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Review Article

Ras farnesyltransferase inhibitors

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CONTENTS

Introduction 1099
Development of farnesyltransferase inhibitors 1100
Inhibitors based on the CAAX motif 1100
Inhibitors from compound library screening 1102
Conclusions 1104
References 1104

Introduction

The recent development of farnesyltransferase (FTase) inhibitors impairing the growth of Ras-transformed cells demonstrates that membrane association of Ras is important for its biological functions. In the late 1980s, it was established that Ras is modified at the C-terminus leading to its membrane association and tumorigenicity (1-3). The first step in this modification is the addition of a 15-carbon farnesyl group to Ras utilizing farnesyl pyrophosphate (FPP) as the prenyl donor. The farnesylation reaction, which is catalyzed by FTase, provides cytosolic Ras with requisite hydrophobicity for membrane attachment (4, 5).

All Ras proteins share a common consensus sequence, termed the CAAX motif at their C-termini. This CAAX box is characterized by a cysteine residue preceding 2 aliphatic amino acids and a terminal “X”, which in many cases is methionine, serine, cysteine, alanine or glutamine. Attachment of a farnesyl group occurs via a thioether linkage to the cysteine residue in the CAAX region. The 3 C-terminal amino acids are then cleaved by a protease (6-9) followed by carboxymethylation of the C-terminal cysteine (10, 11). In some cases, membrane attachment is further promoted by palmitoylation of a cysteine located just upstream of the farnesylated cysteine (H-ras) or the presence of a polylysine stretch upstream of the CAAX region (K-ras 4B) (2, 12, 13).

In addition to FTase, the protein prenyltransferase family is comprised of 2 related enzymes, geranylgeranyltransferase (GGTase) I and II. GGTase I, recognizing protein substrates with a leucine or phenylalanine at the C-terminus, is structurally and functionally very similar to FTase; both enzymes are heterodimeric. While FTase and GGTase I share a common α-subunit, the β-subunit is highly homologous. The crystal structure of the enzyme FTase was published at 2.25 Å resolution (14). The active site consists of 2 clefts which are at the junction of a bound zinc ion. More recently, the crystal structure of acetyl-Cys-Val-Ile-selenoMet-OH and α-hydroxyfarnesyl phosphonic acid (α-HFP) complexed with rat FTase was also reported (15). The α-HFP was shown to be in an extended conformation in the active site cavity. The backbone of the bound CAAX peptide was extended as well and its side chains interact with both FTase and α-HFP. The cysteine sulfur coordinates with the zinc ion. This ternary complex structure showed major rearrangement of the enzyme with the side chains of several residues in the active site being in a different location when compared to the unliganted FTase structure indicating that the enzyme can change conformation upon binding of the inhibitor. The GGTase I structure has not been reported.

Pharmaceutical interest in FTase stems from its critical role in promoting Ras functionality. Mutations in the CAAX sequence render *ras*, which is otherwise oncogenic, to a nontransforming state. Oncogenic ras has been implicated in a wide variety of human cancers including colon, pancreatic, breast, lung, liver, renal, ovarian and stomach carcinomas (16). Inhibition of Ras FTase represents a potential therapy for the treatment of proliferative diseases including cancer, and interest in FTase inhibitors has steadily increased since the early 1990s when reports of their identification first appeared. At that time, FTase inhibitors were envisioned as a potential means to selectively target oncogenic Ras. However, we now know that tumors need not possess a *ras* mutation to have their growth impaired by treatment with a FTase inhibitor (17). A recent study indicating that FTase inhibitors may have gain-of-function effects with respect to geranylgeranylated RhoB further point to the complexity of understanding the mechanism of FTase inhibitors (18).

Multiple FTase inhibitors are now being evaluated in clinical trials. We will present an overview of the various classes of FTase inhibitors reported to date and will highlight the progress made in this field of research.

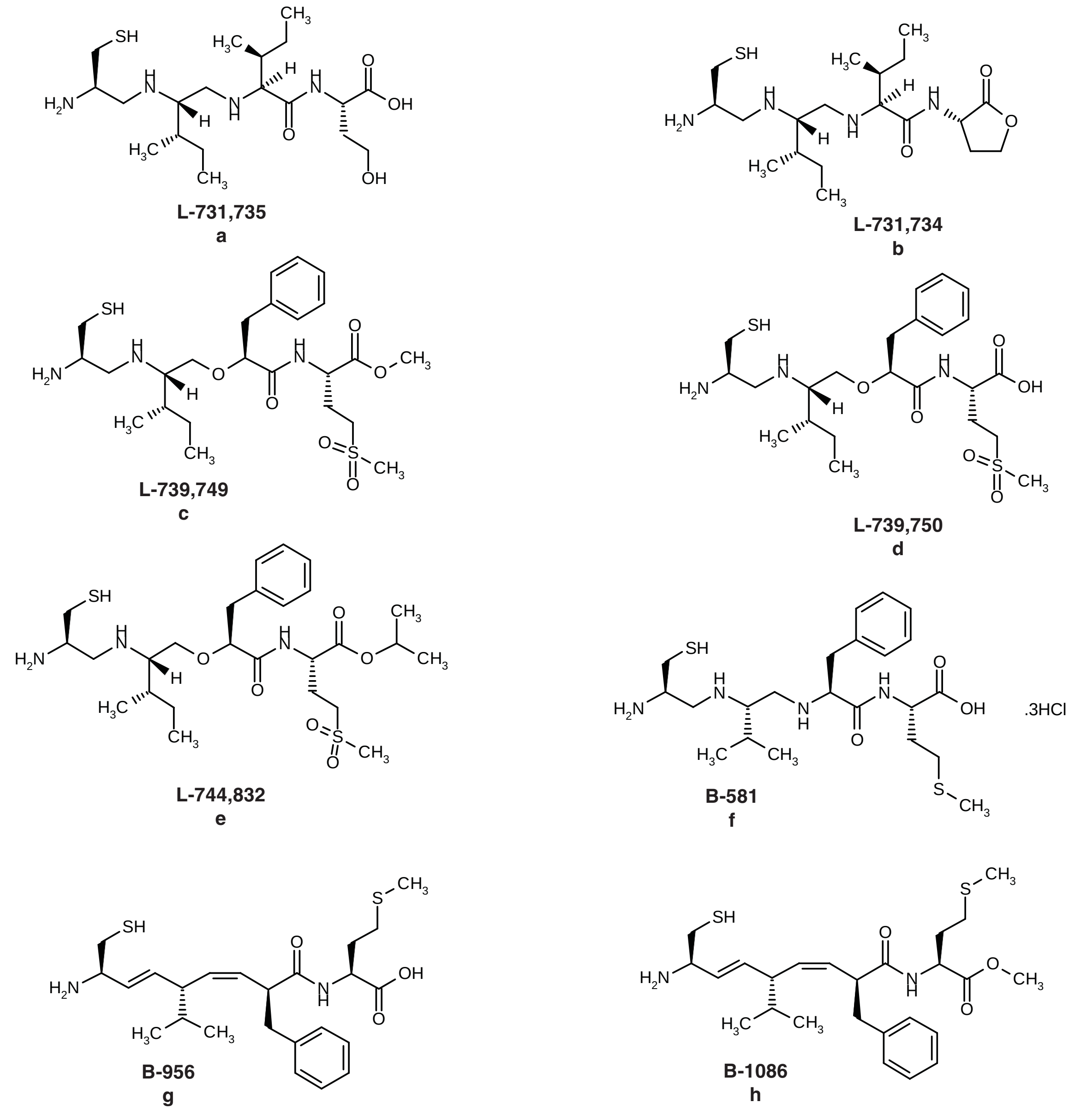


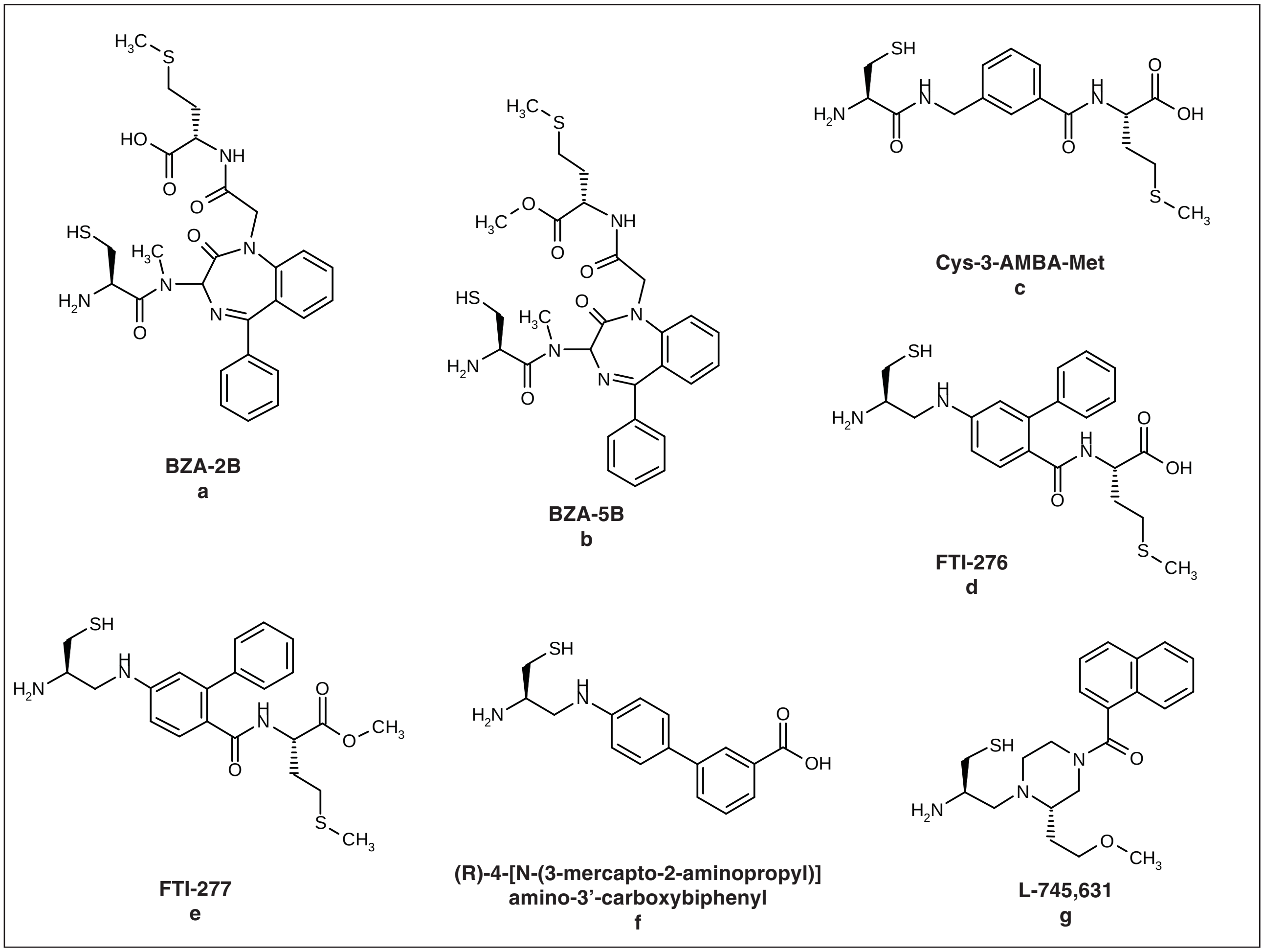
Fig. 1. CAAX peptidomimetic inhibitors of FTase.

Development of farnesyltransferase inhibitors

Many classes of FTase inhibitors have been discussed in the literature with most of the work focusing on mimetics of the CAAX box of the ras protein. A second class of compounds which have been successful with advancement to clinic has been inhibitors which were developed from compounds identified from compound library screening. These two classes of inhibitors will be the focus of this review. Other reviews can be consulted for inhibitors based on the second substrate of FTase, namely FPP mimetics and inhibitors from natural product sources (19, 20).

Inhibitors based on the CAAX motif

The first reported FTase inhibitors were based on the CAAX motif of the natural p21 H-ras substrate CVIL. The tetrapeptide itself was found to be a competitive inhibitor with p21 ras (5). Modifications of the tetrapeptide where isoleucine was replaced by the aromatic amino acid phenylalanine led to a potent ras FTase inhibitor that was not a substrate and exhibited an IC_{50} value of 25 nM against bovine FTase (21). Further modifications have also been reported. Introduction of reduced bond isosteres and homoserine for methionine led to L-731,735 (Fig. 1a) which has an IC_{50} of 18 nM against



BZA-2B
a

BZA-5B
b

Cys-3-AMBA-Met
c

FTI-276
d

FTI-277
e

(R)-4-[N-(3-mercapto-2-aminopropyl)] amino-3'-carboxybiphenyl
f

L-745,631
g

Fig. 2. Constrained analogs of CAAX peptidomimetic inhibitors of FTase.

bovine FTase. The corresponding homoserine lactone, L-731,734 (Fig. 1b), characterized by an IC$_{50}$ of 280 nM, was active in cells. L-731,734 inhibited Ras processing in v-*ras* transformed cells and growth in soft agar, and reduced ras farnesylation by 50% in NIH 3T3 fibroblasts in culture at 50 μM (22, 23). Both pseudopeptides were found to be selective for FTase (IC$_{50}$ for GGTase I, > 10 μM) (22).

L-739,749 (Fig. 1c), the methyl ester analog of L-739,750 (Fig. 1d), is also a pseudopeptide based on the CAAX motif. It is a potent selective inhibitor of bovine FTase with an IC$_{50}$ of 240 nM (24). In cells, it was found to inhibit Ras farnesylation by 50% at a concentration between 0.1 and 1 μM (25). Complete inhibition of growth in soft agar was obtained with 10 μM of L-739,749 and partial inhibition with concentrations as low as 2.5 μM (24). However, it had no effect at concentrations up to 10 μM on growth of rat-1 cells transformed by v-raf and v-mos. *In vivo* efficacy of L-739,749 was further demonstrated as evidenced by a 51% and 66% reduction in the average weight of N-ras- and H-ras-dependent tumors (24). Another CAAX mimetic, L-744,832 (Fig. 1e), after daily administration for 2 weeks, resulted in complete regressions of MMTV-v-ras tumors (26). H-Ras tumor-bearing transgenic mice that have lost the p53 tumor suppressor gene also regressed upon similar treatment with L-744,832 (27).

Systematic modifications of the CVFM tetrapeptide by replacement of the amino terminal amide bonds led to the inhibitor B-581 (Fig. 1f) (28), which has an IC$_{50}$ of 0.021 μM against bovine FTase. It was shown to inhibit Ras farnesylation in cells with an IC$_{50}$ of 50 μM and was reported to be 40-fold selective towards FTase *versus* GGTase I *in vitro* and in transformed cells (29). Further modifications led to the inhibitor B-956 (Fig. 1g) which inhibits H-ras farnesylation with an IC$_{50}$ of 11 nM (30). B-956 and its methyl ester B-1086 (Fig. 1h), inhibited colony formation of 14 human tumor cell lines grown in soft agar at concentrations between 0.2 and 60 μM (30). B-956/B-1086 at 100 mg/kg inhibited tumor growth of EJ-1 human bladder carcinomas by about 60% (30).

Replacement of the 2 aliphatic amino acids by a benzodiazepine mimicking a peptide turn, BZA-2B (Fig. 2a), gave a potent inhibitor of FTase with an IC$_{50}$ of 0.85 nM against recombinant rat FTase (31). The corresponding methyl ester, BZA-5B (Fig. 2b) was found to be active in cells. At μM concentrations, it was able to restore a nontransformed growth phenotype to *ras*-transformed cells and was subsequently shown to interrupt the MAP kinase activation pathway in H-ras transformed cells (31, 32). It was reported that BZA-5B blocks farnesylation of lamin proteins with an IC_{50} comparable to that obtained for p21 ras (33). However, it did not interfere with a variety of cellular functions expected to be farnesylation-dependent that might affect cell growth and viability (33). This report suggested that inhibition of Ras farnesylation may represent an effective means to specifically target malignant cells. Although these results showed no interference with normal cellular function, the blockade of farnesylation of other proteins should still be of concern. As more potent agents are developed and tested in various *in vivo* models, it will become of utmost importance to monitor closely the modification of prenylated proteins involved in normal cellular functions (*e.g.*, rhodopsin kinase, transducin). However, as will be addressed below, clinical candidates have not yet revealed an unacceptable toxicity profile.

The 2 aliphatic residues have also been replaced by a hydrophobic spacer, 3-amino-methyl benzoic acid (3-AMBA) (Fig. 2c) (34). Cys-3-AMBA-Met inhibits p21 ras FTase purified from human colon carcinoma (Colo-205) and Burkett's lymphoma (Daudi) cells with IC_{50} values of 60 and 120 nM, respectively (34). Substitution of the 2 aliphatic residues was also carried out with 4-AMBA and 3- and 4-amino benzoic acid (3- and 4-ABA) (35). Cys-4-AMBA-Met was 17-fold less potent than the corresponding 3-AMBA analog and Cys-3-ABA-Met also showed reduced activity (36). However, Cys-4-ABA-Met had an IC_{50} of 50 nM. Introduction of functionality such as N,S-di-Cbz-Cys-3-AMBA-Met-OCH_3 led to compounds which could penetrate NIH 3T3 cells and disrupt p21 ras plasma membrane association (36). Substitution at the 2-position of the 4-aminobenzoic acid moiety with a phenyl group led to FTI-276 (Fig. 2d), a potent inhibitor of ras FTase with an IC_{50} of 0.5 nM against the human enzyme, which exhibited 100-fold selectivity when assayed against human GGTase I (37). This compound, when dosed parenterally, blocked the growth in nude mice of a human lung carcinoma expressing oncogenic K-ras (38). The corresponding methyl ester FTI-277 (Fig. 2e) had an IC_{50} of 50 nM against human FTase and inhibited cellular farnesylation of H-ras with an IC_{50} of 100 nM. Furthermore, FTI-277 induced accumulation of cytoplasmic nonfarnesylated H-ras that was able to bind raf, forming a ras/raf complex where raf was not activated (37). This compound did not inhibit processing of rap by GGTase I at concentrations as high as 10 μM (37). Further design led to the replacement of the AAX tripeptide with biphenyl derivatives (39). The analog (*R*)-4-[*N*-(3-mercapto-2-aminopropyl)]amino-3'-carboxybiphenyl (Fig. 2f) was found to inhibit rat brain FTase with an IC_{50} of 50 nM, while it inhibited GGTase I with an IC_{50} of 100 μM (39). It inhibited Ras farneslyation at a concentration of 50 μM in H-ras transformed cells (39). Further modifications of FTI-276 were carried out and recently reported (40). Replacement of the cysteine moiety by a pyridyl ether group and substitution of the phenyl ring led to potent FTase inhibitors (IC_{50} values, < 1 nM) (40).

A piperazine analog, L-745,631 (Fig. 2g), was reported to suppress tumor growth in nude mice (41, 42). It was found to be potent against bovine FTase (IC_{50} = 5 nM) and selective against GGTase I (IC_{50} = 10 μM). It was shown to inhibit Ras farnesylation with an IC_{50} of 0.5 μM and was noncytotoxic to NIH 3T3 cells at concentrations up to 100 μM. In H-ras fibroblast tumors, s.c. dosing of 40 mg/kg suppressed tumor growth by 75% (41).

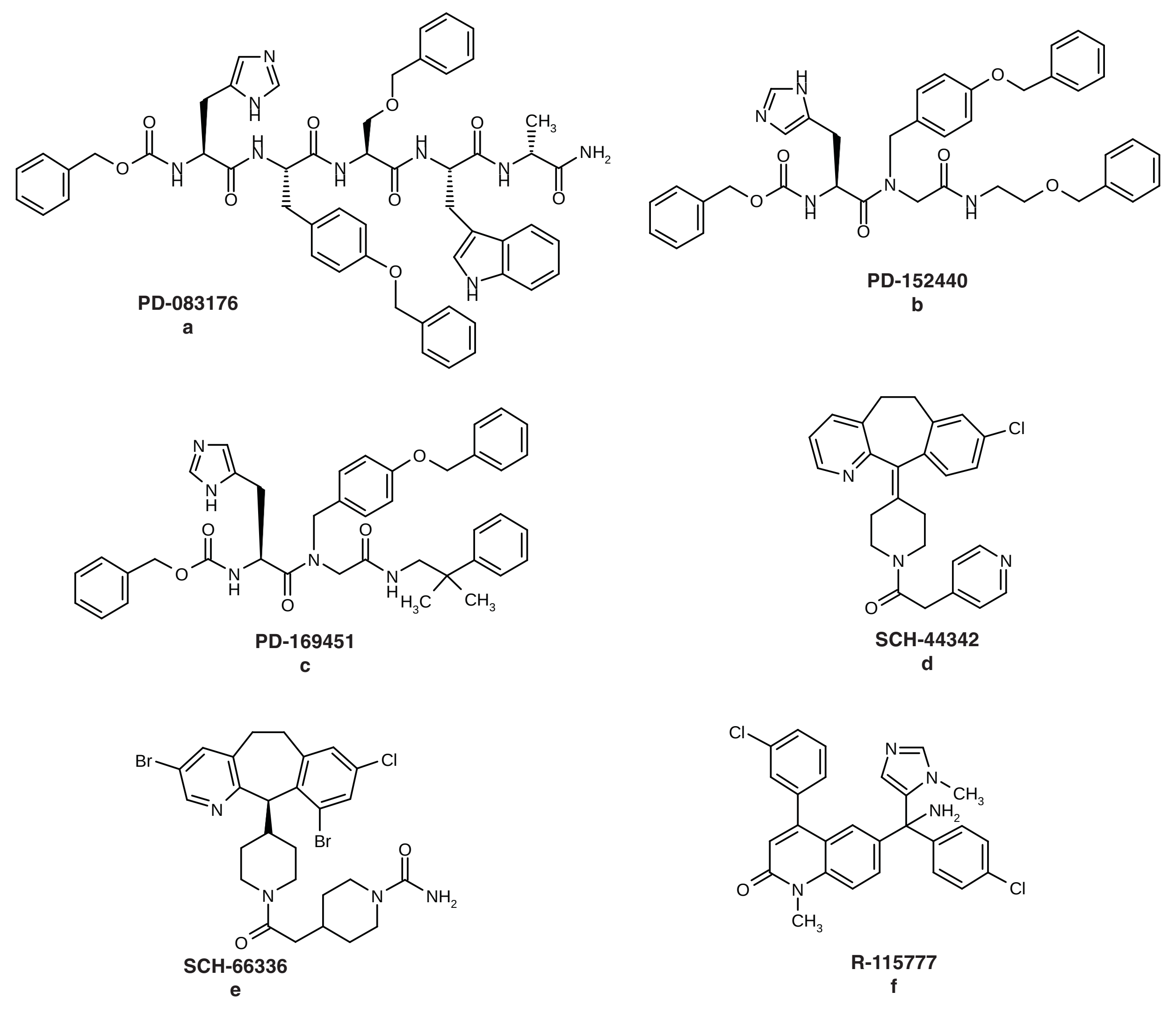
While extensive work has been carried out with CAAX-based inhibitors, no clinical candidates have been reported to date. It is likely that their peptidomimetic nature results in an unacceptable pharmacokinetic profile.

Inhibitors from compound library screening

Through high volume screening, PD-083176 was identified as a potent and selective FTase inhibitor (Fig. 3a), exhibiting IC_{50} values of 17 nM and 1.25 μM against Ras FTase and GGTase I, respectively (43). PD-083176 when injected into *Xenopus* oocytes was shown to significantly impair their Ras-dependent maturation in response to insulin. PD-083176 was shown to be competitive against farnesyl pyrophosphate, a unique feature of this class of inhibitors.

Since PD-083176 lacks any significant cellular activity presumably due to poor cellular permeability, truncation of the pentapeptide was carried out. Deletion of the Trp-D-Ala moiety from the pentapeptide led to a tripeptide inhibitor with an IC_{50} of 0.42 μM (a 25-fold loss in activity compared to PD-083176) (44, 45). Substitution of the serine residue by *O*-benzylethylamine, followed by transposition of the Tyr(OBn) side chain to the α-nitrogen, was then carried out. The resulting compound, PD-152440 (Fig. 3b), proved to be active against FTase with an IC_{50} of 0.26 μM and inhibited cellular farnesylation of Ras at a concentration of 1 μM (46).

Structure-activity relationship studies carried out on PD-152440 focused on the C-terminus portion (47). Replacement of the *O*-benzyl group with a phenyl group resulted in a compound 10-fold more potent than PD-152440 at inhibiting cellular farnesylation (0.1 μM). The selectivity towards FTase against GGTase I was also increased. Further work led to PD-169451 (47) (Fig. 3c), a compound exhibiting an IC_{50} of 4 nM and inhibiting cellular H-ras farnesylation at 0.05 μM. This inhibitor, which was 4500-fold selective for FTase, was also competitive against FPP with a K_i value of 0.74 nM. Evaluation of the *in vivo* antitumor activity of this compound showed it to be significantly active against s.c. ras fibroblast xenografts. Treatment with PD-169451 (i.p.) resulted in dose-dependent tumor growth inhibition with 88% inhibition observed at the high dose of 150 mg/kg/day (47). This agent was subsequently shown to be equally effective against a panel of human tumors (48, 49).



PD-083176
a

PD-152440
b

PD-169451
c

SCH-44342
d

SCH-66336
e

R-115777
f

Fig. 3. FTase inhibitors from screening of compound library.

A series of nonpeptide tricyclic FTase inhibitors have been reported. SCH-44342 (Fig. 3d) inhibits rat brain FTase with an IC_{50} of 0.25 μM and is reported to be selective against GGTase I (IC_{50} = 144 μM) (50). In Cos-7 monkey kidney cells transiently expressing H-ras [Val^{12}], ras processing was inhibited by SCH-44342 with an IC_{50} of 3 μM (50). SCH-44342 was 21% orally bioavailable. However, its half-life was less than 10 min. When administered orally to nude mice bearing SW-620 colon tumors, SCH-44342 reduced tumor weight by 42% (51, 52). An extensive structure-activity relationship study on this class of FTase inhibitor was recently reported (53).

Further modification of the tricyclic ring system, with incorporation of bromine at the 7- or 10-position, led to compounds with increased potency against FTase (54, 55). This series of new analogs were found to have improved pharmacokinetic profiles, with longer half-lives upon oral administration. *In vitro* and *in vivo* evaluation of selected compounds from this series led to the identification of SCH-66336 (Fig. 3e), which inhibits FTase with an IC_{50} of 1.9 nM (54). SCH-66336 administered orally to rodents and primates resulted in 40% and 76% inhibition, respectively, in the growth of DLD-1 colon tumors xenografts (54). It was also found to significantly inhibit the growth of human pancreatic MIA-PaCa-2 and HCT-116 colon tumors (54). This compound is currently undergoing clinical evaluation.

A series of imidazolyl methyl quinolinones, exemplified by R-115777, have also been reported to be potent FTase inhibitors (56, 57). R-115777 (Fig. 3f) has been shown to inhibit H-ras and K-ras FTase with IC_{50} values of 2 and 7.9 nM, respectively. It is selective for FTase, as evidenced by only 40% inhibition of GGTase I at 50 μM (58). In nude mice bearing LoVo human colon tumors, R-115777 (100 mg/kg p.o. b.i.d.) inhibited tumor growth by 81% (59). A similar response was seen with CAPAN-2 human pancreatic tumors. Furthermore, no signs of toxicity were reported (59). R-115777 is also currently undergoing clinical evaluation.

Conclusions

Extensive work on the design and synthesis of various classes of FTase inhibitors has been carried out during the last decade and these compounds have been shown to demonstrate therapeutic utility in antitumor models. Preclinical testing of antitumor models has consistently demonstrated potential therapeutic utility for these inhibitors as well an encouraging lack of toxicity. While Janssen and Schering-Plough have progressed through phase I trials, a number of other pharmaceutical companies are now entering the clinical testing stage. The potential of FTase inhibitors as cancer therapeutics remains an exciting and challenging unknown. As results from the clinical trials begin to emerge, a better understanding of the safety and efficacy of these inhibitors for cancer will unfold.

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