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5-Imidazolyl-quinolinones, -quinazolinones and -benzo-azepinones as Farnesyltransferase Inhibitors

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Abstract—The evaluation of structure–activity relationships associated with the modification of the R115777 quinolinone ring moiety displaying potent in vitro inhibiting activity is described.

Oncogenic mutations of the *ras* gene are frequently found in approximately 30% of human cancers including a substantial proportion of pancreatic and colon adenocarcinomas.[1,2] By preventing attachment of the Ras protein to the cell membrane, inhibitors of the enzyme farnesyl protein transferase (FPT) prevent a key step in the post-translational processing of Ras and the subsequent propagation of the cell growth promoting signals.[3–10] Hence it was hypothesized that farnesyl protein transferase inhibitors (FTI's) could be very useful as anticancer agents for tumors in which Ras contributes to transformation. With the advent of potent FTI's it has become clear that the antitumor activity of the class is quite complex involving other farnesylated proteins such as Rho B, centromere associated proteins or modulation of transcriptional events.[11–14]

The discovery and progression to phase III clinical studies of R115777 (**1**)[15,16] have spawned in our group extensive structure–activity relationship (SAR) studies for this class of compounds. One identified in vivo metabolite[17] of R115777 is the *N*-demethylated quinolinone **2** (Fig. 1) which displayed a 10-fold lower in vitro potency on cells than **1**.

In attempts to block or attenuate this metabolization, 1,8-annelated quinolinones were developed.[18] Furthermore the attempts for solubility, polarity and pharmacokinetic profile improvements of R115777 led us to several other modifications of the quinolinone backbone. We report here features that are consistent with maintaining high enzyme activity and cellular potency and show their impact on in vivo results in murine models.

The pyrrolo[3,2,1-*ij*]quinolin-4-one **14**, **18** and the 2,3-dihydro-1*H*,5*H*-benzo[*ij*]quinolizin-5-one **15**, **19** were prepared as described in Scheme 1. 2,3-Dihydro-1*H*-indole **3** and tetrahydroquinoline **4** were acylated and cyclized into tetrahydro-quinolinone and-quinolizinone **8** and **9**. Then Friedel–Craft acylation of **8** and **9** gave preferentially isomers **10** and **11**. Oxidation of **10** and **11** was followed by addition of the lithio derivative of 1-methylimidazole onto the ketone to provide **14** and

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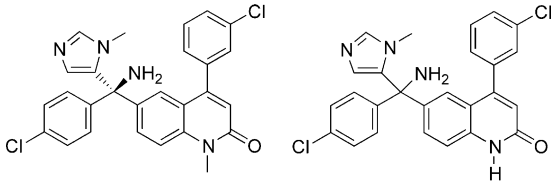
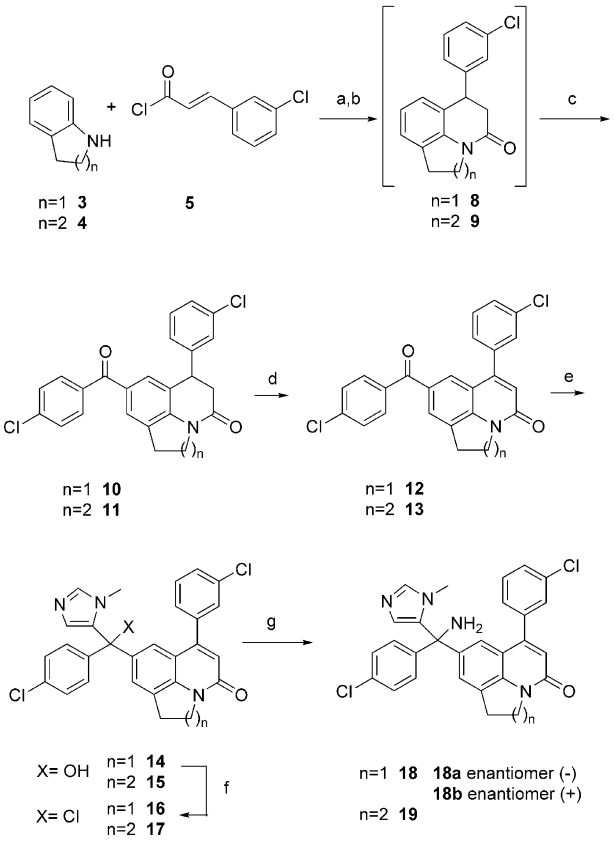


Figure 1. Structure and FPT inhibitory potency of R115777 **1** and its desmethyl metabolite **2** (racemic form).

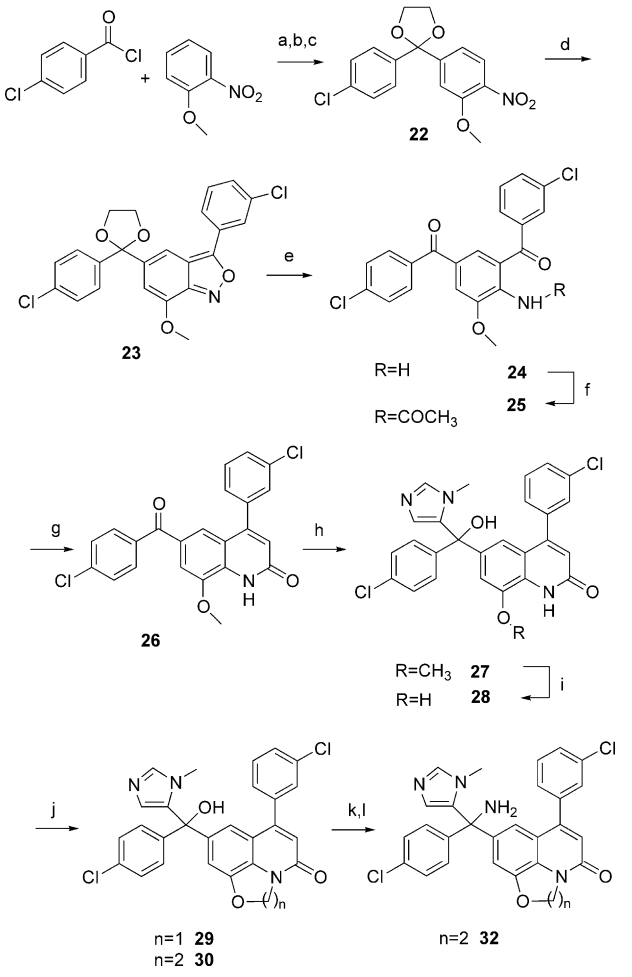


Scheme 1. Reagents and conditions: (a) Et_3N, CH_2Cl_2, rt, 73–98%; (b) PPA, 140 °C; (c) 4-chlorobenzoic acid, PPA, 140 °C, 20–45%; (d) Br_2, PhBr, 160 °C, 22–43%; (e) (i) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (ii) *n*-BuLi, −70 °C, 30–80%; (f) $SOCl_2$, 40 °C; (g) NH_4OH, THF, 5 °C, 23–38%.

15. Substitution of the hydroxy group by a chlorine and subsequent condensation of ammonia gave finally compounds **18** and **19**.

The same scheme could not be applied to the synthesis of oxazolo[5,4,3-*ij*]quinolin-4-one **29** or pyrido[1,2,3-*de*]-1,4-benzoxazin-5-one **32** as Friedel–Craft acylation of intermediates of type **8**,**9** did not give the correct isomer. Therefore we stepped back to the formation of the 8-methoxyquinolinone intermediate **26** taking advantage of the experience on quinolinone chemistry gained with the R115777 project (Scheme 2).[19–21]

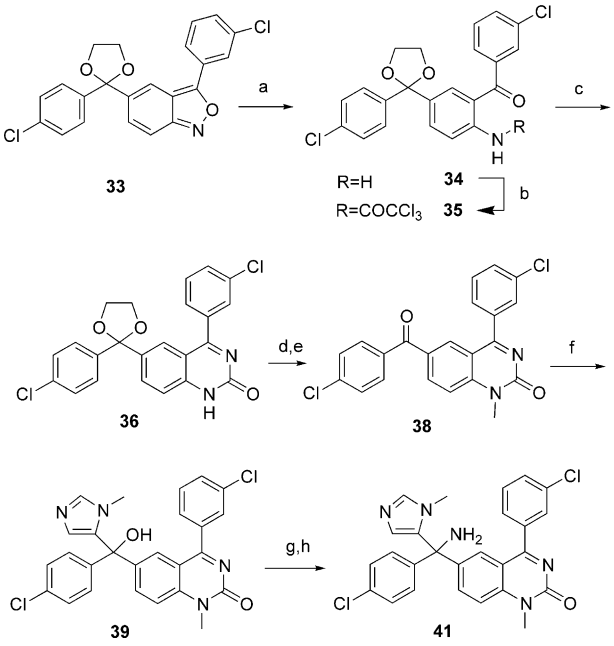
Acylation of the 1-methoxy-2-nitrobenzene by the 4-chlorobenzoyl chloride gave the (4-chlorophenyl)(3-hydroxy-4-nitrophenyl)methanone **20** which was then alkylated into **21** and ketalized. Reaction of **22** with



Scheme 2. Reagents and conditions: (a) $AlCl_3$, CH_2Cl_2; (b) CH_3I, K_2CO_3, CH_3CN; (c) ethyleneglycol, APTS, Toluene, 50%, 3 steps; (d) 3-chlorobenzyl cyanide, NaOH, MeOH, 27%; (e) H_2, Pt/C, HCl conc., THF, 84%; (f) Ac_2O, toluene; (g) tBuOK, DME, 78%; (h) (i) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (ii) *n*-BuLi, −70 °C, 88%; (i) BBr_3, CH_2Cl_2; (j) $Br–(CH_2)_n–Br$, K_2CO_3, DMF, 31%; (k) $SOCl_2$, RT; (l) NH_3/*i*PrOH, THF, 10 °C, 50%.

3-chlorobenzyl cyanide in presence of a base provided the benzisoxazole **23** which was converted into the corresponding *ortho*-aminobenzophenone **24** by hydrogenation under acidic conditions. Acylation and base-catalyzed cyclization into quinolinone **26** were followed by condensation of the lithio derivative of 1-methylimidazole onto the ketone to provide **27**. Demethylation of **27** by BBr_3 gave the quinolinone **28** which was converted by intramolecular alkylation into quinolin-4-one **29** and benzoxazin-5-one **30**.

Benzisoxazole chemistry was also used as starting point to synthesize the quinazolinone **41**, a strict analogue of R115777. **33**[22] was converted into *ortho*-amino benzophenone **34** by hydrogenation and the amino moiety acylated by trichloroacetyl chloride to afford **35**. Cyclization of **35** into a quinazolinone was achieved by heating it in the presence of ammonium acetate in a polar solvent. Subsequent cleavage of the ketal ring followed by *N*-alkylation provided the ketone **38**. Compound **41** was then obtained in three steps following the chemistry presented in Scheme 3.

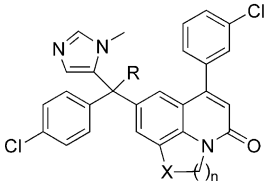


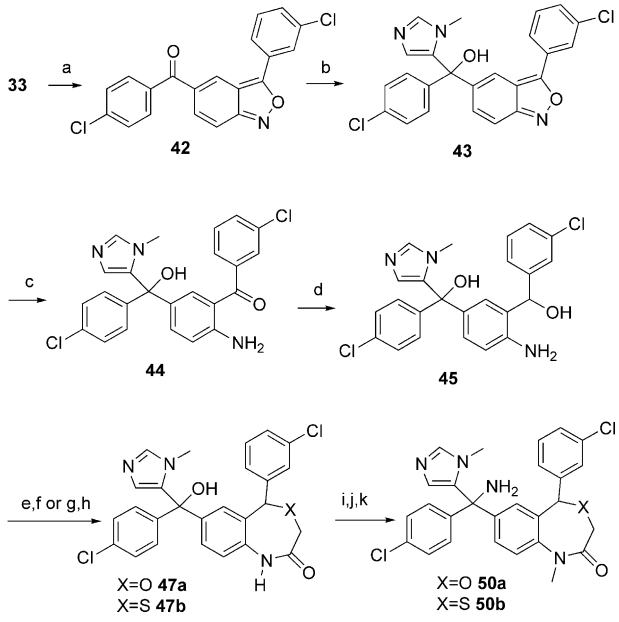
Scheme 3. Reagents and conditions: (a) H_2, Pd/C, MeOH; (b) $ClC(O)CCl_3$, Et_3N, CH_2Cl_2; (c) $AcONH_4$, HMPT, 100 °C, 76%, three steps; (d) HCl 3 N, MeOH, 95%; (e) CH_3I, NaH, DMF, 65%; (f) (i) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (ii) *n*-BuLi, −70 °C, 35%; (g) $SOCl_2$, 40 °C; (h) NH_3/*i*PrOH, THF, 10 °C, 50%.

As some benzodiazepines[23] have shown in the past some FPT inhibitory effect the ring enlargement of the quinolinone into benzoxa-, benzothia-, or benzo-diazepinones was naturally envisioned. To prepare the 1,5-dihydro-1-methyl-4,1-benzoxazepin-2(3*H*)-one **50a** or benzothiazepin-2(3*H*)-one **50b** we first condensed the 1-methyl-5-imidazolyl moiety onto the 5-(4-chlorobenzoyl)-benzisoxazole **42**, obtained from **33** by cleaving the ketal moiety in acidic media, to provide **43**. The derivative **43** was converted into the corresponding *ortho*-aminobenzophenone **44** as already described and the keto function was reduced into the key alcohol **45**. Bromoacetyl bromide or thioacetic acid were then condensed on the amino and alcohol functions of **45** to provide the heterocycles **47a** and **47b**. *N*-methylation of the amide nitrogen was followed by the chlorination and amination steps already depicted to afford **50a** and **50b** (Scheme 4).[24]

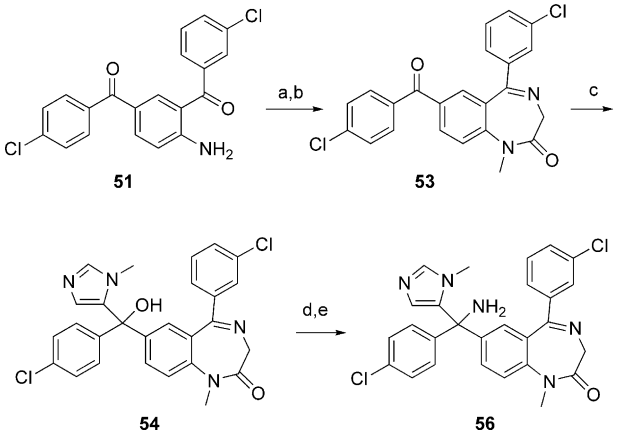
Benzodiazepinone **56** was prepared in a similar way from [2-amino-5-(4-chlorobenzoyl)phenyl](3-chlorophenyl)-methanone **51**[25] except that the imidazole was introduced after heterocycle formation as depicted in Scheme 5.

The structure–activity relationships for these compounds are presented in Tables 1–3.





Scheme 4. Reagents and conditions: (a) HCl 3N; (b) (i) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (ii) *n*-BuLi, −70 °C, 58%; (c) $TiCl_3/H_2O$ THF, 5 °C, 69%; (d) $NaBH_4$, MeOH; (e) $BrC(O)CH_2Br$, K_2CO_3, CH_2Cl_2; (f) tBuOK, *i*PrOH, 65%; (g) $HOC(O)CH_2SH$, HCl 6 N, 98%; (h) toluene, $MgSO_4$, 30%; (i) CH_3I, NaH, DMF, 13–19%; (j) $SOCl_2$, 5 °C; (k) NH_3/*i*PrOH, THF, 27–45%.



Scheme 5. Reagents and conditions: (a) ethylglycinate, pyridine, mol. sieves, 88%; (b) NaH, CH_3I, DMF, 43%; (c) (i) 1-methylimidazole, $ClSiEt_3$, *n*-BuLi, THF, −70 °C; (ii) *n*-BuLi, −70 °C, 38%; (d) $SOCl_2$, 5 °C; (e) NH_3/*i*PrOH, THF, 5 °C, 48%.

A survey of 1,8-annelated analogues of R115777 (Table 1) revealed that X = C or O had only a minor impact on enzyme activity, whereas X = O significantly decreased cellular activity compared to R115777. As **18** showed the best enzyme and cellular potency, it was separated into its enantiomers **18a** and **18b**.[27] Stereochemistry clearly has an influence on in vitro potency and **18b** was tested in murine models (Table 2). Relative to R115777, metabolism was slightly reduced as foreseen. But **18b** showed a decrease in activity at a dose of 25 mg/kg probably due to several factors including a lesser solubility and a reduction of the free circulating fraction in plasma.

We turned our attention towards more drastic modifications of the R115777 skeleton, still keeping intact the

Table 1. FPT inhibition results for compounds **14–32**

Compd	X	*n*	*R*	FPT (enz) IC_{50}, nM[a]	FPT (cells) IC_{50}, nM[b]	GGPT IC_{50}, μM[c]
1	—	—	NH_2	0.8	1.7	10
2	—	—	NH_2	3.5	23	nt
14	CH_2	1	OH	1	9	nt
18	CH_2	1	NH_2	0.5	4	1.5
15	CH_2	2	OH	4	54	nt
19	CH_2	2	NH_2	1.7	9	nt
29	O	1	OH	6	45	51% inh@10 μM
30	O	2	OH	6	62	22% inh@10 μM
32	O	2	NH_2	1.6	>500	38% inh@10 μM
18a[d]	CH_2	1	NH_2	31% inh@0.1 μM	nt	nt
18b[d]	CH_2	1	NH_2	0.5	3.3	0.6

nt, not tested.
[a]The concentration required to reduce the FPTase-catalyzed incorporation of [*3H*]-farnesylpyrophosphate into a biotinylated laminB peptide by 50%.
[b]See ref 26.
[c]The concentration required to reduce the Geranylgeranylprotein transferase-I (GGPTase-I) catalyzed incorporation of [^{3}*H*]-geranylgeranylpyrophosphate into a biotinYRASNRSCAIL substrate by 50%.
[d]ee > 98%.[27]

Table 2. Comparison of in vivo results for compounds **R115777** and **18b**

Compd	Metabolization[a] (%)	In vivo[b] % inhib.	Protein binding[c] % free
R115777	66	37	0.66
18b	74	28	0.25

[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 injected with T24 NIH 3T3 cells, percentage of tumor weight inhibition.[29]
[c]Percentage of free circulating fraction of drug in plasma.

Table 3. FPT inhibition results for compounds **41**, **50a**, **50b** and **56**

Compd	FPT (enz) IC_{50}, nM[a]	FPT (cells) IC_{50}, nM[b]	GGPT IC_{50}, μM[c]
41	6.5	64	nt
50a	48% inh@0.1 μM	nt	nt
50b	29	nt	nt
56	22% inh@0.1 μM	nt	32% inh@10 μM

[a,b,c]See corresponding footnotes in Table 1. nt, not tested.

basic features previously identified as crucial, namely, the C5-linked imidazole, the two chloro-phenyl moieties and the *N*-methyl–amide bond.[30]

Quinazoline analogue **41** displayed a reduced activity in vitro (Table 3) but owing to its lower calculated logP value (3.4 compared to 4.2 in **1**), we considered that the introduction of another nitrogen atom in R115777 backbone could result in improved solubility. However, no clear in vivo activity could be detected at a dose of 25 mg/kg.

Quinoline ring enlargement into benzo-azepinone close analogues of R115777 was our next attempt of modification. However the three compounds synthesized showed inferior in vitro enzymatic profile than R115777 and therefore they were not considered as valuable candidates for further studies.

Modification of the R115777 backbone has led us to derivatives showing similar but not superior in vitro and in vivo FPT inhibiting potency. In vitro selectivity of R115777 for FPTase versus GGPTase I has also been conserved within the tested compounds. Further chemical efforts in these series are being focused on improving activity, solubility and pharmacokinetic parameters.

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26. Cellular assays for H-*ras* transforming function: T24

F1H-*ras* transfected NIH 3T3 cells were inoculated into six-well cluster dishes at an initial density of 200,000 cells per well in 3 mL of complete growth medium. Test compounds were added at concentrations ranging from 0.5 to 500 nM in dimethylsulfoxide. Cells were allowed to proliferate to high saturation densities beyond confluence for 4 days. Cell numbers were quantified by detaching cells in 1 mL of trypsin/EDTA and counting cell suspensions on a Coulter particle counter. IC_{50} (cells): The concentration required to decrease the cell number by 50% as compared to a control (untreated) sample.

27. (12.4 g) was separated and purified by chiral column chromatography over Chiracel OD® (eluent: 100% CH_3OH). Two pure fraction groups were collected and the solvent was evaporated. The first fraction was crystallized from 2-propanol/DIPE. The precipitate was filtered off and dried, yielding 4.4 g of (−)-8-[amino(4-chlorophenyl)(1-methyl-1*H*-imidazol-5-yl)methyl]-6-(3-chlorophenyl)-1,2-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one **18a**. The solvent of the second fraction group was evaporated. The second fraction was crystallized from 2-propanol/ DIPE. The precipitate was filtered off and dried, yielding: 4.1 g of (+)-8-[amino(4-chlorophenyl)(1-methyl-1*H*-imidazol-5-yl)methyl]-6-(3-chlorophenyl)-1,2-dihydro-4*H*-pyrrolo[3,2,1-*ij*]quinolin-4-one **18b**.

28. Metabolism: Compounds were incubated at 30 μM with human subcellular liver fractions (12,000 g supernatants) during 120 min at a final microsomal protein concentration of 1 mg/mL in a presence of an NADPH-generating system. Reactions were stopped by freezing the incubates in dry ice, and samples were stored at $<18\,^{\circ}C$ until analysis. Samples were analysed for unchanged compounds by HPLC-UV. The compounds were also incubated with boiled 12,000 g fractions to check the compound stability and recovery.

29. Tumor studies in nude mice: Tumor cell lines maintained as monolayer cultures were detached by tripsynisation. Tumor cell suspensions were pooled after trypsin was inactivated by the addition of serum-containing medium. Cells were collected by centrifugation and washed once in HBSS. Cell suspensions were adjusted to a final concentration of 1×106 cells/0.1 mL of HBSS. Mice were inoculated with a single s.c. injection of 0.10 mL of tumor cell suspension in the inguinal region of the thigh. Mice were housed five per cage, with 15 mice randomly assigned to treatment groups. Three days after tumor inoculation, treatment with R115777 **1** or **18b** was initiated. Compounds were administered once daily by oral gavage in a 20% β-cyclodextrin vehicle as a volume of 0.10 mL of solution/10 g of body weight. Control groups received the same dosage/volume of the 20% β-cyclodextrin vehicle. Body weight and tumor size as determined by caliper measurements were monitored weekly. At the end of the study mice were sacrificed by CO_2 asphyxiation. Tumors were excised, weighted, and fixed immediately in 4% paraformaldehyde. ANOVA, mean values for treatment groups, and SE for in vivo parameters were calculated on a VAX computer using IMSL subroutines compiled by the Science Information Department of the R.W. Johnson Pharmaceutical Research Institute. A value of $p < 0.05$ was considered significant.

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