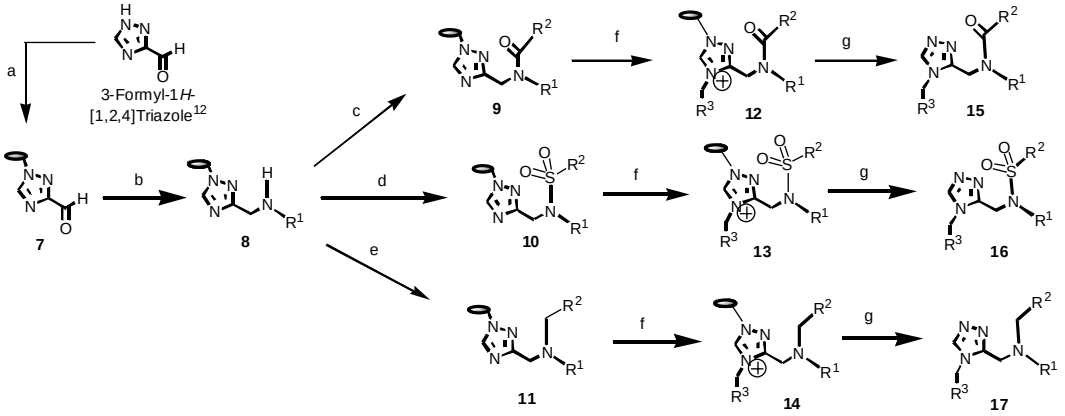


Figure 2. JRF libraries of imidazole and triazoles.

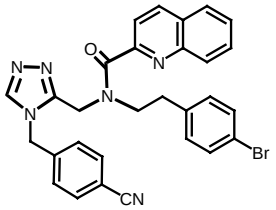
strategy that would allow preparation and testing of a broad range of structures.

Our earlier published synthesis of the imidazole derivatives on solid support involved immobilization of 4-formyl imidazole on polystyrene resin followed by functionalization of the formyl group and subsequent derivatization by parallel synthesis. A similar strategy for triazoles required 3-formyl-1,2,4-triazole **6** as a key building block, which unlike the imidazole was not commercially available. We were able to synthesize this aldehyde in multi-gram amounts according to a published synthetic procedure.[12] With this key building block in hand, we next investigated its stability under the conditions of resin loading on 2-chlorotrityl resin (Nova Biochem). We were delighted to find that this reaction (Scheme 1) went extremely well and multi-gram quantities of the resin-bound triazole **7** could be easily prepared and safely stored. Reductive amination of the resin-bound formyl group with a variety of amines by sodium triacetoxy borohydride in 1,2-dichloroethane was investigated next. The triazole formyl group reacted with ease with a broad variety of amines as evidenced by LC–MS and NMR spectra of resin products cleaved in small amounts by 5% TFA in CH_2Cl_2. Derivatization of the resulting secondary amines **8** with a variety of carboxylic acids in the presence of coupling agents such as HATU or PyBOP gave resin-bound amides **9**. Sulfonylation with aryl sulfonyl chlorides also went well producing sulfonamides **10**. Reductive alkylation of the secondary amines **8** with a variety of aromatic aldehydes similarly went without any difficulty producing tertiary amine products **11**. Having optimized the conditions for library generation, we turned our attention to the development of a synthetic methodology for the generation of *N*-(4-cyanobenzyl) derivatives. We hypothesized that resin-bound triazole derivatives **9**, **10**, and **11** may react with benzyl triflates generated in situ to produce quaternary triazole salts **12**, **13**, and **14**. Representative resin-bound library members were treated with freshly generated 4-cyanobenzyl triflate at −78 °C under argon followed by warming to room temperature and washes with methylene chloride. The resultant resin samples were then subjected to cleavage with weak TFA mixtures producing desired triazole derivatives **15**, **16**, and **17** in excellent overall yields after RP-HPLC purification.

The above-described findings allowed for the generation of libraries of 3,4-disubstituted triazole derivatives with at least three diversity generating elements (amines, carboxylic acids, sulfonyl chlorides or aldehydes and benzyl alcohols). Utilizing parallel synthesis equipment and techniques described in our earlier publication,[13] we generat-



Scheme 1. Synthesis of 3,4-disubstituted triazole library molecules. Reagents and conditions: (a) 2-Cl Trityl resin, DMF/DCE/Et_3N; rt, 48 h; (b) R_1NH_2, $NaBH(OAc)_3$, 1% HOAc-DCE, overnight; (c) R_2CO_2H, PyBoP or HATU, DIEA, DMF, 3–6 h; (d) $ArSO_2Cl$, DIEA, CH_2Cl_2, overnight; (e) R_2CHO, 1% HOAc-DCE, $Na(OAc)_3BH$, overnight; (f) R_3CH_2OH, Tf_2O, DIEA, −78 °C to rt, 30 min; (g) 10% TFA-CH_2Cl_2, 1–2 h.



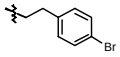
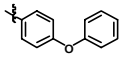
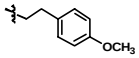
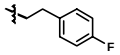
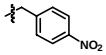
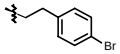
15a: **FTase** IC50 = 26 nM

Figure 3. Structure of first hit molecule from triazole library.

ed focused libraries of disubstituted triazoles beginning with 0.1 mmol of starting 2-Cl-trityl polystyrene resin for each discreet molecule. The entire sequence of synthetic steps, followed by preparative HPLC purification, yielded multi-milligram amounts of each product for screening against FTase. Overall isolated yields ranged from 10 to 60%.

Initially, screening was conducted at a single concentration of 100 nM of test compound in a high-throughput screen using a K-Ras peptide substrate (biotin-KKKKKKSKCVIM). A full dose response curve was obtained for compounds meeting the initial potency criteria in the single point screen (% inh. >75% at 100 nM) to obtain IC_{50} values. Triazoles without the 4-cyanobenzyl group had no notable activity. Unlike many of the Merck type analogs which contained a tertiary amine, none of our *tert*-amines **17** had any significant inhibitory activity. To our delight however, we observed potent inhibition of K-Ras farnesylation by several members of our carboxamide **15** and sulfonamide **16** derivatives. In general, carboxamides were more potent and we focused mainly on these analogs. Structure of the first carboxamide hit molecule is shown in Figure 3. This compound with a quinoline 2-carboxamide and 4-bromo phenethyl side chains **15a** had an IC_{50} of 26 nM. Several members of our initial library had activity and a sense of SAR was immediately apparent as shown in Table 1. For example, the analog with 4-methoxyphenethyl chain (**15c**) showed weaker inhibition. On the other hand, 4-fluorophenethyl analog (**15d**) had comparable potency. When we prepared an analog with the 4-phenoxyphenyl, a group commonly seen in some literature FTIs, it did not show improved potency. The benzyl groups with electron-withdrawing substituents (**15e**, **15f**, and **15g**) showed improvement with the nitrobenzyl analog **15g** demonstrating the best IC_{50} value of 1.6 nM. Several of our initial library members with the 4-bromophenethyl chain had groups other than the quinoline carboxamide seen in hit molecule **15a**. Presence of a methoxy group on the quinoline ring (**15i**) as well as the benzothiophene analog (**15j**) was threefold less active. The 3,4-methylenedioxyphenyl analog (**15k**) as well as the benzofuran (**15l**) were weaker but closer to the hit molecule in potency, while the naphthalene analog (**15m**) was equipotent, indicating that the quinoline N-atom played no particular role in potency. A brief investigation for replacement of the 4-cyanobenzyl group was then conducted. The data revealed that 4-cyano group is indeed a must. Only other benzyl substituent on the triazole N-atom with any notable in vitro activity was the 4-carboxymethyl group, while fluoro, chloro, and alkyl substituents were poorly active (data not shown). Analog **15f** (IC_{50} = 3.5 nM; only fourfold weaker than R115777) emerged as the molecule of choice from this

Table 1. Preliminary SAR data from triazoles of general structure **15**

Compound	R^1	R^2	K-Ras FTase IC50 (nM)
15a			26
15b		Same as above	44
15c		Same as above	75
15d		Same as above	19
15e		Same as above	5
15f		Same as above	3.5
15g		Same as above	1.6
15h			99.3
15i	Same as above		96.5
15j	Same as above		89.3
15k	Same as above		61
15l	Same as above		53.2
15m	Same as above		28.3

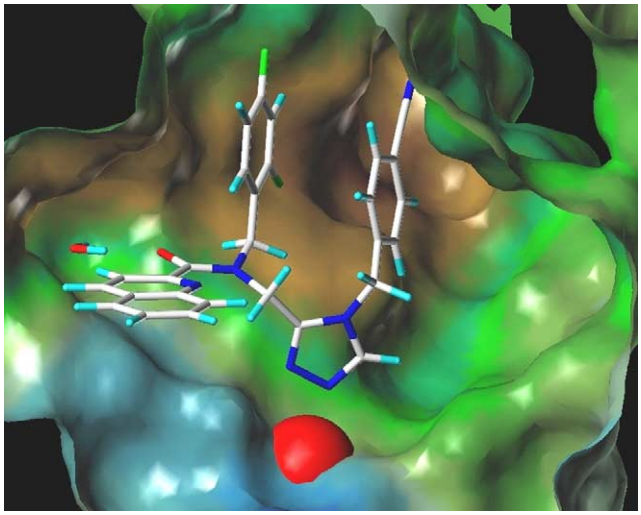


Figure 4. Docking model of compound **15f** within the FTase catalytic site. The inhibitor and the structured water molecule are represented in capped sticks, the HFP ligand in wire, and the zinc atom by a red sphere. The molecular lipophilic potential has been mapped onto the Connolly surface of the cavity (blue coloring represents polar regions and brown represents hydrophobic regions). The two benzyl substituents point backward; the quinoline moiety is oriented toward the reader.

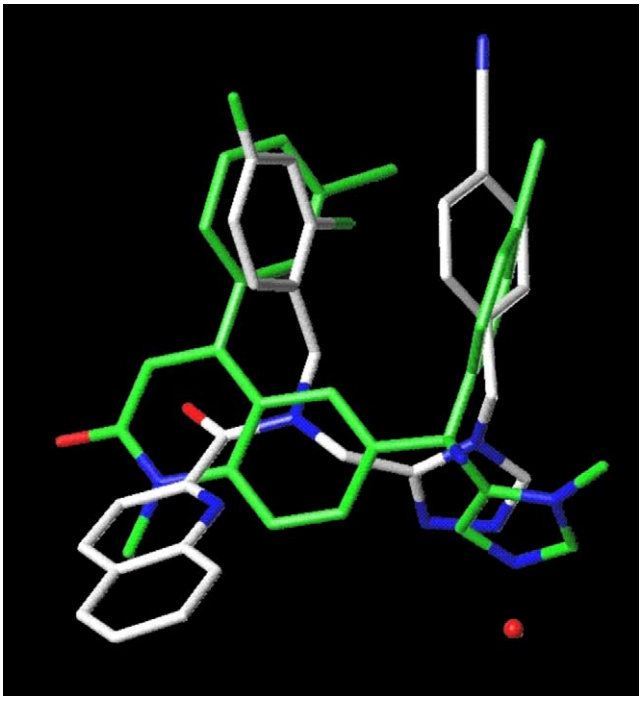


Figure 5. Comparison of the binding modes of compound **15f** (white, docking model) and Tipifarnib (green, X-ray structure). Only the enzyme backbone atoms of both complexes have been used to superimpose the structures (not shown). The zinc atom is represented by a red sphere.

first phase of lead exploration for additional optimization studies.

To aid optimization, we initiated a molecular modeling study whereby **15f** was docked in the FTase active site (PDB code: 1QBQ)[14,15]. Compound **15f** sits across the entry of the catalytic site, precluding any endogenous ligands to bind (Fig. 4). Hydrophobic benzyl substituents are buried within the cavity, while the quinoline moiety is oriented toward the solvent in the exit groove. Key pharmacophoric requirements for strong binding include—(a) coordination of triazole-N1-atom with the zinc atom, (b) interaction of carboxamide with a structured water molecule which forms a H-bond with Phe360, (c) hydrophobic interaction of 4-cyanobenzyl ring with the tail region of farnesyl pyrophosphate with the cyano group itself occupying a positively charged region lined by Arg202, and (d) a double edge-to-face pi stacking of the difluorophenyl ring residues Trp106 and Trp102.

This docking model was validated indirectly by comparison with the recent X-ray structure of the ternary complex FTase/Tipifarnib/FPP (PDB code: 1SA4).[9] Although shorter than Tipifarnib, the scaffold of **15f** manages to simultaneously anchor the zinc atom and the structured water molecule (Fig. 5). The two benzyl substituents of compound **15f** match closely the corresponding chlorophenyl rings in Tipifarnib, while the 4-cyano group inserts more deeply into the binding cavity. Finally, the quinoline ring in compound **15f** exploits the exit groove, while Tipifarnib does not.

To further define this in vitro SAR in light of the docking hypothesis, we undertook an optimization effort involving small changes to the structure of **15f** via either solid-phase or solution-phase chemistries. The corresponding naphthalene **15n** was prepared and was found to have similar potency as was seen with our earlier hit SAR (Fig. 6). With a goal of potentially improving physical characteristics such as solubility, the 5-cyano-2-pyridyl derivative **18** was synthesized. This molecule, although significantly more polar than **15f** (c log *P* 1.66 vs. 3.87), turned out to have potency similar to that of the lead triazole **15f**.

We were interested in going back and synthesize the exact imidazole analog of **15f**. This compound **20** was synthesized utilizing chemistry identical to that for **15f**, except starting with 4-formyl imidazole. It turned out to be

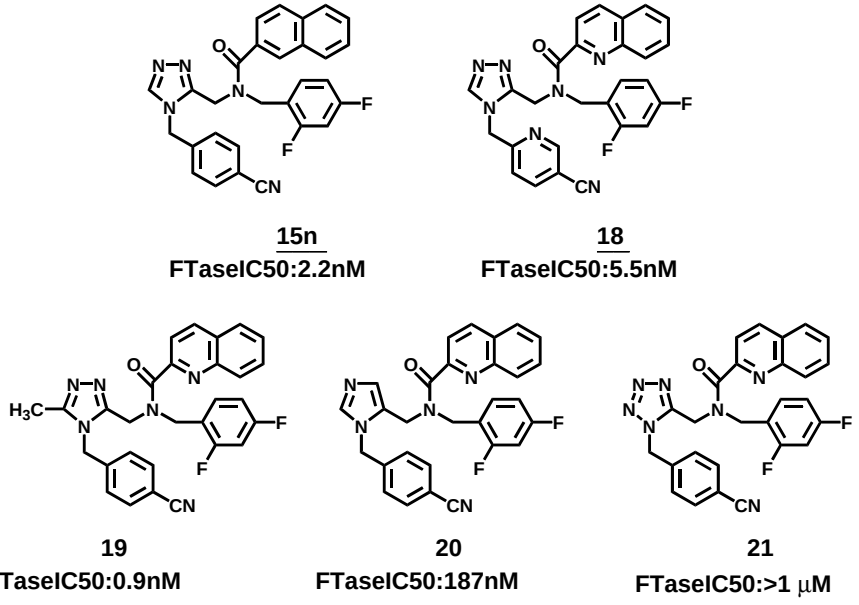


Figure 6. Advancement of SAR from lead structure **15f**.

nearly 50-fold less active with an IC_{50} of 187 nM (Fig. 6). We then synthesized the corresponding tetrazole derivative **21**, utilizing available solution-phase chemistries,[18] which was found to be completely inactive. The 5-methyl triazole **19** was synthesized beginning with a 3-formyl-5-methyltriazole building block (synthesis not shown) to fully assess the SAR around the triazole moiety. This analog demonstrated improvement in FTI potency identical to that of R115777 of 0.9 nM. The stronger potency of this 5-methyl triazole is in keeping with our docking model (Fig. 5), whereby the triazole-5-carbon atom is seen pointing in the same direction as that of the imidazole ring methyl in Tipifarnib.

In summary, we have synthesized a series of novel 3,4-substituted triazoles utilizing a rapid and highly efficient solid phase and parallel synthesis protocol. Although we undertook library generation in a target focused manner, this novel synthetic scheme possesses the potential for generation of thousands of interesting small molecules for lead generation purposes. Screening of our triazole library has led to identification of potent inhibitors of Ras FTase with IC_{50} value identical to clinical candidate R115777 as well as other published inhibitors. There were no reports of triazoles as FTIs in the literature at the time of our investigations. In fact, we explored the replacement of the imidazole with the triazole in the 2-quinolone series from which R115777 is derived with very promising results.[19] Further investigations on the potential of the non-quinolone series disclosed here as a potential drug candidate will be reported shortly.

Acknowledgments

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15. The conformer of compound **15f** with the lowest energy was docked manually within the FTase catalytic site. The CVIM peptide in this complex was removed whereas the alpha-hydroxyfarnesyl-phosphonic acid (HFP), an analog of the farnesyl pyrophosphate needed for farnesylation, was preserved. The triazole ring was positioned such as to coordinate the zinc atom, while the carboxamide carbonyl group was moved as close as possible to a structured water molecule known to form a hydrogen bond with other FTase inhibitors.[16] Molecule **15f** was then rotated around the triazole...C–O axis to orient the two benzylic substituents toward the hydrophobic back-wall of the binding cavity. Finally, the structure of the FTase/**15f**/HFP ternary complex was minimized using the Tripos force field[17] and the Kollman All-Atom charge set.
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